ZURICH UNIVERSITY OF APPLIED SCIENCES ZHAW
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Stream biofilm response to an increasing number of non-flow periods

Bachelor Thesis

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Bachelor's Degree Program 2015/2016 | Natural Resource Sciences
Due date: February 06th, 2020

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Imprint

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Citation Etter, A., & Fischer, S. (2020). Stream biofilm response to an increasing number of non-flow periods. Bachelor Thesis. Zurich University of Applied Sciences, Wädenswil.

Keywords Intermittent waterways, intermittency, non-flow period, artificial streams, stream biofilm, frequency, duration, impact, recovery, autotrophic and heterotrophic activities, function, biomass

Picture front page Part of the Experimental Stream Facility. (Etter, 2019)

Abstract

Anthropogenic impacts and climate change are considerably altering freshwater systems. Most significant consequences of this are changes to flow regimes as well as the transformation of permanent into temporary waterways. This is problematic, as despite their role in supporting biodiversity and ecosystem processes, temporary waterways are undervalued and poorly understood. In fact, although the effects of non-flow periods are known, there is a lack of knowledge regarding how changes in frequency and duration of non-flow periods influence temporary waterways. Therefore, the goal of further research is to extend the current knowledge surrounding the effects of temporal components on the aquatic ecosystem. This bachelor thesis aims to identify how the frequency of non-flow periods affects autotrophic and heterotrophic stream biofilm. With this objective, an experiment at the Experimental Stream Facility of Catalan Institute for Water Research (ICRA) was performed. The treatments consisted of one drought duration (28 days) and three frequencies (1 period of 28, 2 periods of 14, 4 periods of 7 non-flow days). The development of the autotrophic and heterotrophic stream biofilm was measured during flow periods by means of yield of photochemistry, aerobic respiration, ecosystem metabolism and ash free dry mass. The hypotheses were that with increasing frequency, the effects on stream biofilm are less because the number of subsequent non-flow days is smaller and thus the biofilm is less stressed, and that autotrophs in the epipsammic biofilm recover more slowly than in the epilithic biofilm, because the amount of water retained in sand is higher. Both hypotheses were partially confirmed, as an increasing frequency only lessened the effect on epipsammic biofilm and only autotrophic function recovered more slowly in sand than on cobbles. In addition, the majority of the variables experienced the most severe impact and thus the quickest recovery in the same treatment. Nevertheless, at the end of the experiment none of the differences persisted. Therefore, the frequency of non-flow periods only had an effect on a short-term but not on a long-term scale and consequently, both hypotheses were discarded.

Zusammenfassung

Anthropogene Einflüsse und der Klimawandel führen zu erheblichen Veränderungen von Süsswassersystemen. Dabei zählen die Veränderung des Abflussregimes sowie der Wechsel von permanenten zu temporären Fliessgewässern zu den nennenswertesten Folgen. Trotz ihrer Wichtigkeit für die biologische Vielfalt und die Ökosystemprozesse werden temporäre Fliessgewässer bis heute unterbewertet und nur unzureichend verstanden. Die Auswirkungen von Trockenperioden sind zwar bekannt, jedoch ist nicht klar, wie Änderungen der Frequenz und Dauer von Trockenperioden die temporären Fliessgewässer beeinflussen. Es besteht ein Forschungsbedarf bezüglich der Auswirkungen von temporalen Komponenten auf das aquatische Ökosystem. Das Ziel der vorliegenden Bachelorarbeit war es deshalb, zu untersuchen wie die Frequenz von Trockenperioden den autotrophen und heterotrophen Biofilm in Fliessgewässer beeinflusst. Dazu wurde ein Experiment in den künstlichen Fliesskanälen (Experimental Stream Facility) des Katalanischen Instituts für Wasserforschung (ICRA) durchgeführt. Der Versuch bestand aus einer Trockenperiode (28 Tage) und drei Frequenzen (1 Periode von 28, 2 Perioden von 14, 4 Perioden von 7 Trockentagen). Die Reaktion des autotrophen und heterotrophen Biofilms wurde während der Fliessperioden anhand der photosynthetischen Aktivität, der aeroben Atmung, des Ökosystem-Metabolismus und des aschefreien Trockengewichts gemessen. Die dabei verfolgten Hypothesen besagen erstens, dass mit zunehmender Frequenz die Wirkung auf den Biofilm geringer ist, weil die Anzahl der aufeinanderfolgenden Trockentage kleiner ist und somit der Biofilm weniger belastet wird. Zweitens, dass sich der autotrophe Biofilm im Sand langsamer erholt als auf den Steinen, da die im Sand zurückgehaltene Wassermenge grösser ist. Beide Hypothesen wurden teilweise bestätigt, da mit zunehmender Frequenz die Reaktion nur beim epipsammischen Biofilm vermindert war und sich nur die autotrophe Funktion im Sand langsamer erholte als auf den Steinen. Bei der Mehrheit der Variablen zeigte sich zudem, dass umso grösser die Auswirkung war, desto schneller war auch die Erholung innerhalb des entsprechenden Versuchs. Am Ende des Experiments bestanden jedoch keine Unterschiede mehr, was zur Schlussfolgerung führte, dass die Frequenz von Trockenperioden nur kurzfristige aber keine langfristigen Effekte hatte. Folglich wurden beide Hypothesen verworfen.

Acknowledgements

We wish to thank various people for their time, their support and their contribution to this bachelor thesis. We would first and foremost like to thank our supervisor Dr. Vicenç Acuña of the Catalan Institute for Water Research in Girona, Spain, who made it possible for us to work at the Institute. He was always available to answer our questions regarding the research and provided us with valuable inputs for this thesis. As well, our thanks go out to Prof. Dr. Michael Döring of the Zurich University of Applied Sciences in Switzerland for supervising this thesis and giving relevant feedback. We also wish to acknowledge our co-workers Anna Freixa, Julio César López Doval, Núria Perujo who supported us in expanding our knowledge on various subjects. A special thanks go out to Carme Font, as without her help we would not have been able to carry out any statistical analyses. Furthermore, we would like to express our thanks to Jason Parry for his proofreading and for his constructive comments. Finally, we are grateful for all those who supported us at one point or another during the time we were writing this thesis.

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Abbreviations

AR	Aerobic respiration
AFDM	Ash free dry mass
COST	European Cooperation in Science and Technology
Diving-PAM	Submersible pulse amplitude modulated fluorometer
EL	Epilithic compartment
ER	Ecosystem respiration
EPA	Environmental protection agency
EPS	Extracellular polymeric substances
ES	Epipsammic compartment
ESF	Experimental Stream Facility
EU	European Union
Ft	Minimum fluorescence yield in light-adapted state
GPP	Gross primary production
ICRA	Catalan Institute for Water Research
IPCC	Intergovernmental Panel on Climate Change
NEP	Net ecosystem production
PAR	Photosynthetic active radiation
RMA	Resource management act
SMIRES	Science and management of intermittent rivers and ephemeral streams
WC	Water content
WFD	Water Framework Directive 2000/60/EC
Yeff	Effective quantum yield of photochemistry

1 Introduction

Intermittent streams and rivers are waterways whose flow ceases at some point in time and space, often experiencing a periodic loss of some or all surface water and thus creating fragments of pools and dry sections (Acuña et al., 2014; Datry et al., 2014) (Figure 1). They dominate in, but are not restricted to arid, semi-arid and Mediterranean areas. Flow intermittency is caused naturally, as well as by human activities and is influenced by the on-going issue of climate change. According to Datry et al. (2014) intermittent waterways constitute more than half of the global river network. In accordance with current research, the number of intermittent waterways is projected to increase in the future, altering hydrologic connectivity and more importantly flow regimes (Döll & Zhang, 2010; Jaeger et al., 2014; Raymond et al., 2013). Flow regimes are characterized by spatial and temporal components. According to Richter et al. (1996) the spatial dimension refers to changes in water conditions such as the previously mentioned flow cessation in a particular location. Further, it is suggested that five temporal characteristics are essential: timing, rate of change, frequency, duration and magnitude. These components are crucial to the integrity of river ecosystems, since not only do they influence specific biophysical structures, but their ecological processes and services also carry economical and societal values (Acuña & Tockner, 2010; Datry et al., 2014; Datry et al., 2017a). To quantify this influence, stream biofilm can be used as an impact indicator, due to its essential role in the bottom-up supply of energy and its major contribution to a well-functioning ecosystem (Anderson-Glenna et al., 2008; Sabater et al., 2007).

Figure 1: Examples of intermittent waterways during a non-flow and flow period. (received from Acuña, 2019)

1.1 Impact of climate change on freshwater ecosystems

According to the Intergovernmental Panel on Global Change (IPCC), during the past decade (2006–2017) the global surface temperature has on average risen by 0.87°C above the pre-industrial level. Following this trend, the warming will reach 1.5°C relative to the pre-industrial temperature between 2030 and 2052 (IPCC, 2018).

Climate change has a substantial influence on terrestrial and freshwater ecosystems. The freshwater ecosystems, however, are considered to be among the most endangered ecosystems worldwide and are therefore significantly more affected (Dudgeon et al., 2005). For instance, during the 20th century alone, over 50% of the freshwater ecosystems in Australia, New Zealand, Europe and North America were degraded (MEA, 2005a). The IPCC's (Settele et al., 2014) fifth assessment report determined that the main stressors to freshwater ecosystems are very likely to continue to be dominated by human actions as the demand for water resources grows, irrigated agricultural areas expand and urbanization increases (Malmqvist et al., 2008). It is, however, also emphasized that climate change will have a significant additional impact. Consequently, it is essential to pay attention to both the direct and indirect impact on freshwater ecosystems that is caused by climate change, as well as the intensification of human stressors and their co-occurrence, which leads to complex interactive effects (Griffith & Gobler, 2019; Settele et al., 2014). Direct influences include changes in precipitation rates and the rising temperature, which result in habitat shifts or changes in the phenology and physiology of freshwater species, ultimately altering freshwater ecosystem dynamics and food web structures (Ledger et al., 2012; Walther et al., 2002). Indirect effects via the geomorphology of aquatic ecosystems are shown, for instance, in changes in the acidification, salinization or eutrophication of water bodies (Day et al., 2008; Settele et al., 2014). The magnification of human stressors induced by climate change can be seen in nutrient polluted rivers and lakes, due to agricultural practices and wastewater (Schindler et al., 2016). Such problems are aggravated by the previously mentioned direct influences, which impact nutrient loads from catchment areas (Couture et al., 2018).

There is, of course, a certain amount of variability between the estimated impacts of various climate scenarios, in particular at a regional scale (Döll & Zhang, 2010; EEA, 2005). Nonetheless, trends concerning the overall magnitude and the direction of change are visible. For instance, the change in temperature and precipitation is forecast to increase the irregularity of spatiotemporal characteristics of flow regimes. Arid, semi-arid and Mediterranean regions, which are already low on water resources, experience droughts frequently, and show a high imbalance between water availability and water demand, are thus especially vulnerable (Christiano et al., 2017; Estrela et al., 2014).

1.2 Intermittent waterways

Since the number of intermittent waterways is growing, the reason they are still mostly considered to be a second-class rather than a unique ecosystem needs to be addressed (Acuña et al., 2017). In this chapter, recent findings are reviewed, current challenges are addressed, and gaps in knowledge which need to be further developed to improve scientific understanding are identified.

1.2.1 Importance of intermittent streams and rivers

Intermittent streams and rivers are of importance because they i) support biodiversity, ii) act as conduits for lateral and longitudinal exchange and are iii) essential to people's well-being (Acuña et al., 2014; Koundouri et al., 2017). The biodiversity of aquatic species which experience non-flow periods regularly and promote life-history adaption which favours resistance and resilience to droughts is facilitated by such waterways (Bogan et al., 2014; Lytle & Poff, 2004). In addition, dry riverbeds are ecologically valuable, as they are used as egg and seed banks and act as ecotones between terrestrial and aquatic phases. They also provide refuge in moist depressions and under leaf litter for various organisms. Nevertheless, if the dry period lasts too long, these refuges will cease to exist (Chester & Robson, 2011; Sabater et al., 2017; Sánchez-Montoya et al., 2016; Steward et al., 2012).

Intermittently flowing streams and rivers can uphold their role as a conduit for lateral and longitudinal exchange even without visible surface water (Acuña et al., 2014). This is essential because lateral connectivity, which allows for the movement of nutrients, is needed to maintain and regenerate riparian and floodplain ecosystems. Also, scientists have found that riparian vegetation were able persist due to the still present subsurface flow in the streambeds (Boulton et al., 2017; Fonesca & List, 2013; Gonzaléz et al., 2017). Concerning longitudinal connectivity, intermittent waterways are still able to maintain the transport of water, materials, energy and organisms without surface flow (Acuña & Tockner, 2010; Larned et al., 2010).

Flow intermittency supports valuable economical and societal services. Dry streambeds supply local people with wood and timber, they are used by livestock for foraging, and they provide additional space for agricultural practices (Goméz et al., 2005; Steward et al., 2012). Furthermore, they provide nonmaterial benefits such as cultural services. This includes educational and recreational services like fishing or hunting or inspirational and spiritual services, such as religious practices at or near intermittent waterways (Koundouri et al., 2017; MEA, 2005b).

1.2.2 Issues and challenges of intermittent streams and rivers

According to Acuña et al. (2014), intermittent waterways mainly face the following challenges: i) a lack of laws and ii) public recognition which is related to iii) a limited scientific understanding which results in iv) poor management.

The legal status of intermittently flowing streams and rivers varies around the world. Despite being the dominant feature of many streams and rivers, they might not be legally recognized as such (Datry et al., 2014). For example, the European Union (EU) Water Framework Directive 2000/60/EC (WFD) (EC, 2000) states that EU Member States and other countries committed to the WFD have to attain no less than a 'good' ecological status or 'good ecological potential' in all

surface waters. However, depending on the regional water body classification, intermittent streams and rivers may or may not be identified as such (EC, 2003). Nevertheless, there are some places which acknowledge intermittent waterways as an independent ecosystem. For instance, the Resource Management Act (RMA) of New Zealand defines a river as a 'continually or intermittently flowing body of fresh water' (RMA, 1991). Nonetheless, a global consensus on the definition of intermittently flowing streams and rivers will probably never be found because they show great variation concerning the cessation of flows, completely dry periods and timing during the year (Datry et al., 2017a).

Moreover, society does not adequately appreciate the ecosystem services that are provided, mostly due to a lack of knowledge. This is, for instance, reflected in the interviews done by Armstrong et al. (2012). They concluded that most landowners, who live in a small Pennsylvanian catchment, lack concern and label non-flow periods as useless. Likewise, the scientific interest in intermittent waterways was minimal and scientist communities predominantly focused on the higher valued perennial streams and rivers (Boulton, 2014; Koundouri et al., 2017). Accordingly, contemporary concepts and methods concerning hydrology, ecology, water chemistry or geomorphology are not adapted to intermittent waterways (Datry et al., 2014; Koundouri et al., 2017). For instance, flow regimes are characterized by hydrological metrics such as duration, timing or frequency. These are measured using gaging stations which are usually sparsely distributed along intermittent waterways (Datry et al., 2017b). In addition to the insufficient amount of data being collected, the data is rarely robust because of the equipment's inability to measure fine-scale nuances in flow regimes (Leigh et al., 2016; Snelder et al., 2013). Therefore, the data measured during non-flow periods and under dry conditions are often merged. Another issue which arises is that the hydrological metrics are limited in their spatiotemporal dimension and do not match with the current collected ecological data (Datry et al., 2017b).

The management of intermittent waterways is an ongoing debate without any suitable solutions. Since strategies have to consider various political, social, economic and environmental aspects, contemporary concepts and models are lacking (Datry et al., 2017b). As a result, intermittent waterways are often managed as a part of wet terrestrial ecosystems or as a part of perennial waterways (Acuña et al., 2017). If intermittently flowing waterways are managed like wet terrestrial ecosystems, they are frequently buried and prepared for agricultural cultivation. If they are managed like perennial streams and rivers, non-flow and dry periods are mostly ignored or transformed to permanent waterways by wastewater discharges or flow augmentation (Chiu et al., 2017; Luthy et al., 2015). These actions might lead to further negative consequences such as the introduction and distribution of alien and invasive species (Bunn & Arthington, 2002; Hamdan & Stromberg, 2016; Téllez et al., 2008).

1.2.3 Research opportunities regarding intermittent streams and rivers

Although there have been recent scientific advances, many gaps in understanding these unique ecosystems remain. Datry et al. (2017b) recommends that future research should focus on investing in i) adapting current concepts used for perennial rivers to intermittent conditions, ii) developing and inventing methods and models to study intermittent waterways adequately and iii) closing present gaps in regional and discipline-specific knowledge.

One of the prevailing gaps is the extent to which current concepts of freshwater ecosystems fail to adapt to intermittent conditions of streams and rivers. Several contemporary conceptual models have been tested and modified to varying degrees. One example is the attempt to adjust the disturbance theory in order to comprehend the variability in the characteristics of intermittent waterways such as severity, timing or intensity (Datry et al., 2017b). This has raised various questions, including how flow cessation and rewetting represent natural disturbances to the aquatic biota or if these processes play a crucial role in defining habitat patches (Lake, 2000; Larned et al., 2010). Nevertheless, it is just as important to closely relate conceptual research opportunities to available methodologies and their suitability to examine flow intermittency.

Methodological limitations restrict research on intermittent streams and rivers and have a significant effect on their successful management (Datry et al., 2017b; Gallart et al., 2016; De Girolamo et al., 2018). Since most sampling and modelling techniques were developed to be used in perennial streams and rivers, they must be either dismissed or carefully implemented and modified. This includes a detailed analysis regarding their sensitivity and uncertainty (Ivkovic et al., 2013; Sheldon, 2005). Scientists agree that to be able to study intermittently flowing waterways more extensively, the number of experiments needs to be increased. One solution is to develop inexpensive equipment which is suitable for researching flow intermittency (Datry et al., 2017b). In addition, rapidly advancing technology allows for the development of new methodological approaches such as remote camera image processing or techniques which track geomorphological processes (Costigan et al., 2017; Tooth, 2012). Nowadays, scientists are investigating novel approaches like citizen-science programs which can produce long-term data on the state of streams and rivers or track the advancement of alien species in intermittent waterways (Allen et al., 2019; Turner & Richter, 2011). This approach has additional benefits because it educates the public and allows for the observation of a larger geographical region (Dickinson et al., 2010; Dickinson et al., 2012).

Although intermittently flowing streams and rivers are an essential contributor to the integrity of river ecosystems around the world, the breadth of knowledge differs greatly depending on the region and the discipline in question. Most of the research has been carried out in the United States, Spain and Australia and thus has been published in English. Meanwhile, countries in which intermittent waterways are prevalent produce only a small number of studies. This geographical

imbalance of research and depth of knowledge increases the difficulty of comparing data sets and impedes the development of policies, laws and management strategies (Boulton, 2014; Datry et al., 2017b). Furthermore, the geographical imbalance is reflected in discipline-specific knowledge. For example, the biochemistry of intermittent streams and rivers has mainly been researched in Mediterranean areas, while there is simultaneously limited knowledge regarding the biochemical responses to intermittency in Africa or South America (Larned et al., 2010; Von Schiller et al., 2017). It seems that this geographical and disciplinary gap is hard to close. Nevertheless, first solutions have been found though scientist initiated projects such as the '1000 Intermittent River Project'. This is an international collaborative network which seeks to explore the same ecosystem processes in as many intermittent waterways as possible to cover a broad geographical range (Datry et al., 2015). A second example is the 'Science and Management of Intermittent Rivers and Ephemeral Streams' (SMIRES) initiated by the European Cooperation in Science and Technology (COST). This project combines research across disciplines in an effort to connect fragmented pieces of knowledge, transforming them into useful tools for management strategies and policy making (COST, 2016).

1.3 Stream biofilm

According to Flemming & Wingender (2010) biofilm is 'the oldest, most successful and widespread form of life on Earth'. Although, several types of biofilm are known, they are generally characterized as communities of microbes that are attached to wet surfaces on which different auto- and heterotrophic species such as bacteria, algae, fungi and protozoa co-exist (Mora-Gómez et al., 2016). In general, the microbes organize themselves in self-produced matrixes built by extracellular polymeric substances (EPSs). With a potential dry mass percentage of >90%, the EPS matrix supports the biofilm and enables communication and interaction among the microbes, therefore, appearing to be a multicellular organism (Flemming & Wingender, 2010; Morisaki, 2016). Due to the spongy and porous structure of the biofilm, adsorption of dissolved and particulate inorganic and organic material may occur very easily, promoting the complex organic matrix (Meyer-Reil, 1996). Interstitial water among the EPSs (90–99% of total wet mass), which forms the immediate habitat of the microbes, affects their existence in a crucial way (Melo, 2005; Morisaki, 2016). For instance, desiccation can be buffered by the retained water, allowing for a certain degree of tolerance (Flemming & Wingender, 2010). The drying surface of the biofilm forms a control layer with low water transport. This hydraulic decoupling reduces the hydraulic conductivity and slows down the drying process within the matrix (Flemming & Wingender, 2010; Or et al., 2007). Furthermore, Roberson and Firestone (1992) observed that in response to desiccation more EPS is produced. The expansion of the matrix allows for more time to adapt to the new external conditions (Roberson & Firestone, 1992).

Since non-flow periods of intermittent waterways manifest as a disturbance, biofilm can be used to assess the effect of flow intermittency and its temporal characteristics. Such biofilm is characterized by its ability to respond to the recurring phases of desiccation and rewetting. The response comprises of two components: i) resistance, which refers to the ability to persist during the disturbance and ii) resilience, as a measure of the capacity to recover after the disturbance (Lake & Barmuta, 1986; Lake, 2000; Nimmo et al., 2015). Both components include various adaption strategies which can be expressed in alterations of life cycles, morphology or physiological traits. For instance, some communities remain dormant on dry streambeds until the rewetting, allowing for the fast recovery of metabolic activities, while other communities have developed resistance structures like spores or thickened cells (Bogan et al., 2017; Sabater et al., 2017; Timoner et al., 2014a).

The composition of biofilm is affected by the substrate on which it grows (see Table 1). For instance, the biofilm that develops on cobbles, which is called epilithic biofilm, is mainly dominated by algal biomass and autotrophic activity and is characterized by a more complex structure than the biofilm that develops in sand. This is the so called epipsammic biofilm, which mainly partakes in the decomposition of organic matter, thus being more heterotrophic than autotrophic (Romani & Sabater, 2001).

Table 1: Considered types of biofilm in the experiment and their community composition depending on the substrate. (Mora-Gómez et al., 2016, modified)

Substrate type	Cobbles	Sand
Name	Epilithic biofilm	Epipsammic biofilm
Community composition	Mainly algae (autotrophic), cyanobacteria, bacteria, fungi, protozoa	Mainly bacteria (heterotrophic), cyanobacteria, archaea, algae, fungi, protozoa

Epilithic and epipsammic biofilm is used in this experiment, because it is of relevance to aquatic ecosystems (Morisaki, 2016). In open rivers, mainly bottom-up processes are prevalent, and biofilm is known to be at the base, functioning as nutrition (primary production) for predators and consumers of higher trophic levels (Anderson-Glenna et al., 2008; Coundoul et al., 2014). Additionally, biofilm participates in the carbon and nitrogen cycle and stores nutrients by retaining them and increasing hydrodynamic transient storage (Battin et al., 2003; Coundoul et al., 2014). The microbial extraction and oxidation of inorganic and organic material from air, water and soil is fundamental to keeping habitats clean and eliminating harmful substances (Meyer-Reil, 1996). Furthermore,

biofilm is known to stabilize the sediment of a riverbed and mitigate its disturbance (Piqué et al., 2016). To summarize, biofilms are involved in leading ecosystem processes and thus important for a well-functioning system. Consequently, due to the major role biofilm plays in the nutrient cycles and the physiological variety of its microbes, it is often considered to be an indicator of the impact of disturbance (Sabater et al., 2007).

1.4 Recent studies

As previously written, flow intermittency requires new investigation methods and strategies. Simultaneously, it creates unique aquatic-terrestrial ecosystems which provide various opportunities to test new and existing theories in a variety of disciplines, as seen in the following recent experiments and studies (Datry et al., 2017b).

1.4.1 Findings concerning intermittent waterways

Datry et al. (2018) argue that flow intermittency contributes substantially to the global carbon cycle. Litter decomposition was evaluated in various climatic zones and CO_2 emissions were quantified during rewetting events. It was found that a single rewetting event contributes up to 10% of the daily CO_2 from perennial streams and rivers and therefore should be included in the global carbon cycle. Additionally, similar results were published by Gómez-Gener et al. (2016). They stated that a single non-flow period can emit a significant amount of CO_2 and therefore needs to be included in the carbon balance of fluvial networks. In other studies, scientists mainly focused on local effects. For example, invertebrate diversity of desert streams in Arizona has been studied by Schriever et al. (2015). Of interest was the relationship between river systems with a high spatiotemporal flow variability and the functional and taxonomic composition of communities. As expected, with a prolonged non-flow period and an increased frequency of non-flow events, the functional and taxonomic richness declined. Furthermore, Datry (2012) found similar results in a study investigating benthic and hyporheic invertebrate assemblage along a flow intermittency gradient in France. An increased duration reduced benthic and hyporheic density and taxonomic richness and the invertebrate assemblage composition diverged. Finally, Jaeger et al. (2014) set out to determine how an increase in flow intermittency associated with climate change threatens hydrological connectivity and thus endangers endemic fish species in southwestern America. It was concluded that with an increase in frequency and duration, the number of isolated stream fragments grows. Consequently, this limits the number of available habitats for native fishes to reproduce and to take refuge.

1.4.2 Findings concerning the effect of flow intermittency on stream biofilm

Within the framework of this thesis, the main focus lies on the temporal components of the intermittent flow regime. As Colls et al. (2019) highlights, not many studies concerning the temporal components of flow intermittency exist and there are even fewer studies with regard to stream biofilm. An overview of the current breadth of knowledge is presented by the following studies: investigating an intermittent Mediterranean stream in Spain, Timoner et al. (2012) observed that non-flow periods have a visible effect on the function of stream biofilm. Autotrophic biomass was reduced by 80% but recovered quickly after the flow returns. In contrast, heterotrophic biomass decreased by only 20% and was thus more resistant, especially in the epipsammic biofilm. Timoner et al. (2014a) stressed in their research that bacterial communities in epilithic biofilm are more affected than they are in epipsammic biofilm because cobbles are more exposed and sediments, in particular sandy sediments, are able to retain moisture which increases the chance of survival. Timoner et al. (2014b) found that algal assemblages have a low resistance to desiccation but recover quickly when flow returns, indicating a high resilience. It was also noted that algae assemblage shows a low resistance to flow intermittency, which was indicated by a 60-90% decrease in biomass represented by chlorophyll-a during the non-flow period. These low values suggest that algae enter a dormant phase during desiccation. However, they are highly resilient, as chlorophyll-a recovers quickly after the flow resumes. In particular, chlorophyll-a recovers most rapidly in epilithic biofilm whereas the concentration of chlorophyll-a in epipsammic biofilm remains low during the rewetting.

Until today, there is only a small number of studies which specifically research the individual and relative role of temporal components. Most of them stress the importance of the duration of non-flow periods and their effect on stream biofilm. This is seen in Acuña et al. (2015), who used artificial streams to research the impact of the duration of non-flow periods on stream biofilm. They showed that duration influences the balance between autotrophic and heterotrophic activities, promoting heterotrophy. However, this shift is limited to the non-flow period, as one week after the flow returned the balance was restored. A most recent study by Colls et al. (2019) researched the effects of duration, frequency and severity of the non-flow periods on stream biofilm. They studied Mediterranean streams of the Iberian Peninsula and found that duration decreased autotrophic biomass and gross primary production. The latter was also negatively affected by the severity of the event. However, there was no significant influence present, which was based on the frequency of non-flow periods. Similarly, Muñoz et al. (2018) published a study concerning the effect of the severity of non-flow periods on ecosystem structure and functions. They found that different severity treatments led to no alterations in ecosystem structure while ecosystem functions responded to rehydration and in some cases persisted until the end of the non-flow period. This implies a decoupling between structure and function. Due to an increase in function and photosynthetic and enzymatic activities, more CO_2 was emitted and water-extractable organic matter in sediments

decreased. The key message is that the effect of non-flow periods on autotrophic and heterotrophic biofilm is inconsistent and the structure and function of autotrophs may be more affected than heterotrophs (Sabater et al., 2017; Timoner et al., 2012).

1.5 Research hypotheses

The previous chapters introduced the main topics of climate change, intermittent waterways and stream biofilm, elaborating a need for research.

This bachelor thesis is part of a study concerning intermittent waterways at the Catalan Institute for Water Research (ICRA) in Girona, Spain. During the past years, ICRA has been using the Experimental Stream Facility (ESF) to conduct studies on flow intermittency and related topics such as research surrounding the severity of non-flow periods and its effects on ecosystem structure and function (Muñoz et al., 2018). However, until now it was not possible to clearly separate the influence of temporal flow regime components from the complex system of other ecological variables such as pH or air temperature. Therefore, the objective of the ICRA's ESF experiment is to identify the effect of the interaction between duration and the frequency of non-flow periods in intermittent flows as well as their individual and relative roles and their joint effects on the biofilm. To further narrow the research objective, the main focus of this thesis lies on the assessment of non-flow period's frequency and the respective response of autotrophic and heterotrophic stream biofilm.

According to Colls et al. (2019), the frequency of non-flow periods does not affect biofilm metabolism and biomass, though the duration of non-flow periods has a significant impact. To confirm, this thesis analyses three frequency levels, and to exclude the effect of duration, the total amount of non-flow days remains the same in all treatments. Based on this, the first hypothesis states that the effects on autotrophic and heterotrophic biofilm are less if the frequency of non-flow periods is higher, because the number of subsequent non-flow days is smaller and thus the biofilm is less stressed. The second hypothesis is based on a study by Timoner et al. (2012), which found that during non-flow periods, autotrophs in epilithic biofilm were highly affected and showed a biomass loss of 80% but were able to recover quickly. Meanwhile, autotrophs in epipsammic biofilm recovered slowly but showed steadier values of photosynthetic efficiency. Therefore, it is hypothesized that autotrophs in epipsammic biofilm recover more slowly than in epilithic biofilm if the frequency of non-flow periods increases, because the amount of water retained in sand is higher.

2 Materials and methods

This chapter describes the Experimental Stream Facility (ESF) as well as the experimental design used to research the effects of non-flow period frequency on stream biofilm. Further, the analytical and statistical methods which are required to collect and analyse the data of the variables are explained.

2.1 Experimental Stream Facility

To determine the effects of non-flow periods' duration and frequency in intermittent waterways, the following experiment was conducted in artificial streams. The streams are part of the indoor ESF of the Catalan Institute for Water Research (ICRA) in Girona, Spain.

The ESF was launched in May 2012 and is unique in Europe. It is inspired by a similar facility which was built by the Environmental Protection Agency (EPA) in Cincinnati, Ohio (USA). The ICRA's ESF comprises of 24 independent methacrylate channels which are divided into four functional units (Figure 2). One channel has a length of 2 m, a width of 10 cm and a rectangular cross section of 50 cm^2. Depending on the experiment, its slope can be adjusted manually, and further conditions can be manipulated as well. For instance, regarding hydraulics, the flow can be set between laminar and turbulent (0.01–0.1 L/s, 2–50 minutes travel time), and can be switched between flow-through, recirculation or hybrid operation. Each channel has a 70 L water tank in order to recirculate water. Sandy streambeds, glazed ceramic tiles or cobbles can be used as a substrate. The air and water temperatures can be controlled between 4–40 ºC. Since the system is computer-based, harvested rainwater, nutrients or dissolved organic carbon can be added automatically. Furthermore, variables such as hydraulics, light cycles, discharge, dissolved oxygen etc. can also be controlled and monitored automatically. (Acuña et al., 2019; Experimental Streams Facility; ICRA (undated))

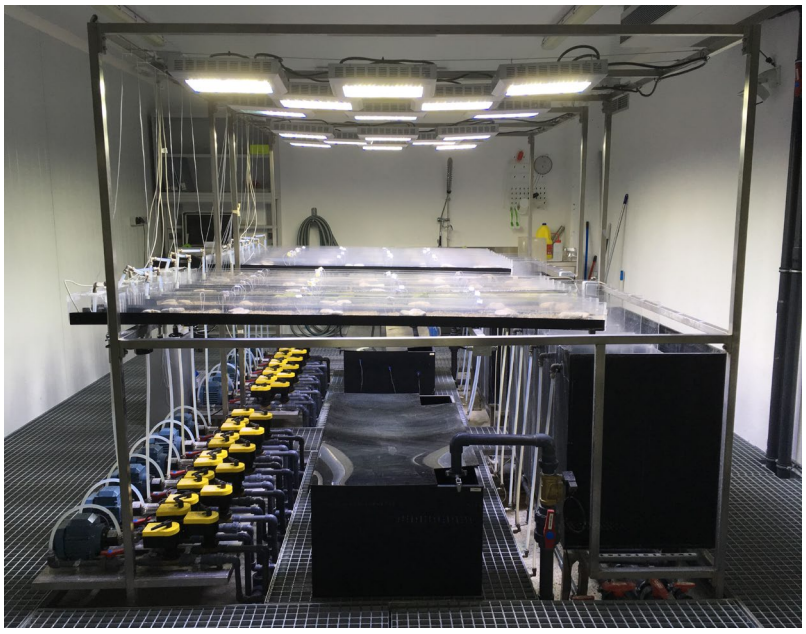

Figure 2: Two functional units of the Experimental Streams Facility at the Catalan Institute for Water Research. (Etter, 2019)

The experiment was conducted between September 2nd and December 11th, 2019. The ESF comprises of 18 stream channels grouped into three functional units:

- Unit A with channels A1–A6
- Unit B with channels B1–B6
- Unit D with channels D1–D6

Six treatments were applied, defined by two different drought durations (D28 = 28 days, D56 = 56 days) and three different frequencies of non-flow periods (F1 = one non-flow period, F2 = two non-flow periods, F4 = four non-flow periods). Note that the duration indicates the total number of days during which no water flowed and the frequency specifies how these days were divided. The treatments were assigned to three channels (replications), so that each treatment was represented once per unit, following a randomized complete block design. Since this bachelor thesis specifically focuses on the frequency of non-flow periods, the three relevant treatments involved only one drought duration (D28) and three different frequencies:

- F1 with 1x 28 non-flow days
- F2 with 2x 14 non-flow days
- F4 with 4x 7 non-flow days

Thus, only the nine channels of the treatments D28F1 (channels A1, B2, D3), D28F2 (channels A2, B3, D4) and D28F4 (channels A3, B4, D5) were analysed in the context of this thesis (Figure 3).

To mimic natural conditions, substrate was taken from the Riera de Llémena. The stream surfaces in the mountains of Finestres and passes the plain of Sant Gregori, before flowing into the Ter river (Valldellemena, undated). It is characterized by well conserved riverside vegetation, good water quality and a diverse fauna (Generalitat de Catalunya, undated).

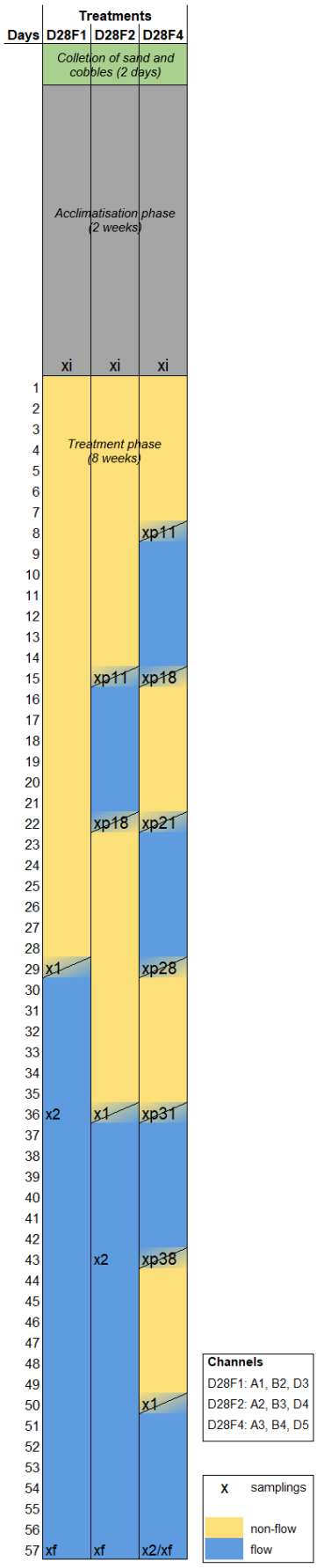

Figure 3: Experimental design and sampling schedule. For the description of the samplings see Table 2.

The stream can be described as a specially intermitted complex. This means that this stream falls dry only in some sections and thus promotes a large pool of species because of the habitat's diversity and oligotrophic conditions (Acuña, oral information, 2019). The stream flows evenly through woodland and is shaded by relatively dense riparian vegetation. The stream course is characterized by differently sized pools and riffles (Figure 4).

Figure 4: Riera de Llémena near Sant Esteve de Llémena, September 2, 2019. (Etter, 2019)

During three days in early September 2019, sand and cobbles were collected at two unpolluted sections situated about 1 km northwest of Sant Esteve de Llémena (WGS84: N 42.071705, E 2.603396 and N 42.067453, E 2.609995). While sand was extracted from the pools with hand shovels and sieved on site (d_{max} = 8 mm), flat fist-sized cobbles (d_{max} = 7 cm) with a recognisable biofilm were collected in the riffles separately.

The collected material was brought back to ICRA and within three hours it was distributed randomly in the channels. In each channel a levelled sand bed of 3.5 cm depth was created and a slope of 1.5% was set. On average 11 cobbles were placed throughout each channel (Figure 5).

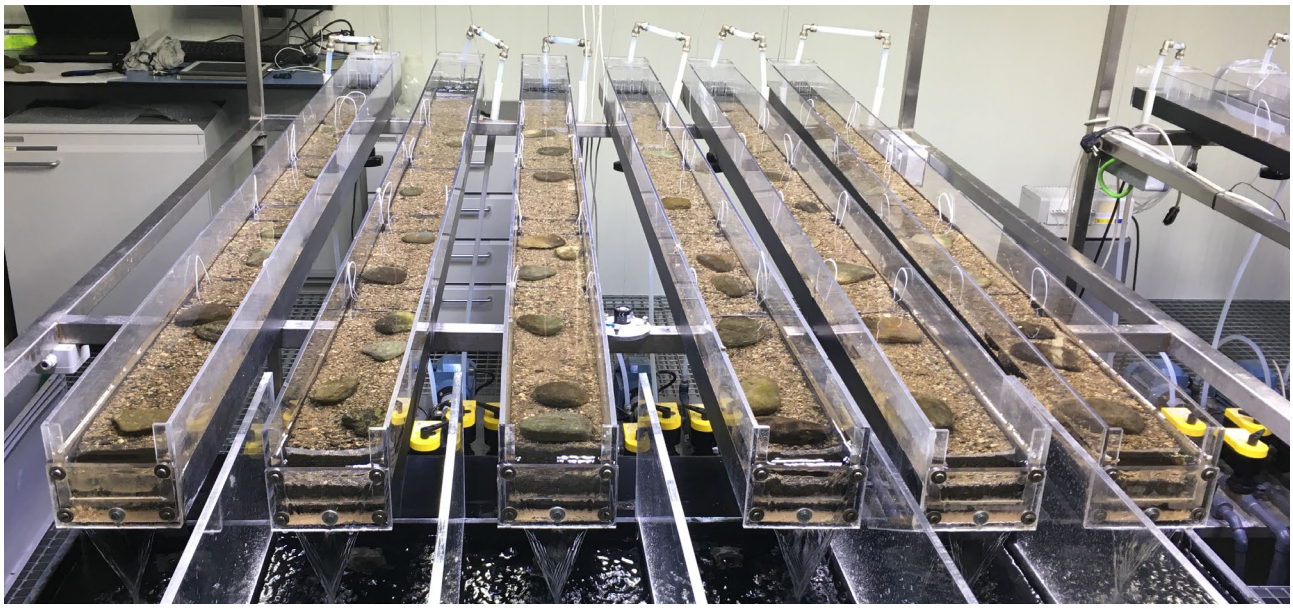

Figure 5: One unit of the Experimental Streams Facility with six channels after set-up, September 3, 2019. (Etter, 2019)

After two days of set-up, the acclimatisation phase started, which lasted two weeks, allowing the biofilm to grow and adapt to the new environmental conditions. The biofilm developed from the inoculum in the sand and cobbles. All channels had a constant water flow of 10 mL/s, with a water depth between 2.5–5.5 cm (increasing from beginning to end of the channel). Harvested rainwater from the collection tank on top of the ICRA, which was filtered with active carbon filters, was used. The water circulation was set for flow-recirculation, preventing the inoculum from being washed away. According to natural conditions, the air temperature was constantly held at 16 ºC with a humidity level of 65–70% and thus the water temperature was between 16.4–18.8 ºC. Additionally, 14 hours of light (10 AM–12 PM) and 10 hours of darkness were set as the daily cycle of photosynthetic active radiation (PAR) during the acclimatisation phase. Daylight was simulated by LED lights (120 W, Lightech, Girona, EU) allowing a constant PAR value of 173.99 ± 33 µE/m·s. These light conditions emulated average natural conditions of the Riera de Llémena in late summer. PAR was monitored using one quantum sensor (LI-192SA, LiCOR Inc., Lincoln, USA) per unit of channels. Moreover, every three days, nutrients in the form of a concentrated solution were added automatically to all channels using a peristaltic pump (IPC pump: Ismatec, Glattbrugg, Switzerland) to maintain a final concentration of 0.5 mg/L of PO_4, 0.5 mg/L of NH_4 and 10 mg/L of NO_3. This concentration corresponds with a moderate to good ecological status according to the implementation guides of the WFD (EC, 2000).

At the beginning of the treatment phase, the water flow was stopped in all channels. The daily PAR cycle was changed to 12 hours of light (10 AM–10 PM) and 12 hours of darkness, in accordance with the average natural light conditions in autumn. The water circulation was altered to an hourly sequence consisting of a combination of flow-recirculation (58 minutes) and flow-open (2 minutes),

resulting in an exchange rate of 8.57% per hour and the water volume being fully replaced twice a day. The remaining conditions stayed the same as during the acclimatisation phase. Following the experimental design shown in Figure 3, new flow or non-flow periods started on Wednesday at 10 AM when the lighting period began. During a flow period a constant water flow of 70 mL/s was maintained, thereby emulating low-flow conditions of the Riera de Llémena in autumn. Similar to the acclimatisation phase, nutrients were automatically added. However, they were more frequent (every hour for two minutes) and only added to channels during flow periods.

2.2 Analytical methods

The response of the epilithic and epipsammic biofilm to different frequencies was assessed in terms of water content (WC) and ash free dry mass (AFDM), ecosystem metabolism (net ecosystem production (NEP), gross primary production (GPP) and ecosystem respiration (ER)), heterotrophic activity (aerobic respiration (AR)) as well as yield of photochemistry (Yeff) and fluorescence yield (Ft). Furthermore, environmental conditions were monitored.

The different samplings took place at defined points in time in the concerned channels, as shown in Table 2. Each sampling time is given its own acronym, representing the described state in the column 'time of sampling' at the mentioned condition in the column 'condition in channel'. The experimental design, which can be found in Figure 3 and in detail in appendix I, serve to classify the sampling times as well.

Table 2: Description of the six defined points in time of the sampling: *xi* (initial state), *xp* (preliminary state), *d* (daily monitoring), *x1* (end of final non-flow period), *x2* (one week after final non-flow period) and *xf* (final state).

Acronym	Time of sampling	Condition in channel
xi	**Initial state:** samples were taken one day before the start of the treatment phase during morning hours.	wet
xp	**Preliminary state:** samples were taken before starting the next flow or non-flow period, respectively. Regarding numeration: the first digit after *xp* refers to the number of flow periods while the second digit refers to the day of the flow period in the respective treatment, for instance *xp28*: sampling of *xp* at the last day of the second flow period.	dry or wet
d	**Daily monitoring:** samples were taken from Monday to Friday during flow periods during morning hours. Regarding numeration: the first digit after *d* refers to the number of flow periods while the second refers to the day of the flow period in the respective treatment, for instance *d33*: sampling of *d* at the third day of the third flow period.	wet
x1	**End of final non-flow period:** samples were taken at the end of the total number of non-flow days before starting the last flow period.	dry
x2	**One week after final non-flow period:** samples were taken one week after the end of the total number of non-flow days during morning hours.	wet
xf	**Final state:** samples were taken at the end of the treatment phase during morning hours.	wet

2.2.1 Physico-chemical parameters

The physico-chemical parameters pH, dissolved oxygen, conductivity and water temperature were measured in order to assess the environmental conditions in the channels.

pH is defined by the lime-carbonic acid equilibrium and the geochemical conditions in the catchment area. In addition, temperature and biological processes such as photosynthesis and degradation of organic material play a major role as well. If the temperature is low, the solubility of CO_2 increases, which leads to a reduction in the pH. During photosynthesis, CO_2 and HCO_3^- are removed from the water, resulting in an increase in the pH. In natural watercourses, the pH should range from 6.5 to 8.5. The collected rainwater at the ICRA had a pH of 8. (Liechti, 2010)

Oxygen concentration is defined by temperature, and the gas exchange between water and atmosphere, as well as photosynthesis, respiration and mineralisation of organic substances. The solubility of oxygen decreases with increasing temperature. The lowest concentration of oxygen is usually observed at the end of the night when more oxygen is used than produced. Additionally, increasing decomposition of organic matter (mineralisation) causes an increasing oxygen demand. (Liechti, 2010; BAFU, 2016)

Conductivity is an indicator of the dissolved salt content and refers to ion concentration. It is primarily influenced by water hardness (calcium, magnesium, bicarbonate) and further parameters such as nitrate, nitrite, ammonium and phosphate. Similar to pH, conductivity is affected by the lime-carbonic acid equilibrium and thus by both temperature and biological processes. Therefore, an increase in the water temperature causes an increase in conductivity. Conductivity rates between 150 and 500 µs/cm are typical for healthy streams. (EPA, undated; Liechti, 2010)

Physico-chemical parameters were measured with hand-held probes (dissolved oxygen: YSI, Yellow Springs OH, USA; pH, conductivity and temperature: WTW, Weilheim, Germany). During the acclimatisation phase, the channels were monitored daily (*d*, see Table 2), 60 minutes after the start of the lighting period to check that the biofilm in all channels was developing under the same conditions. Daily monitoring was also maintained during the treatment phase in the wet channels, 20 minutes after the lighting period began.

2.2.2 Water content and ash free dry mass

The WC describes the amount of water within the sediment (Campbell & Campbell, 2005). Depending on the pore size and type of soil, the water retention capacity varies. With a permeability value of >25.4 cm/h coarse sand, similar to the sand used in the experiment, drains very rapidly (O'Geen, 2013). The WC is of importance because it influences the oxygen concentrations and

nutrient availability (Drenovsky et al., 2004). The AFDM represents the weight of the organic matter and is essential because it allows for the estimation of the samplings' total biomass (Aristi, 2016).

To determine WC, sand samples were taken at *xi*, *xp*, *x1*, *x2* and *xf* (see Table 2). For each channel, a sample of 1 cm^3 of sand was extracted randomly with a trimmed syringe. The samples were weighed and then dried in a furnace for 72 hours at 60 ºC. Afterwards, the difference between wet and dry weight was calculated to determine the WC, expressed as percentage in relation to the wet weight.

To determine the AFDM, the same sand samples were used. In addition, a slurry with tank water and scraped biofilm from two stones was prepared per channel (15 mL of slurry, consisting of 30 mL tank water and scraped biofilm). To scrape the cobbles, a toothbrush was used, and to determine the scraped surface, the scraped area was traced on aluminium foil, which subsequently was cut and weighed. The weight was used for the calculation of the surface by means of a linear regression. The scraped cobbles were marked with a rubber band and returned to the channels but not used for further measurements. The slurry samples were taken at *xi*, *x1, x2* and *xf* (see Table 2). Just as the sand samples, the slurries were weighed and dried in the furnace for 72 hours at 60 ºC and then weighed once more. Afterwards, sand and slurry samples were combusted in a muffle furnace for 4 hours at 450 ºC. The differences between the dry and the mineral matter weight was evaluated to determine the AFDM expressed as mg/cm^2 for sand and mg/cm^3 for cobbles.

2.2.3 Ecosystem metabolism

Ecosystem metabolism is defined as the production and respiration of organic matter within a stream. CO_2 is reduced to C by photosynthetic aquatic organisms, whereas mainly heterotrophic aquatic organisms oxidize C to CO_2. The total amount of fixed C is termed as gross primary production (GPP) and the amount of mineralized C as ecosystem respiration (ER). These two processes are summarized as net ecosystem production (NEP), which is defined as

$$\mathrm{NEP} = \mathrm{GPP} - |\mathrm{ER}| \,.$$

GPP and ER are key processes in specifying an ecosystem's mass and energy balance. Thus, metabolism can be used to assess the activity of biofilm and the ecosystem's health. To measure metabolism in streams, it is common to use oxygen, which is relative to CO_2. The advantage of oxygen lies in its low concentration, allowing for simple detection of fluctuations caused by production and respiration. (Acuña et al., 2015; Fellows et al., 2006; Hall, 2016; Lovett et al., 2006; Rodríguez-Castillo et al., 2018; Tank et al., 2010)

To assess the metabolic rates, a sand sample (tray of 160 cm^3) and a sample of two cobbles per channel were assessed separately at *xi*, *x1*, *x2* and *xf* (see Table 2). The trays were part of the sand bed of the channels (Figure 6). They were removed for the measurements and then returned. However, for every sampling another tray was used. The same process was applied for the cobbles. The samples were place in cylindrical recirculating chambers (acrylic glass, volume of 0.96 L) to monitor the oxygen production and consumption in the absence of any exchange with the atmosphere. A submersible pump provided an even distribution of the oxygen in the water (Figure 7). The chambers were placed in an incubator (Radiber AGP-700-ESP, Barcelona, Spain) at a constant temperature (20 ºC). The oxygen concentration was logged at 15-s intervals with an oxygen sensor (PreSens OXY-10mini, Regensburg, Germany) for 90 minutes, first for 45 minutes under dark conditions and afterwards for 45 minutes under light conditions (PAR value of 168 ± 2 µE/m·s). Under dark conditions, the change in oxygen concentration reflects the ER, whereas NEP is linked to lighting conditions. Both were calculated as

$$R = \frac{SL \times V \times 10'000}{S},$$

with R being the respiration/consumption rate ($gO_2/m^2 \cdot d$), SL being the slope calculated by means of a linear regression of the 360 logged interval data, multiplied by 1440 ($gO_2/L \cdot d$), and V being the water volume in the chamber (L) and S being the 'active' surface of the stone (cm^2). Assuming the respiration rate to be the same under light conditions, the GPP was calculated as sum of NEP and ER.

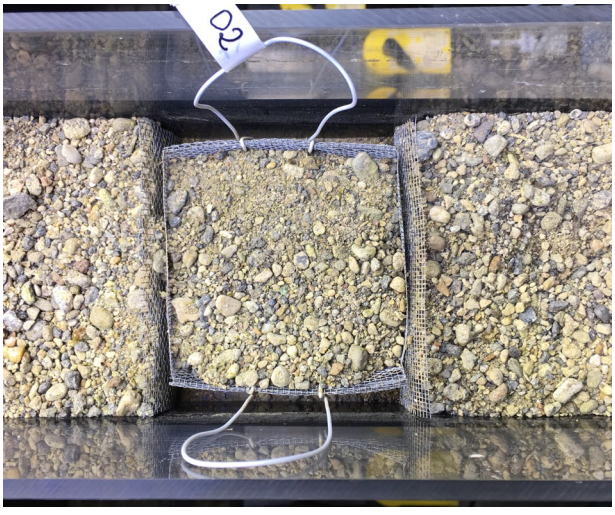

Figure 7: A tray filled with sand as part of the channel bed. (Etter, 2019)

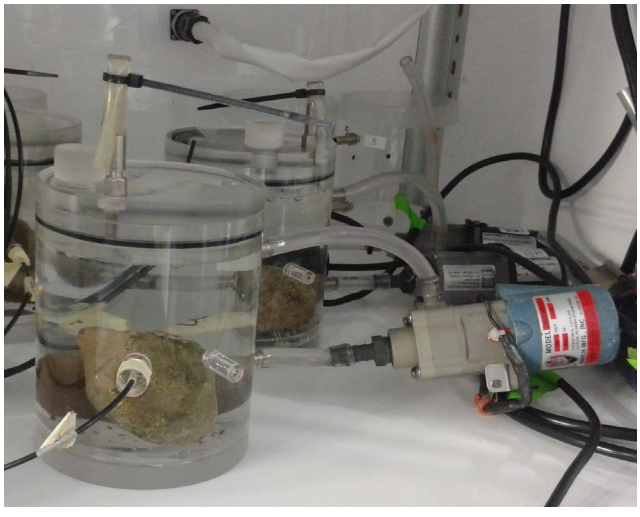

Figure 6: Cylindrical recirculating chambers with two cobbles connected to a submersible pump. (Etter, 2019)

2.2.4 Heterotrophic metabolic activity (aerobic respiration)

Resazurin, a chemical compound, is used to make existing bacterial activity, notably aerobic respiration, visible. If there is any activity and thus reducing conditions, the dye resazurin (blue in colour) is reduced irreversibly to resorufin (pink in colour) by emitting an oxygen ion (Figure 8). The resazurin conversion and the oxygen consumption are directly proportional. The transformation mainly occurs in the surface and hyporheic zones, where metabolic activity is prominent. Since the resorufin is strongly fluorescent, it is quantifiable by fluorimetry. (González-Pinzón et al., 2012; Haggerty et al., 2008; Haggerty et al., 2009; McNicholl et al., 2007; Peroni & Rossi, 1986)

Figure 8: Tubes with sand, tank water and resazurin after four hours of incubation. The purple colour indicates that a reduction to resorufin took place due to aerobic respiration. (Etter, 2019)

The resazurin assay was performed only with sand samples at *xi*, *xp, x1*, *x2* and *xf* (see Table 2). For each channel, a sample of 1 cm^3 of sand was extracted randomly with a trimmed syringe and put in a tube. After the sampling, each tube was filled with 0.5 mL of the sample resazurin solution (see Table 3) and 4.5 mL of tank water (reaching a final resazurin concentration of 0.03 mM). In addition, two extra tubes were prepared as blank samples: one with 0.5 mL of the sample resazurin solution and 4.5 mL of tank water and the other one with sediment and tank water. A phosphate buffer was added to ensure a pH >8 (see Table 3). This stabilizes the fluorescence of resazurin and resorufin and therefore, makes it less error-prone and easier to detect. (Bueno et al., 2002; Kangasniemi, 2004)

Table 3: Composition of the sample solution of resazurin and the phosphate buffer.

Sample resazurin solution	**Phosphate buffer**
0.0126 g of resazurin salts were dissolved in 50 mL of phosphate buffer, creating the initial resazurin solution. This solution was diluted to a resazurin concentration of 0.3 mM (15 mL of initial resazurin solution and 35 mL of phosphate buffer).	The phosphate buffer with a pH ±8 contains 1M NaH2PO4·H2O + 1M NaOH (1:1).

After the preparation, all tubes were stored for 4 hours at 20 ºC and shaken (200 rpm) in an incubator. After incubation, the fluorescence of the resorufin was measured in a fluorescence spectrophotometer (F-7000, Hitachi High-Technologies Corporation, Tokyo, Japan). The maximal excitation and emission wavelength were set at 570 nm and 583 nm, respectively. The detected values of the two blanks were deducted from the samples' values because of the sand and water autofluorescence, which could distort the value of resorufin fluorescence.

A standard curve with final concentrations was used to estimate the concentrations of resorufin (regression analysis with $R^2 = 0.9659$ and $y = 31.746x + 14.471$, for more details see appendix II). Finally, the concentration of resorufin (nmol/cm^3·h), which acts as a proxy of aerobic respiration, was calculated as follows:

$$AR = \frac{C_{RRU} \times V_w}{V_s \times t_i},$$

with C_{RRU} being the concentration of resorufin (µM), V_w the volume of added tank water (mL), V_s the volume of added sand (cm^3) and t_i the incubation time (h).

2.2.5 Yield of photochemistry

The light absorbed by chlorophyll molecules (e.g. of photosystem II pigments of algae) is used for photochemistry (photosynthesis), dissipated as heat or re-emitted as chlorophyll fluorescence. The three processes compete with each other. Hence, if one rate increases, the other two decrease. For example, during the photosynthesis process, electron transport occurs in the photosystem II causing the affected reaction centres to be closed. The efficiency of photochemistry is reduced and thus the yield of fluorescence increases. Therefore, by measuring the chlorophyll fluorescence, assumptions regarding the photochemistry of autotrophic organisms can be made. (Baker, 2008; Maxwell & Johnson, 2000)

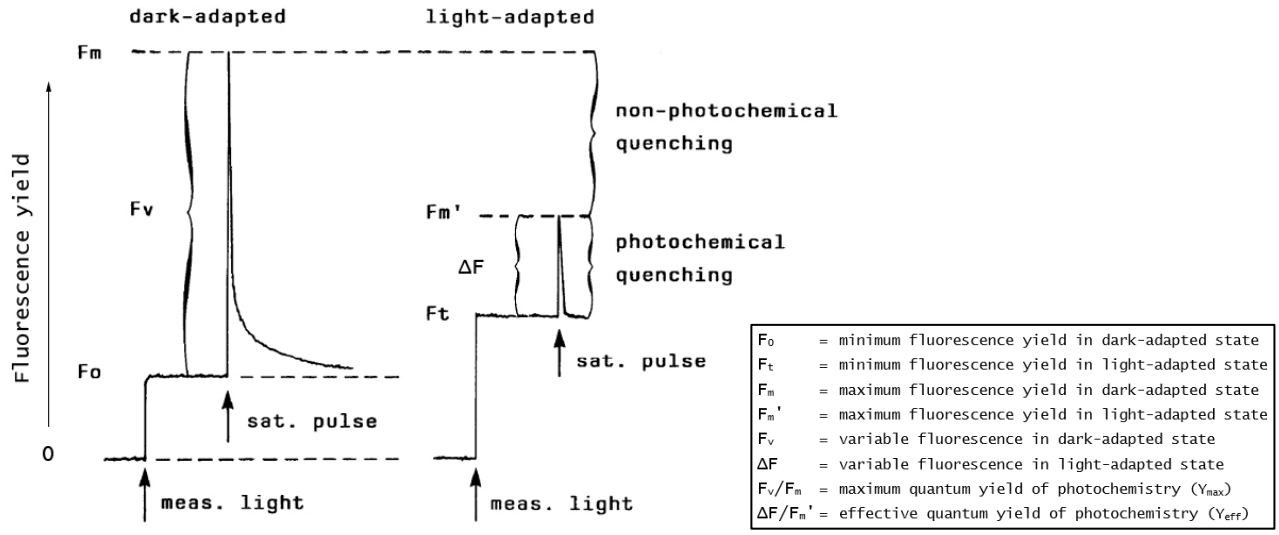

Figure 9: Different variables of fluorescence measurements under dark and light conditions. (Heinz Walz GmbH, 1998, modified)

Photosystem II is the main origin of chlorophyll fluorescence and can be measured in situ with a photosynthesis yield analyser in dark- and light-adapted states, using differing measurement values, indicated as fluorescence units (f.u.) (Figure 9). Under dark conditions, heat dissipation is at its minimum and photosystem II reaction centres are fully oxidised and open. In the presence of a weak measuring light, the steady-state or minimum chlorophyll fluorescence yield (F0) can be measured. Furthermore, after a light saturation pulse, which induces all reaction centres to close, the maximum chlorophyll fluorescence yield (Fm) can be measured. Knowing F0 and Fm, the variable fluorescence (Fv) and therefore the maximum quantum yield of photochemistry (Ymax), describing the potential photochemical capacity, can be calculated as follows:

$$\mathrm{Ymax} = \frac{\mathrm{Fm} - \mathrm{F0}}{\mathrm{Fm}} = \frac{\mathrm{Fv}}{\mathrm{Fm}}.$$

Under light conditions, so called fluorescence quenching effects occur, resulting in a lower variable fluorescence (ΔF). Consequently, the effective quantum yield of photochemistry (Yeff) is lower. This describes the effective efficiency of photochemistry on the basis of the proportion of absorbed energy used. Yeff is calculated in the same way as Ymax, but using the light-adapted equivalent minimum fluorescence yield (Ft) and the maximum fluorescence yield (Fm'):

$$\mathrm{Yeff} = \frac{\mathrm{Fm}' - \mathrm{Ft}}{\mathrm{Fm}'} = \frac{\Delta \mathrm{F}}{\mathrm{Fm}'}.$$

Like with F0, Ft was used to estimate the algal biomass (Acuña et al., 2019). As long as actinic light is present, photosynthesis takes place and most of the reaction centres are closed. However, due to the increase in the conversion rate of the photochemical energy during the adaption time,

which means that electrons are passed on faster, the fluorescence yield decreases. This phenomenon is called photochemical quenching. Light also leads to heat dissipation which prevents photodamage because of excess exiting energy, lowering the fluorescence as well. This is called non-photochemical quenching. (Baker, 2008; Genty et al., 1989; Heinz Walz GmbH, 1998; Maxwell & Johnson, 2000; Schreiber, 2004)

In this experiment a submersible pulse amplitude modulated fluorometer (Diving-PAM: Walz, Effeltrich, Germany) was used to assess photosynthetic performance and stress of the biofilm's algae (Baker, 2008). For instance, stress becomes visible through a lowered Fv/Fm ratio (= Ymax) (Kraus & Weis, 1991; Murchie & Lawson, 2013). The variables Ymax, Yeff, F0 and Ft, Fm and Fm' as well as the fluorescence quenching coefficients (qP, qN and NPQ) were quantified. For each measurement, three locations in the sand and three on the cobbles (pseudo replicates) were examined per channel, resulting in an average value per channel, one for sand and one for cobbles. During the acclimatisation phase, the channels were monitored every day (*d*, see Table 2) under light conditions (around one hour after the start of the lighting period) by measuring the Ft variable with a distance of 10 mm between fiberoptics and the sample. The MEAS-INT and GAIN were set at 12. This monitoring was used to observe the development of the biofilm. The daily monitoring was also maintained during the treatment phase in the wet channels, 20 minutes before and after the start of the lighting period (with MEAS-INT and GAIN set at 8). Thereby, Fm and Ymax (in dark) and Ft and Yeff (in light) were measured. In addition, weekly measurements (with MEAS-INT and GAIN set at 8) also took place at *xi*, *xp*, *x1*, *x2* and *xf* (see Table 2). Under dark conditions F0, Fm and qP, qN and NPQ were measured, followed by the measurement of Ft and Yeff under light conditions (light was switched on manually and biofilm had an adaption period of 20 minutes prior to measurement).

2.3 Data analyses

The effects of the frequency of non-flow periods on the measured variables were determined by means of impact and recovery. Impact was calculated as the difference between the measurement values of *x1* and *xi* (see Table 2), showing the biofilm's resistance to non-flow conditions. Recovery was calculated as the difference between *x2* and *x1* (see Table 2), representing the biofilm's resilience after the disturbance (see chapter 1.3). Impact and recovery were analysed as absolute and as relative changes. (Lake & Barmuta, 1986; Lake, 2000; Nimmo et al., 2015)

All analyses were performed using R (version 1.2.5001, RStudio, Inc., Boston, USA) with an assigned significance of $p < 0.05$. The following variables were considered: AFDM, Ft, Yeff, NEP, ER and GPP in epipsammic and epilithic biofilm and AR and WC only in the epipsammic biofilm. Ft, Yeff and GPP represent the autotrophic and AR and ER the heterotrophic biofilm, whereas AFDM and NEP stands for both. In addition, the biofilm's biomass (structure) is represented by AFDM and Ft and the function (activity) by Yeff, AR, NEP, ER and GPP. WC describes the environmental condition and was statistically used only for the initial independence test.

Before the start of the treatment phase, the independence of the units as well as the uniformity of the initial biofilm was tested. A one-way analysis of variance (ANOVA) was used with channel as a random factor and unit as a fixed factor (randomized block design). If a significant difference was detected, a post-hoc Tukey Test was applied, and the variable was not used for further statistical analyses (except for the final ANOVA). Provided that the difference did not persist until the end of the final non-flow period (*x1*), the variable was consulted as a descriptor variable instead, to improve the understanding of the development and the relationship with other variables. If the difference persisted until the end of the final non-flow period (*xi*), the initial state had a greater influence on the variable than the treatment. Therefore, the variable was excluded. To determine the uniformity of the biofilm, the focus was on the robust variables (Ft, Yeff, NEP and ER), as Ft and Yeff were measured three times per channel, acting as pseudo replicates, and for the measurement of NEP and ER the largest quantity of substrate was used.

A further one-way ANOVA with condition (impact or recovery) as a random factor and treatment as a fixed factor was conducted to test for significant differences between treatments regarding impact and recovery of the variables in epipsammic and epilithic biofilm. In the case of a significant difference, a post-hoc TukeyC Test was applied to locate which treatments differed significantly.

A pearson correlation was used to assess the strength and the direction of the relationship between impact and recovery of each variable. The results were expressed as a coefficient of determination (R^2) and as a regression slope to determine whether the relationship is positive or negative. R^2 values between 0 and 0.19 indicated no relationship, whereas values between 0.20 and 0.39 stood for a weak and values >0.39 for a strong relationship. Additionally, it was used to compare different variables and make a predication regarding their relationship.

Lastly, the state at *xf* was compared between the treatments. All variables were included, similar to the initial uniformity one-way ANOVA. The difference was statistically tested with a one-way ANOVA with channel as a random factor and treatment as fixed factor. Conceding that there was a significance, a post-hoc TukeyC Test was applied.

3 Results

In this chapter, the results are given for all relevant variables which are needed to answer the hypotheses. For the sake of simplicity, treatment names D28F1, D28F2 and D28F4 are abbreviated as F1, F2 and F4. Additionally, value names are extended with a subscripted ES for epipsammic (e.g. $Yeff_{ES}$) and with an EL for epilithic (e.g. $Yeff_{EL}$) compartment.

3.1 Analysis of physico-chemical parameters

Table 4 shows the average value of each physico-chemical parameter per channel during the acclimatisation and treatment phase. Both phases showed approximately the same averages. pH averaged between 8.09 and 8.49, O_2 content averaged between 88.10 and 97.75% as well as between 8.44 and 9.26 mg/l and the average of conductivity was between 332.94 and 435.50 µs/cm. In addition, temperature ranged from 16.64 to 18.83 ºC. However, the averages during the acclimatisation phase were higher than during the treatment phase with the exception of the conductivity levels in F1 with the channels A1 (425.60 µs/cm; 434.40 µs/cm), B2 (430.60 µs/cm; 437.20 µs/cm) and D3 (424.40 µs/cm; 434.40 µs/cm). Further, it is apparent that the average temperature of channels D3 (16.70 ºC) and D5 (16.64 ºC) were lower compared to the other channels (average of 18 ºC) during the treatment phase. In addition, nutrients were added regularly, but they were not evaluated within the context of this thesis.

Table 4: The average values of physico-chemical parameters measured throughout the acclimatisation and treatment phase per channel (n = 1): F1 (channels A1, B2, D3), F2 (channels A2, B3, D4) and F4 (channels A3, B4, D5).

Acclimatisation phase		**F1**			**F2**			**F4**		
		A1	**B2**	**D3**	**A2**	**B3**	**D4**	**A3**	**B4**	**D5**
pH		8.49	8.46	8.44	8.48	8.47	8.44	8.45	8.48	8.45
O_2	**[%]**	97.23	97.10	96.95	97.35	97.28	96.73	97.75	97.13	96.85
O_2	**[mg/l]**	8.98	9.03	9.20	8.99	9.05	9.26	9.01	9.03	9.25
Conductivity	**[µs/cm]**	425.60	430.60	424.40	414.00	431.00	415.00	409.00	435.40	424.00
Temperature	**[°C]**	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*

Treatment phase		**F1**			**F2**			**F4**		
		A1	**B2**	**D3**	**A2**	**B3**	**D4**	**A3**	**B4**	**D5**
pH		8.16	8.15	8.13	8.09	8.09	8.11	8.15	8.11	8.09
O_2	**[%]**	88.80	89.60	88.10	90.66	90.33	90.36	92.82	93.06	90.85
O_2	**[mg/l]**	8.37	8.50	8.59	8.51	8.44	8.59	8.77	8.85	8.82
Conductivity	**[µs/cm]**	434.40	437.20	434.40	377.63	376.13	377.88	333.00	332.94	333.59
Temperature	**[°C]**	18.48	18.18	16.70	18.83	18.78	18.24	18.30	17.96	16.64

3.2 Uniformity of the initial state of the biofilm

The three units were independent from each other and the initial biofilm was uniform at *xi* (for data per treatment see appendix III), as the variables Yeff, Ft, ER, AR and WC showed no significant difference (see Table 5). In contrast, a significant difference was noted in the variables GPP_{ES} (p-value = 5.3 E-5), NEP_{EL} (p-value = 0.036), GPP_{EL} (p-value = 0.048) and $AFDM_{EL}$ (p-value = 0.050). Nevertheless, since the initial differences were not maintained during the experiment, the variables are used as descriptors. Solely, $AFDM_{ES}$ (p-value = 0.005) showed a significant difference which persists and therefore, this variable is excluded.

Table 5: Results of the statistical analysis regarding the independence between units A, B and D and the uniformity at the initial state of the epipsammic (ES) and epilithic biofilm (EL). The concerned variables (n = 9) are quantum yield of photochemistry ($Yeff_{ES}$, $Yeff_{EL}$), minimum fluorescence yield (Ft_{ES}, Ft_{EL}), net ecosystem production (NEP_{ES}, NEP_{EL}), gross primary production (GPP_{ES}, GPP_{EL}), ecosystem respiration (ER_{ES}, ER_{EL}), aerobic respiration (AR), ash free dry mass ($AFDM_{ES}$, $AFDM_{EL}$) and water content (WC).

		ANOVA			Tukey (p-value)		
		Df	F-value	p-value	A-B	A-D	B-D
ES	Yeff	2	1.063	0.403	-	-	-
	Ft	2	2.894	0.132	-	-	-
	NEP	2	4.984	0.053	-	-	-
	GPP	2	126.0	***5.3E-05***	0.0526	***0.0001***	***0.0001***
	ER	2	1.748	0.266	-	-	-
	AR	2	0.394	0.866	-	-	-
	AFDM	2	14.09	***0.005***	***0.0077***	***0.0103***	0.9570
	WC	2	2.531	0.102	-	-	-
EL	Yeff	2	0.359	0.712	-	-	-
	Ft	2	2.311	0.180	-	-	-
	NEP	2	6.065	***0.036***	***0.0352***	0.6745	0.1044
	ER	2	1.974	0.219	-	-	-
	GPP	2	5.234	***0.048***	0.0527	0.8654	0.1012
	AFDM	2	5.147	***0.050***	0.8138	0.1138	***0.0520***

3.3 Analysis of water content and ash free dry mass

Figure 10 illustrates the amount of WC over the course of the experiment for each treatment. In addition to the measurements at *xi, x1, x2* and *xf*, several preliminary measurements at *xp* are included to highlight the development. At the beginning of the treatment phase, the WC was approximately the same in each treatment (20 ± 2%). At the beginning of a flow period, the WC amounted to 0.35 ± 0.04% in F1 and F2 and to 6 ± 2% in F4, whereas at the end of each period the average WC amounted 25 ± 3%. Finally, the WC value at *xf* in F1 was higher (37 ± 4%) than in F4 (28 ± 10%) and F2 (26 ± 1%).

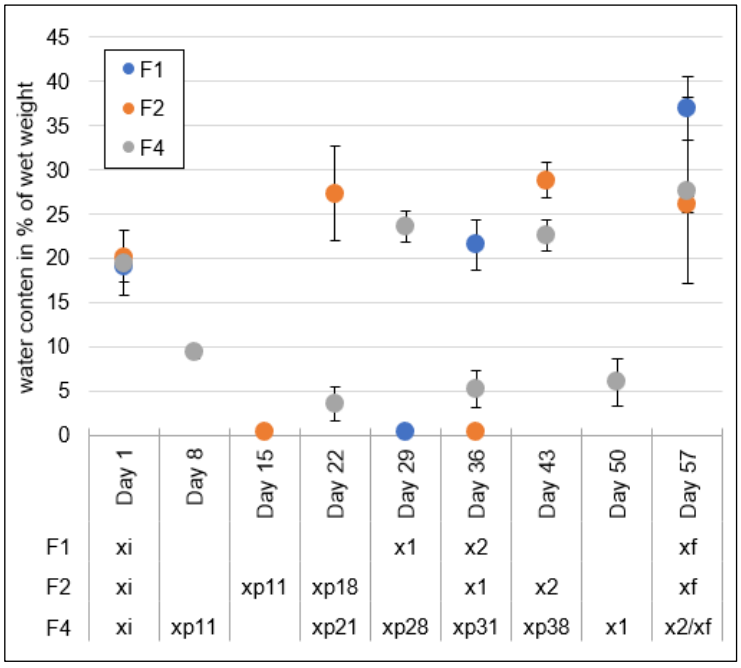

Figure 10: Epipsammic water content (WC) per treatment (n = 3) measured at *xi* (initial state), *xp* (preliminary state), *x1* (end of final non-flow period), *x2* (one week after final non-flow period) and *xf* (final state). There is no available data for *xp18* (last day of the first flow period) in treatment F4.

Looking at the descriptor variable $AFDM_{EL}$, the impact in F1 and F2 showed a decrease (-0.6 ± 0.4 mg/cm^2 and -0.3 ± 0.7 mg/cm^2, respectively), and an increase in F4 (1.1 ± 1.0 mg/cm^2). The recovery showed a reverse behaviour, with an increase in F1 (0.7 ± 0.3 mg/cm^2), in F2 (0.5 ± 0.3 mg/cm^2) and in F4 (0.008 ± 0.4 mg/cm^2) (see Table 6). Impact and recovery were negatively related, displaying a weak linear relationship (R^2 = 0.23).

Table 6: Average and standard deviation of impact and recovery in epilithic biofilm (EL) per treatment (n = 3): ash free dry mass ($AFDM_{EL}$).

		Impact			Recovery		
		F1	F2	F4	F1	F2	F4
EL	AFDM [mg/cm^3]	-0.6 ± 0.4	-0.3 ± 0.7	1.1 ± 1.0	0.7 ± 0.3	0.5 ± 0.3	0.008 ± 0.4

3.4 Analysis of ecosystem metabolism

Analysing the variables NEP, ER and GPP, it is important to consider that NEP_{EL}, GPP_{ES} and GPP_{EL} are only used as descriptors. Due to incomplete measurement results, there are no results available for the impact and the recovery of GPP_{ES} and GPP_{EL} in F1.

With respect to the NEP_{ES}, the impact in F1 and F2 showed a decrease (-24.4 ± 9.7 $gO_2/m^3 \cdot d$ and -1.6 ± 52.2 $gO_2/m^3 \cdot d$, respectively), while in F4 an increase occurred (227.2 ± 113.6 $gO_2/m^3 \cdot d$). During the recovery phase, NEP_{ES} increased in F1 (317.1 ± 114.2 $gO_2/m^3 \cdot d$) and F4 (191.2 ± 114 $gO_2/m^3 \cdot d$) and showed the highest values in F2 (427.6 ± 143.5 $gO_2/m^3 \cdot d$). The highest negative impact value for NEP_{EL} appeared in F2 (-6.5 ± 2.3 $gO_2/m^3 \cdot d$), followed by F1 (-5.8 ± 1.6 $gO_2/m^3 \cdot d$) and F4 (-4.8 ± 2.4 $gO_2/m^3 \cdot d$). Similarly, the recovery values for NEP_{EL} were highest in F2 (15.6 ± 1.8 $gO_2/m^3 \cdot d$), but, in contrast to the impact, were followed by F4 (8.2 ± 8.6 $gO_2/m^3 \cdot d$) and then by F1 (6.3 ± 3.7 $gO_2/m^3 \cdot d$). The impact of ER_{ES} decreased in F1 (-7.3 ± 4.0 $gO_2/m^3 \cdot d$) and F4 (-0.5 ± 39.6 $gO_2/m^3 \cdot d$), while the ER_{ES} values in F2 were positive (17.2 ± 13.8 $gO_2/m^3 \cdot d$). During the recovery phase, positive values were visible in F1 (62.8 ± 40.6 $gO_2/m^3 \cdot d$), F2 (0.3 ± 20.3 $gO_2/m^3 \cdot d$) and F4 (75.2 ± 22.1 $gO_2/m^3 \cdot d$). As in the epipsammic biofilm, ER_{EL} is affected in all three treatments, displaying similar negative impact values in F1 (-1.4 ± 0.3 $gO_2/m^3 \cdot d$), F2 (-1.3 ± 0.3 $gO_2/m^3 \cdot d$) and F4 (-1.1 ± 0.7 $gO_2/m^3 \cdot d$). ER_{EL} recovered in F1 (0.4 ± 0.1 $gO_2/m^3 \cdot d$) and F4 (0.7 ± 0.5 $gO_2/m^3 \cdot d$), while it was further impacted in F2 (-0.3 ± 0.1 $gO_2/m^3 \cdot d$). Lastly, for GPP_{ES} impact values were positive in F2 (15.6 ± 60.3 $gO_2/m^3 \cdot d$) and even higher in F4 (184.0 ± 33.5 $gO_2/m^3 \cdot d$). During the recovery phase, the values continued increasing in F2 (427.6 ± 128.8$gO_2/m^3 \cdot d$) and F4 (266.4 ± 135.8 $gO_2/m^3 \cdot d$). Contrary to the epipsammic variables, the impact of GPP_{EL} was higher in F2 (-7.8 ± 2.5 $gO_2/m^3 \cdot d$ $O_2/m^3 \cdot d$) than in F4 (-5.9 ± 2.9 $gO_2/m^3 \cdot d$).

The same behaviour was visible in the recovery values with F2 (15.3 ± 1.9 $gO_2/m^3 \cdot d$) having higher values than F4 (8.8 ± 9.0 $gO_2/m^3 \cdot d$). Describing the difference between the epipsammic and epilithic biofilm, it is apparent that in F1 the impact and recovery values were higher in the epipsammic biofilm. In F2 and F4 the impact was more severe in the epilithic biofilm while the recovery values were higher in the epipsammic biofilm. Overall, the differences between the treatments regarding impact and recovery were more distinct in the epipsammic than in the epilithic biofilm (see Table 7). Impact and recovery were not related for NEP_{EL} (R^2 = 0.15), while a weak negative relationship was evident for NEP_{ES} (R^2 = 0.20) and ER_{EL} (R^2 = 0.27). For ER_{ES}, GPP_{ES} and GPP_{EL} relationship analyses could not be performed due to incomplete measurement results.

Table 7: Average and standard deviation of impact and recovery in epipsammic (ES) and epilithic biofilm (EL) per treatment (n = 3): net ecosystem production (NEP_{ES}, NEP_{EL}), ecosystem respiration (ER_{ES}, ER_{EL}) and gross primary production (GPP_{ES}, GPP_{EL}). As exceptions, the number of samplings was smaller (n = 2) for the impact of ER_{ES} in F1 and ER_{ES} and GPP_{ES} in F4, as well as for the recovery of ER_{ES} in F1. There is no data available for impact and recovery of GPP_{ES} and GPP_{EL} in F1.

			Impact			Recovery		
			F1	F2	F4	F1	F2	F4
ES	NEP	[$gO_2/m^3 \cdot d$]	-24.4 ± 9.7	-1.6 ± 52.2	227.2 ± 113.6	317.1 ± 114.2	427.6 ± 143.5	191.2 ± 114.0
	ER	[$gO_2/m^3 \cdot d$]	-7.3 ± 4.0	17.2 ± 13.8	-0.5 ± 39.6	62.8 ± 40.6	0.3 ± 20.3	75.2 ± 22.1
	GPP	[$gO_2/m^3 \cdot d$]	*NA*	15.6 ± 60.3	184.0 ± 33.5	*NA*	427.9 ± 128.8	266.4 ± 135.8
EL	NEP	[$gO_2/m^3 \cdot d$]	-5.8 ± 1.6	-6.5 ± 2.3	-4.8 ± 2.4	6.3 ± 3.7	15.6 ± 1.8	8.2 ± 8.6
	ER	[$gO_2/m^3 \cdot d$]	-1.4 ± 0.3	-1.3 ± 0.3	-1.1 ± 0.7	0.4 ± 0.1	-0.3 ± 0.1	0.7 ± 0.5
	GPP	[$gO_2/m^3 \cdot d$]	*NA*	-7.8 ± 2.5	-5.9 ± 2.9	*NA*	15.3 ± 1.9	8.8 ± 9.0

3.5 Analysis of heterotrophic metabolic activity (aerobic respiration)

The Impact of AR decreased in F1 (-3.1 ± 0.6 $nmol/cm^3 \cdot h$) and in F2 (-3.0 ± 1.0 $nmol/cm^3 \cdot h$) while in F4 an increase (0.5 ± 1.2 $nmol/cm^3 \cdot h$) was visible. During the recovery phase, positive values were apparent in F1 as well as in F2 (1.2 ± 0.3 $nmol/cm^3$ and 0.4 ± 0.6 $nmol/cm^3 \cdot h$, respectively) and in F4 (1.4 ± 1.2 $nmol/cm^3 \cdot h$) (see Table 8). Impact and recovery were positively related, having a weak linear relationship (R^2 = 0.21).

Table 8: Average and standard deviation of impact and recovery in epipsammic biofilm (ES) per treatment (n = 3): aerobic respiration (AR).

			Impact			Recovery		
			F1	F2	F4	F1	F2	F4
ES	AR	[$nmol/cm^3 \cdot h$]	-3.1 ± 0.6	-3.0 ± 1.0	0.5 ± 1.2	1.2 ± 0.3	0.4 ± 0.6	1.4 ± 1.2

Figure 11 highlights the changes in AR throughout the treatment phase for better understanding. It is apparent, that AR showed lower values at the end of non-flow periods in comparison to the values sampled at the end of flow periods. The average value for each treatment was between 0.1 ± 0.3 and 2.4 ± 0.8 nmol/cm^3 · h at the end of non-flow periods and between 1.3 ± 0.2 and 3.1 ± 1.0 nmol/cm^3 · h at the end of flow periods. An exception can be noted in F4, in which AR at *x1* (3.8 ± 0.4 nmol/cm3 · h) was higher than at *xp38* (1.1 ± 1.4 nmol/cm^3 · h). Additionally, it is recognizable that AR values were higher at the beginning of each new flow period in comparison to the previous. However, once more, in F4 an exception occurred, given that the values at *xp21* (0.8 ± 1.3nmol/cm^3 · h) were lower than at *xp11* (2.0 ± 0.4nmol/cm^3 · h). It is also noticeable for all treatments that values at *xf*, ranging between 4.4 ± 0.4 and 5.3 ± 1.5 nmol/cm^3 · h, were higher than at *xi*, ranging between 3.2 ± 0.4 and 3.6 ± 0.5 nmol /cm^3 · h.

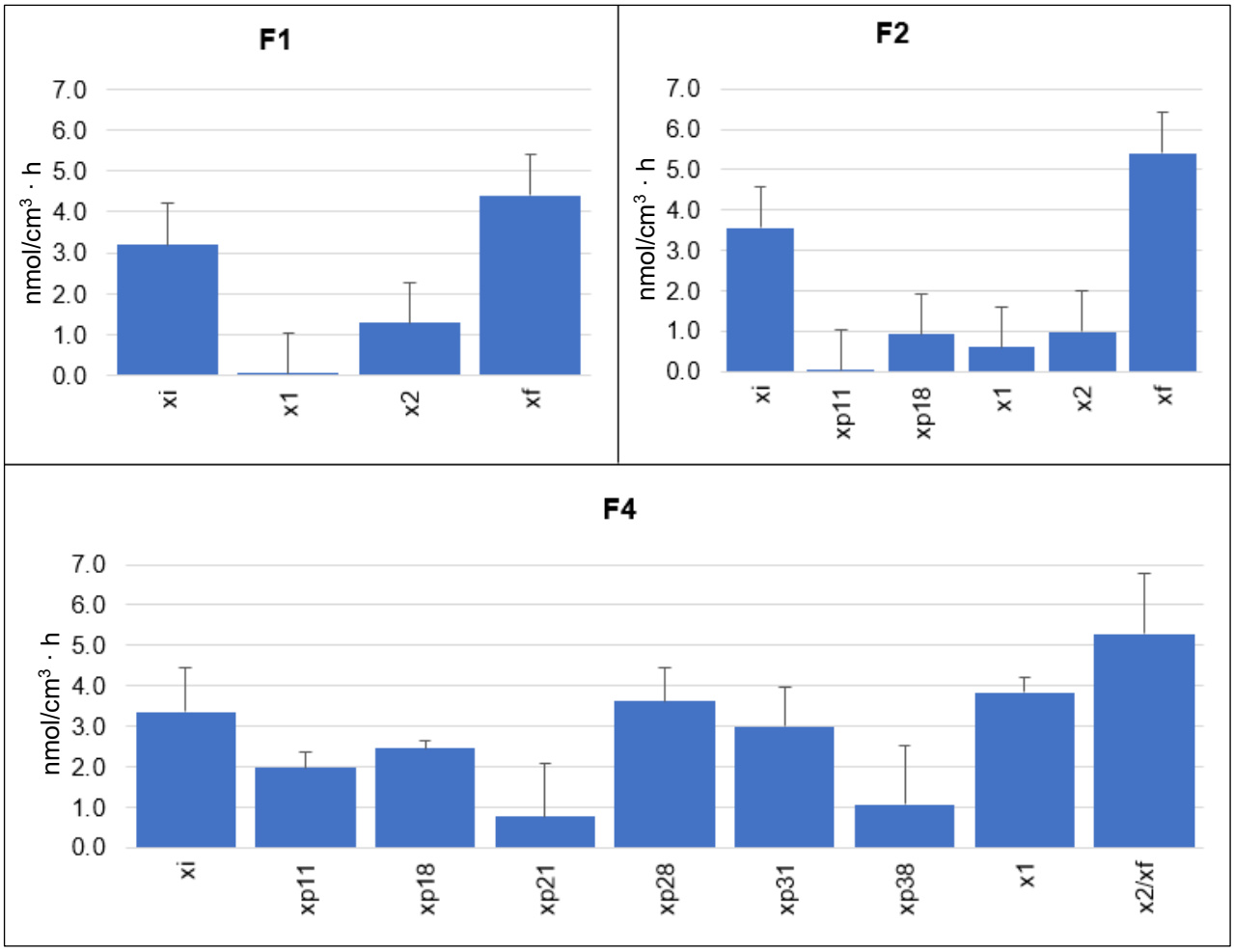

Figure 11: Development of aerobic respiration (AR, as concentration of resorufin) per treatment (n = 3) measured at *xi* (initial state), *xp* (preliminary state), *x1* (end of final non-flow period), *x2* (one week after final non-flow period) and *xf* (final state).

3.6 Analysis of yield of photochemistry

As there is not continuous data for all the variables measured using the Diving-PAM, only the variables Yeff and Ft are considered for the analyses.

As seen in Table 9, Yeff values generally showed higher variations between treatments than Ft values. Regarding the epipsammic variables, the impact of both $Yeff_{ES}$ and Ft_{ES} decreased in F1 ($Yeff_{ES}$: -20 ± 1183 f.u.; Ft_{ES}: -24 ± 11 f.u.) and in F2 ($Yeff_{ES}$: -3080 ± 2964 f.u.; Ft_{ES}: -20 ± 10 f.u.). In contrast, an increase was visible in F4 ($Yeff_{ES}$: 190 ± 1703 f.u.; Ft_{ES}: 24 ± 8 f.u.). In the epilithic biofilm, the impact was negative for all the treatments and highest for $Yeff_{EL}$ in F1 (-2675 ± 2382 f.u.) and for Ft_{EL} in F2 (-164 ± 39 f.u.), respectively. Referring to recovery, both $Yeff_{ES}$ and $Yeff_{EL}$ increased. However, considering the high standard deviations, no clear patterns are evident. The Ft_{ES} values increased during recovery in F1 (22 ± 14 f.u.) and in F2 (21 ± 2 f.u.) while Ft_{ES} in F4 declined (-24 ± 15 f.u.). In the epilithic biofilm, the Ft_{EL} values only increased in F2 (17 ± 9 f.u.) while they decreased in F1 (-1 ± 15 f.u.) and F4 (-8 ± 12 f.u.). The epilithic variables $Yeff_{EL}$ and Ft_{EL} showed higher negative impact values than the epipsammic variables $Yeff_{ES}$ and Ft_{ES}, except in F2, in which the opposite was true for Yeff. Regarding the recovery phase, the patterns are less clear. The epilithic variable $Yeff_{EL}$ in F1 and F4 had higher values than the epipsammic variable $Yeff_{ES}$, whereas in F2 it was reversed. The same applied to Ft in F1 and F2, but with epipsammic values being higher than the epilithic ones. In contrast, Ft_{ES} and Ft_{EL} in F4 did not recover, with Ft_{ES} showing a higher decrease than Ft_{EL}. Impact and recovery were negatively related. For Ft_{EL} a strong linear relationship was apparent (R^2 = 0.53), although it was not pronounced, whereas for Ft_{ES} (R^2 = 0.37), $Yeff_{ES}$ (R^2 = 0.34) and $Yeff_{EL}$ (R^2 = 0.26) the relationships were weak.

Table 9: Average and standard deviation of impact and recovery in epipsammic (ES) and epilithic biofilm (EL) per treatment (n = 3): quantum yield of photochemistry ($Yeff_{ES}$, $Yeff_{EL}$) and minimum fluorescence yield (Ft_{ES}, Ft_{EL}).

			Impact			Recovery		
			F1	F2	F4	F1	F2	F4
ES	Yeff	[f.u.]	-20 ± 1183	-3080 ± 2964	190 ± 1703	1031 ± 3599	5661 ± 834	1132 ± 4066
	Ft	[f.u.]	-24 ± 11	-20 ± 10	24 ± 8	22 ± 14	21 ± 2	-24 ± 15
EL	Yeff	[f.u.]	-2675 ± 2382	-1217 ± 1240	-542 ± 575	2363 ± 2201	4054 ± 4416	2210 ± 4324
	Ft	[f.u.]	-144 ± 29	-164 ± 39	-132 ± 24	-1 ± 15	17 ± 9	-8 ± 12

Figure 12 illustrates the development of Yeff and Ft in the epipsammic and epilithic biofilm throughout the treatment phase for better understanding. There are four trends which need to be addressed.

First, Yeff and Ft experienced a contradictory trend, mainly between the first day of the experiment and the last day of the flow period at *x2*. This is supported by the fact that Yeff showed higher val-

ues on the last day of each flow period compared to the first day of the flow period. For instance, this is seen in F4, where $Yeff_{EL}$ increased from 1586 (*xp21*) to 2563 f.u. (*xp28*). Despite this clear pattern, the values within one flow periods varied from day to day (e.g. see the last trend below). Meanwhile, Ft developed differently depending on the treatment. Ft_{ES} in F1 increased during the flow period from 2 at *x1* to 24 f.u. at *x2* while the Ft_{EL} values remained approximately constant between 34 at *x1* and 32 f.u. at *x2*. F2 presented a pattern in which Ft_{ES} increased during both flow periods and Ft_{EL} decreased at first and then increased in the second flow period. In F4, both the Ft_{ES} and Ft_{EL} generally displayed a decrease in each flow period, except for Ft_{ES} from 31 (*xp11*) to 53 f.u. (*xp18*) during the first flow period and for Ft_{EL} from 42 (*xp21*) to 65 f.u. (*xp28*) during the second flow period.

Secondly, comparing Yeff and Ft values at the beginning and at the end of the treatment phase supports the contradictory relationship between Yeff and Ft which occurred during the flow periods. Yeff showed positive developments in all treatments, for instance in F4 the $Yeff_{EL}$ values increased from *xi* (1467 f.u.) to *xf* (3135 f.u.). The exception is $Yeff_{EL}$ in F1, whose values remained approximately the same at *xi* (2857 f.u.) and *xf* (2637 f.u.). Further, it is apparent that the values in F1, F2 and F4 for $Yeff_{ES}$ were generally higher than for $Yeff_{EL.}$ Regarding Ft in the epipsammic and epilithic biofilm, Ft_{EL} values were considerably higher than Ft_{ES} at *xi*, but seemed to have converged in the last quarter of the treatment phase. This is supported by the fact that Ft_{ES} values in all three treatments were low at the beginning and remained more or less constant until *xf* as seen in F1 where the value at *x1* (25 f.u.) was approximately the same as at *xf* (22 f.u.). In contrast, the Ft_{EL} value at *xi* (177 f.u.) experienced a considerable decline to *xf* (19 f.u.). This trend is applicable to F2 as well as F4.

Thirdly, Yeff and Ft in F1 and F2 seemed to have stabilized after *x2*, meaning that the values at *x2* and *xf* were similar. For example, in F2 the Ft_{ES} value of 24 f.u. at *x2* declined minimally to 29 f.u. *xf*. Since *x2* was at the same time as *xf* in F4, no further development between *x2* and *xf* existed and therefore F4 cannot be compared with F1 and F2.

Lastly, there is a pattern which was only visible during the flow period in the Yeff diagram. Between day 3 and day 6 of each flow, the $Yeff_{ES}$ and $Yeff_{EL}$ values in all treatments decreased. For instance, $Yeff_{ES}$ in F2 declined from 4288 (*d13*) to 3338 f.u. (*d16*) and $Yeff_{EL}$ from 2681 (*d13*) to 2046 f.u. (*d16*). The only exception occurred in the second flow period in F4 during which no measurements were taken for a duration of four days instead of two days. $Yeff_{ES}$ increased from 5488 (*d22*) to 6730 f.u. *(d26)* and $Yeff_{EL}$ from 3426 (*d22*) to 4060 f.u. (*d26*).

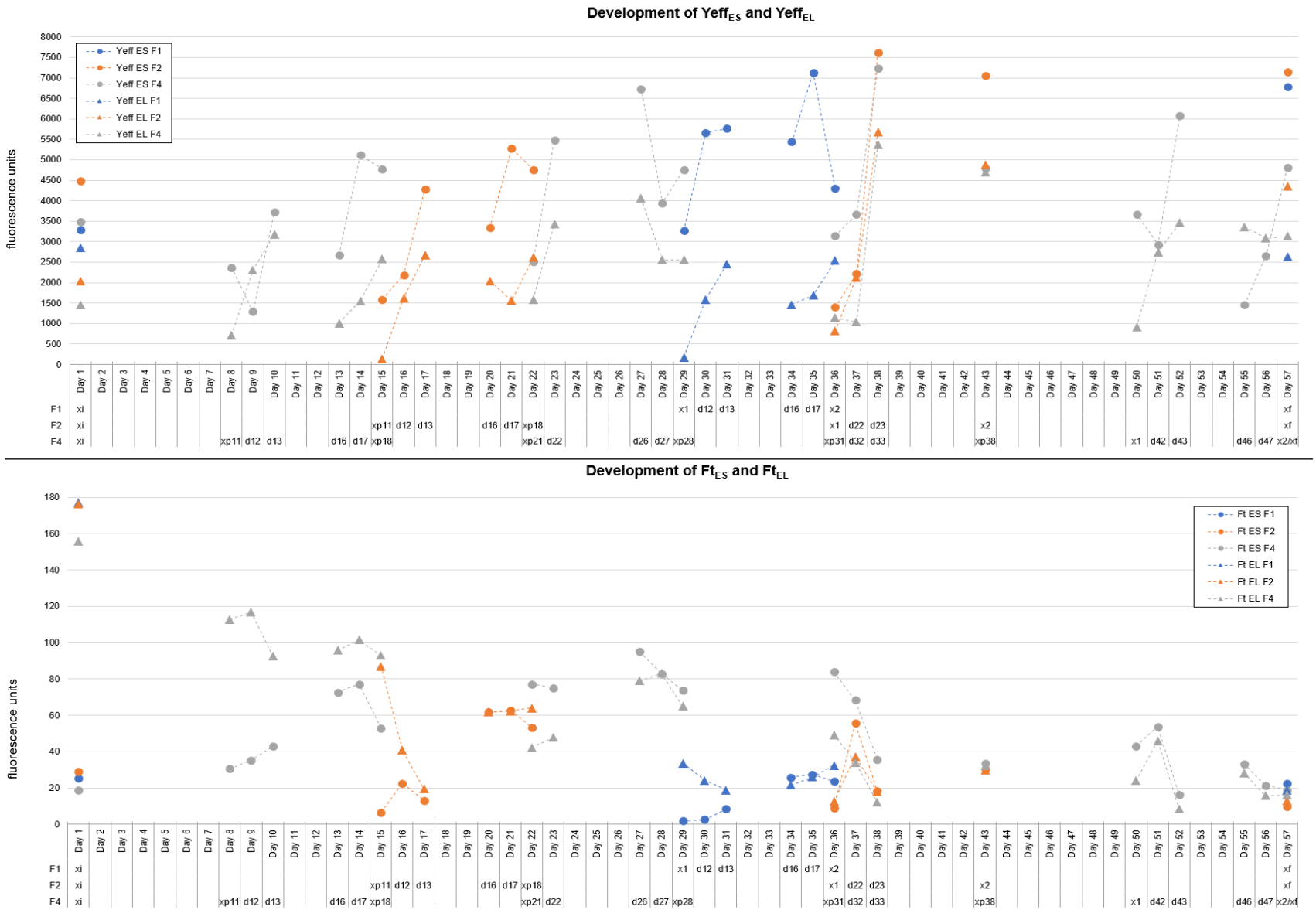

Figure 12: Development of the effective quantum yield of photochemistry (Yeff) and minimum fluorescence yield (Ft) in epipsammic (ES) and epilithic biofilm (EL) per treatment (n = 3) at *xi* (initial state), *xp* (preliminary state), *d* (daily monitoring), *x1* (end of final non-flow period), *x2* (one week after final non-flow period) and *xf* (final state). On days with no values, no measurements were taken due to non-flow conditions or a day off. The standard deviations are not indicated for the sake of clarity.

3.7 Overall patterns of impact and recovery

For better understanding of the overall pattern caused by the three treatments, Figure 13 shows an overview of the impact and recovery expressed as relative change (in %) (for data per treatment see appendix IV). High standard deviations existed for some variables, in particular for NEP, Yeff, GPP and AFDM. Neglecting the standard deviations and considering impact for the epipsammic biofilm, it is apparent that in F1 all values were negative, except for $Yeff_{ES}$ (11 ± 39%), which was slightly positive. In F2 the values were both negative and positive, while the variables in F4 showed the highest values, except for ER_{ES} (-3 ± 113%). Moreover, a pattern was evident in Ft_{ES}, NEP_{ES} and AR: F1 was affected the most, followed by F2 and F4. This pattern was most pronounced in NEP_{ES} (F1: -954 ± 1470%; F2: 726 ± 853%; F4: 1467 ± 1698%). Looking at the epilithic biofilm, it is obvious that the impact was more severe because a decrease occurred in all treatments, except for $AFDM_{EL}$ in F4 (134 ± 121%). Again, F1 was most affected with the exception of Ft_{EL} (F1: -80 ± 5%; F2: -92 ± 4%; F4: -84 ± 11%). Considering recovery, in the epipsammic biofilm, all variables had the highest values in F1, except $Yeff_{ES}$ (F1: 40 ± 106%; F2: 579 ± 354%; F4: 39 ± 117%) and the lowest values in F4, with the exception of ER_{ES} (F1: 852 ± 886%; F2: 3 ± 43%; F4: 620 ± 876%). Regarding epilithic recovery, there were no specific patterns visible.

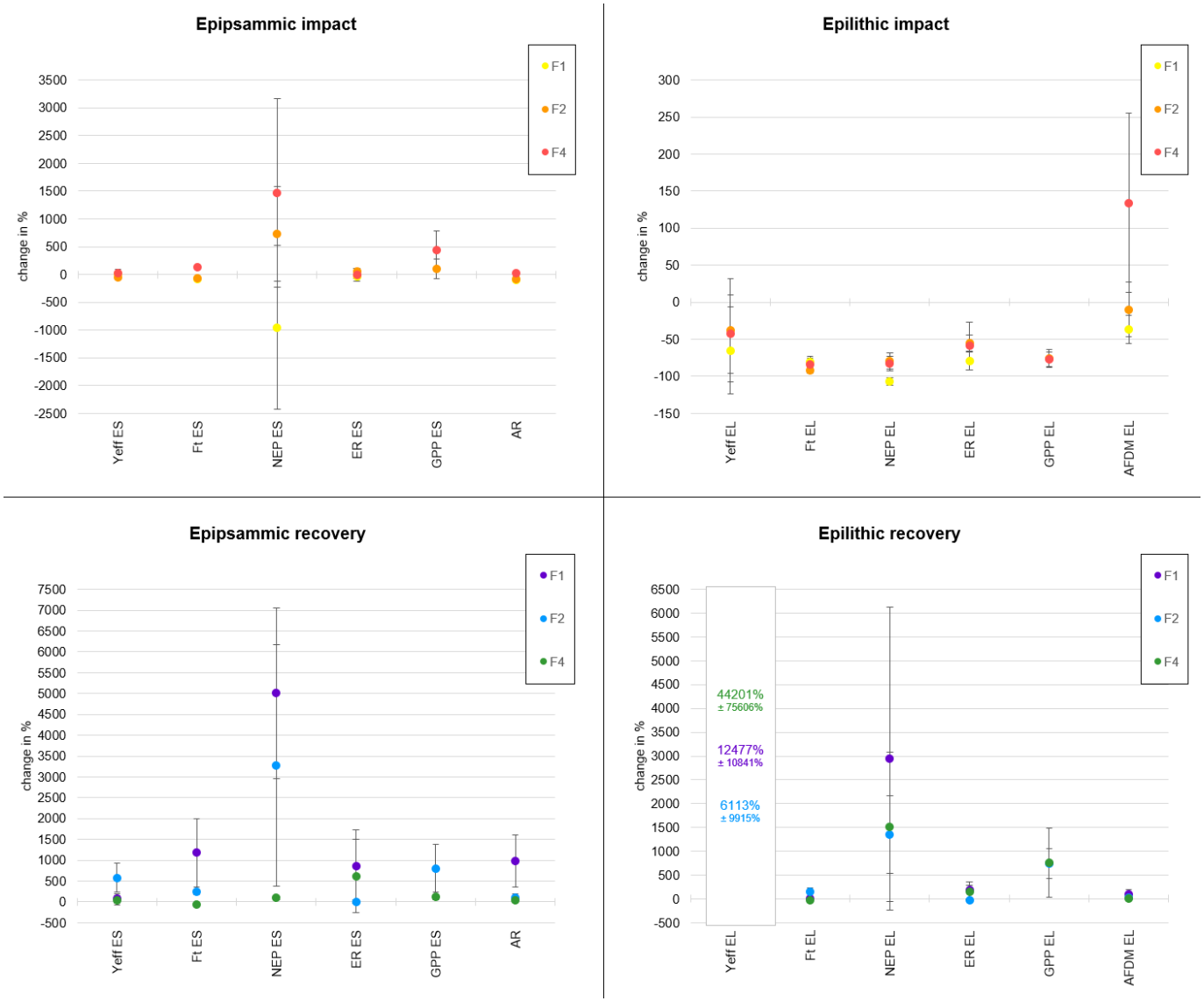

Figure 13: Overview of impact and recovery expressed as relative change [%], including standard deviation in epipsammic (ES) and epilithic biofilm (EL) per treatment (n = 3). The concerned variables effective quantum yield of photochemistry ($Yeff_{ES}$, $Yeff_{EL}$), minimum fluorescence yield (Ft_{ES}, Ft_{EL}), net ecosystem production (NEP_{ES}, NEP_{EL}), ecosystem respiration (ER_{ES}, ER_{EL}), gross primary production (GPP_{ES}, GPP_{EL}), aerobic respiration (AR) and ash free dry mass ($AFDM_{EL}$). As exceptions, the number of samplings was smaller (n = 2) for the impact of ER_{ES} in F1 and ER_{ES} and GPP_{ES} in F4, as well as for the recovery of ER_{ES} in F1. There is no data for the impact and the recovery of gross primary production (GGP_{ES}, GPP_{EL}) in F1. As the effective quantum yield of photochemistry in the epilithic biofilm ($Yeff_{EL}$) has very high values, they are indicated as numbers.

Analysing the relationship between variables, only few correlations are recognizable. Considering the relationship between variables within the epipsammic or epilithic compartment: epipsammic impact values of heterotrophic and autotrophic biofilms' function variables AR and $Yeff_{ES}$ showed a weakly positive relationship (R^2 = 0.32), whereas the recovery values showed no relationship (R^2 = 0.01). This is the same for the epilithic impact values of heterotrophic and autotrophic biofilms' function variables ER_{EL} and $Yeff_{EL}$ (R^2 = 0.03), whereas recovery values showed a weakly positive relationship (R^2 = 0.24). There was no relationship for impact and recovery between the autotrophic biomass and function variables Ft_{ES} and $Yeff_{ES}$ (impact: R^2 = 0.08; recovery: R^2 = 0.07) and Ft_{EL} and $Yeff_{EL}$ (impact: R^2 = 0.08; recovery R^2 = 0.06), as well as for the biofilms' biomass variables $AFDM_{EL}$ and Ft_{EL} (impact: R^2 = 0.12; recovery: R^2 = 0.01). Considering the relationship of variables between the epipsammic and epilithic compartment: A strong negative relationship existed for the impact values between autotrophic function variables $Yeff_{ES}$ and $Yeff_{EL}$ (R^2 = 0.45). In contrast, the recovery values were not related (R^2 = 0.19). Furthermore, there was no relationship between the autotrophic biofilms' biomass variables Ft_{ES} and Ft_{EL} (impact: R^2 = 0.001; recovery: R^2 = 0.001), neither in impact nor in recovery.

To summarize the described results, there were only a few significant differences between the treatments, regarding impact and recovery in epipsammic and epilithic biofilm (see Table 10). The impact of Ft_{ES}, NEP_{ES}, and AR differed significantly between F1 and F4 (Ft_{ES}: p-value = 0.002; NEP_{ES}: p-value = 0.013; AR: p-value = 0.008) and F2 and F4 (Ft_{ES}: p-value = 0.003; NEP_{ES}: p-value = 0.019; AR: p-value = 0.010). Thus, F1 and F2 experienced similar behaviour and did not differ significantly. In addition, the recovery of Ft_{ES} showed a significant difference between F1 and F4 (Ft_{ES}: p-value = 0.007), and between F2 and F4 (Ft_{ES}: p-value = 0.008) as well. In contrast, ER_{ES} and ER_{EL} were only significantly different between F2 and F4 (ER_{ES}: p-value = 0.039; ER_{EL}: p-value = 0.019), whereas the values of F1 and F4 were similar. However, comparing the variables at *xf* (for data per treatment see appendix III), no significant differences between treatments were evident. Thus, the significant differences did not persist.

Table 10: Results of the statistical analysis of the differences between treatments concerning impact and recovery in the epipsammic (ES) and epilithic biofilm (EL). The concerned variables (n = 9) are quantum yield of photochemistry ($Yeff_{ES}$, $Yeff_{EL}$), minimum fluorescence yield (Ft_{ES}, Ft_{EL}), net ecosystem production (NEP_{ES}), ecosystem respiration (ER_{ES}, ER_{EL}) and aerobic respiration (AR).

		Impact						**Recovery**					
		ANOVA			**TukeyC (p-value)**			**ANOVA**			**TukeyC (p-value)**		
		Df	**F-value**	**p-value**	**F1-F2**	**F1-F4**	**F2-F4**	**Df**	**F-value**	**p-value**	**F1-F2**	**F1-F4**	**F2-F4**
ES	**Yeff**	2	2.304	0.181	-	-	-	2	2.086	0.205	-	-	-
	Ft	2	22.88	***0.002***	0.908	***0.002***	***0.003***	2	15.25	***0.004***	0.997	***0.007***	***0.008***
	NEP	2	11.08	***0.010***	0.922	***0.013***	***0.019***	2	2.701	0.146	-	-	-
	ER	2	0.822	0.502	-	-	-	2	6.767	***0.038***	0.103	0.866	***0.039***
	AR	2	13.77	***0.006***	0.974	***0.008***	***0.010***	2	1.541	0.288	-	-	-
EL	**Yeff**	2	1.418	0.313	-	-	-	2	0.219	0.809	-	-	-
	Ft	2	0.798	0.493	-	-	-	2	3.541	0.097	-	-	-
	ER	2	0.302	0.750	-	-	-	2	8.022	***0.020***	0.068	0.578	***0.019***

4 Discussion

In this chapter, the considerations and findings of the experiment are reflected and discussed, and suggestions for future experiments and practical implementations are illustrated as well.

4.1 Considerations on the experiment

The results of a controlled experimental system, such as the ESF, should be cautiously extrapolated to field conditions. While the advantage of a manipulative experiment is the elimination of irrelevant variables and enhancing statistical power to identify the effects, the disadvantage is that the conditions in the real world are potentially more extreme and stressors can co-occur (Colls et al., 2019; Acuña et al., 2015; Acuña et al., 2019). Such co-occurrences can be of human origin, for instance, an increasing water demand, or of natural origin, such as alterations of the flow regime, induced by climate change (Griffith & Gobler, 2019; Malmqvist et al. 2008; Settele et al., 2014). Therefore, it is essential to identify the effects of interest as a first step and subsequently use the acquired knowledge to undertake further experiments under natural conditions. To extrapolate any results, various limitations have to be considered. For instance, it is important to bear in mind that the past hydrological history of stream biofilm, such as its ability to adapt to intermittent flow regime, has great influence on stream biofilm response. It is also important to note that the natural community composition and the geomorphology are more complex and therefore the biota in natural streams are differently affected by disturbances (Muñoz et al., 2018; White & Pickett, 1985). Further constraints are found in the environment of the temporary stream. In this experiment, it was the Riera de Llémena, which is characterized, for example, by a well conserved and shading vegetation. This specific characteristic was not simulated at the ESF. Hence, light conditions and the related availability of nutrients, provided by organic material, differed.

Prior to the following discussion, we would like to point out that the descriptor variables are included in the discussion to have a broader basis for our statements and conclusions. However, in order to not distort the statements regarding patterns, we have decided to exclude GPP from the discussion as there was only data for two treatments, and thus it is not comparable with the development of other response values. Additionally, several variables had large standard deviations, most clearly for autotrophic function. Since standard deviations indicate the data distribution, we assumed that the biofilm was not evenly spread, creating irregular patches in the substrata which influenced our sampling. Patches are caused by dominating flow paths or the resistance to completely dry out and can therefore be described as an ‘ever-changing mosaic’ (Febria et al., 2012; Lake, 2000; Pringle et al., 1988). Each disturbance alters the patchiness patterns in streams and during the recovery phase, they undergo further changes due to recolonization (Lake, 2000). Moreover, although the biofilm was acclimatised for two weeks, which is about the same time range as Muñoz

et al. (2018) applied in their ESF experiment for which biofilm samples from the same intermittent stream were used, the autotrophic variables showed rather low values at the beginning. Thus, the biofilm might have been more affected by the transport and set-up of the ESF than expected or due to a recent drought event in the Riera de Llémena. Considering that some methods, for instance the Diving-PAM, are point measurements and both patches with well-developed or less developed biofilms could be sampled, a high standard deviation might occur. One approach to reduce high standard deviations could be to conduct more measurements per channel, as the standard deviation generally decreases with increasing sample size (Rumsey, 2016).

4.2 Response of the stream biofilm

Regarding relative changes, the impact values of epipsammic, but not of epilithic biofilm decreased with increasing frequency, with the exception of ER_{ES}. It is apparent that heterotrophic and autotrophic biofilm was similarly impacted by increasing frequency, as F1 and F2 significantly differed from F4 for biomass (Ft_{ES}) and function (AR and NEP_{ES}). The values in F4 for Ft_{ES} and AR were positive, continuously increasing instead of experiencing a small (negative) impact, which was unexpected. The same pattern applied as well for the autotrophic function (Yeff), but without significance. Consistent with the first hypothesis, the observed pattern allows for the presumption that frequency only mattered, if the non-flow period was split into several shorter ‘sub-periods’. With higher frequency the total number of non-flow days was divided in shorter durations, leading to both shorter flow and non-flow periods, to which epipsammic biofilm was apparently more resistant. Although duration was not of relevance for this thesis, a link to its importance, as found by Acuña et al. (2015) under artificial and by Colls et al. (2019) under natural conditions, is inevitable. It seemed that even within the total drought duration of 28 days, the duration of the ‘sub-periods’ played a key role, suggesting a threshold between 2 and 4 frequencies (periods of 14 and 7 non-flow days). The threshold indicates a change in conditions during which, despite non-flow conditions, the biofilm continues to grow. As non-flow periods are ramp-patterned disturbances, conditions change steadily, allowing biofilm to adapt (Lake, 2000). For example, it was previously observed by Roberson & Firestone (1992), that biofilm’s EPS were able to retain water which led to a slower dehydration. Thresholds caused by extrinsic factors like hydrology are common for streams, as it characterizes the ecosystem’s structure (Groffman et al., 2006). The evidence for the mentioned threshold is linked to the fact that at high non-flow frequency and shorter non-flow periods, the water content at the beginning of flow periods was approximately 6%, whereas fewer frequency led to complete desiccation between flow periods. As the water content is one of the most important abiotic components, influencing the oxygen concentrations and nutrient availability for the biofilm, it enables a steady growth (Drenovsky et al., 2004). This occurred for instance, in autotrophic biomass in the epipsammic compartment, during the non-flow period at high frequency.

A threshold associated with the cessation of flow was also observed by Acuña et al. (2015) for community respiration, whose resistance values considerably changed between 6 and 12 non-flow days.

An additional observation concerning the relative changes of the response variables was that the recovery level related to the severity of the impact. This was observed for all variables with the exception of the autotrophic function and biomass in epilithic compartment ($Yeff_{EL}$ and Ft_{EL}). The sequence pattern was equal during impact and recovery for each particular variable, although not all variables decreased correspondingly with a higher frequency. An exception can be noted with Ft_{ES} as impact and recovery experienced the same sequence pattern with the highest values in F1 and the lowest values in F4, but the recovery value in F4 was negative, which was unexpected because the autotrophic biomass did recover during the other two treatments. One explanation could be that measurement errors occurred during the sampling. However, this is unlikely because Ft_{ES} as well as Ft_{EL} exhibited a decrease in all channels. Additionally, the biomass value $AFDM_{EL}$ experienced the slowest recovery at the highest frequency as well. It is assumed that other factors were involved, such as community dynamics or the addition of nutrients. A change in the composition and concentration of nutrients might have led to growth inhibition (Harpole et al., 2011). Hence, it is not possible to say with certainty why the autotrophic biomass in F4 decreased during the last flow period. Nonetheless, the pattern of 'the larger the impact, the quicker the recovery' was also observed at an ESF experiment by Acuña et al. (2015). They argue that some autotrophic communities are more resilient, because they were able to use the released space and energy. This is in accordance with Townsend and Hildrew's (1994) description of a disturbance as an event which removes organisms, creating patches of empty space and increasing resources. In addition to the findings in the autotrophic biofilm, Acuña et al. (2015) compared the impact and recovery relationship to the heterotrophic biofilm. They found that the autotrophic disturbance-response relationship was linear while the relationship was sigmoid for heterotrophs. This means that non-flow periods had an immediate effect on autotrophs while the effect on heterotrophs was delayed, indicating that heterotrophs are more resistant and less resilient. The shift to heterotrophy after a disturbance occurred at a duration of non-flow periods longer than 6 days and was limited to the non-flow period and the first weeks of flow return. Considering our results, despite the fact that the 'sub-periods' of non-flow periods in all treatments (7, 14 and 28 non-flow days) lasted longer than these 6 non-flow days, no shift to heterotrophy was observed. The weakly positive relationship of autotrophic and heterotrophic function ($Yeff_{ES}$ and AR) let us assume that they responded similarly.

Comparing impact and recovery of biofilm's function and biomass in the epilithic compartment, it is noticeable that all variables, except $AFDM_{EL}$, were affected by desiccation but not by frequency of non-flow periods. For instance, the autotrophic biofilm's biomass diminished equally in all treatments. The reduction during impact averaged between 80-90%, which corresponds to the decrease of 80% in natural streams observed by Timoner et al. (2012). Analysing the biofilm's recovery values, the variables accordingly showed several differing developments. Overall, the response of the biofilm's biomass and function in the epilithic compartment during impact and recovery stood in contrast to epipsammic compartment, where variables, except ER_{ES}, were affected similarly by the frequency of non-flow periods. Hence, the similar behaviour of the biomass and function accentuate the assumption that humidity, which was present in the epipsammic substrate at highest frequency, was decisive (as mentioned previously).

We further hypothesized that the autotrophic biofilm in the epipsammic compartment recovers more slowly than in the epilithic compartment due to the retained water in the sand. This has only proven to be true for the autotrophic function. Timoner et al. (2012) came to the same conclusion as they found a positive relationship between autotrophic function and water content under natural conditions, while Muñoz et al. (2018) confirmed this development under artificial conditions. A further observation was that the autotrophic function's values in the epilithic compartment were generally higher than in the epipsammic compartment. The strong negative relationship between $Yeff_{ES}$ and $Yeff_{EL}$ supports this statement, meaning the higher the values in the epilithic biofilm are, the lower they are in the epipsammic biofilm. Therefore, we conclude that the epilithic compartment was more affected as it experienced a greater impact and a quicker recovery, indicating low resistance and high resilience. This high capacity to recover, especially in the epilithic compartment, was also studied in earlier works, which showed that autotrophs were able to develop various adaption strategies (Robson, 2000; Sabater et al., 2017). For instance, they had the ability to remain dormant during dry periods, developing drought resistant cell structures or the ability to closely attach themselves to substrata to immediately resume their activities after flow return (McKew et al., 2011; Timoner et al., 2014a). This prevented damage in the photosynthetic apparatus and might partly account for the sensitivity of the autotrophs and their ability to respond to a small pulse of rehydration and thus allow for a rapid recovery (Timoner et al., 2012; Timoner et al., 2014b). Muñoz et al. (2018) confirmed the observation of the autotrophs' sensitivity in natural streams. They found that with a rehydration pulse, autotrophic function increased up to approximately 60%, compared to the initial state. Moreover, a few days after flow return, which followed one week after the rehydration pulse, the autotrophic functions reached roughly the same values as the initial state. Considering our results, we found that after one week under flow condition, the autotrophic function in both compartments reached or exceeded the values of their initial state and seemed to stabilize until the end of the experiment.

Last but not least, while some variables showed a significant difference at the beginning (uniformity) or for impact and recovery during the treatment phase, all variables were uniform at the end of the experiment. Actually, this was well illustrated for instance by the autotrophic and heterotrophic function (Yeff and AR), which responded with an increase in activity with every returning flow period, showing higher values at the final state, compared to their initial state. This pattern was especially visible in F4, in which the duration of a single non-flow period was the shortest, as mentioned previously. Despite this apparent pattern of growth with every flow period during the treatment phase, indicating an effect of the increasing number of frequencies, the frequency of non-flow periods with the same total number of non-flow days had seemingly short-term, but no long-term effects on stream biofilm. This fact suggests, that according to the definition of Holling (1973), the system was resilient enough to recover and that the disturbance was within the tolerable magnitude of the system, allowing the return to the steady-state instead of altering the systems' structure. However, one has to consider Schwalm et al. (2017), who predicted, that if droughts occur more often, ecosystems will be permanently harmed on a long-term scale, as there is not enough time left between two subsequent dry periods to fully recover.

4.3 Future outlook

Contemplating further studies, one approach could be to adapt the experimental design to a higher number of frequencies. To provide further insights into the results, the addition of a frequency with three non-flow periods (F3) might lead to a more precise determination of the threshold. It would also be interesting to research what would occur if the experiment is extended. For instance, if the duration of non-flow periods were to last at least two weeks, would the same short-term patterns appear or does the duration of the non-flow periods have more substantial effects? We would expect similar patterns as we observed in the context of this thesis, but for the threshold to disappear, because it is linked to the retained water in the substrate. Therefore, we also assume that the impact and recovery would be greater in the epipsammic biofilm and that the effect would remain more or less the same in the epilithic biofilm. Furthermore, a prolongation of the experiment would perhaps result in clearer visible patterns regarding relationships between variables. Moreover, various methods and equipment should be applied to improve present methodological limitations and to gain experience (Datry et al., 2017b).

The knowledge acquired by this thesis could be consulted to plan and conduct further experiments in natural temporary waterways. For instance, the threshold under natural conditions could be determined, which is influenced more severely by factors such as climate, temperature, current flow regime or geomorphology (Acuña et al., 2015; Jaeger et al., 2017; McDonough et al., 2011). Therefore, the obtained findings improve the understanding of whether frequency is a decisive factor for stream ecosystems or not. In addition, the determination of thresholds is important to predict

future risks as well as to establish adequate regulatory frameworks, which improve the currently poor management strategies of intermittent streams and rivers (see chapter 1.2.2) (Acuña et al., 2014; Brenden et al., 2008). An adequate management strategy considers the temporal aspects of flow regimes to uphold natural conditions of non-flow periods as far as possible and to avoid exceeding tipping points that could negatively affect flow intermittent ecosystems (Acuña et al., 2015). With regard to this thesis, such a specific management strategy could include solutions to maintain the balance between impact and recovery of stream communities, support conservation or restore of degraded intermittent streams.

5 Conclusion

To summarize, temporary waterways fulfil an essential role in supporting biodiversity and providing various ecological, economic and societal services. The number of intermittent waterways is expected to increase in the future, and it is anticipated that their flow regime will be altered by the ongoing climate change issue and the intensification of anthropogenic stressors (Acuña et al., 2017; Settele et al., 2014). As Colls et al. (2019) stated, only a small number of studies concerning the temporal components of flow intermittency exist and even fewer which research their individual and relative role. Therefore, this bachelor thesis assessed the specific role of frequency on stream biofilm. We hypothesized that the response of the stream biofilm is less if the frequency of non-flow periods is higher, which we were able to prove for the biomass and function of autotrophic and heterotrophic biofilm in the epipsammic compartment. The biofilm's behaviour suggested a threshold between 7 and 14 non-flow days. In contrast, the epilithic biofilm was affected by desiccation regardless of the number of frequencies. We also assumed that autotrophs in epipsammic biofilm recover more slowly than in epilithic biofilm with higher number of frequencies due to an increasing amount of retained water. The experiment showed that the water content was retained at high frequency which was consistent with the observation that autotrophic function in the epipsammic compartment had lower recovery values than epilithic autotrophs. However, the most noteworthy result was that at the end of the experiment none of the differences persisted. This led to the conclusion that frequency with the same total number of non-flow days only had an effect on a short-term but not on a long-term scale. Thus, although the two hypotheses were partially confirmed, in relation to the fact that no long-term effects occurred, the two hypotheses have to be discarded. Nevertheless, these findings could be used to conduct experiments under natural conditions as well as to develop adequate management strategies.

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Plagiarism declaration (Plagiatserklärung)

Mit der Abgabe dieser Bachelorarbeit versichern die Studierenden, dass sie die Arbeit selbständig und ohne fremde Hilfe verfasst haben.

Die unterzeichnenden Studierenden erklären, dass alle verwendeten Quellen (auch Internetseiten) im Text oder Anhang korrekt ausgewiesen sind, d.h. dass die Bachelorarbeit keine Plagiate enthält, also keine Teile, die teilweise oder vollständig aus einem fremden Text oder einer fremden Arbeit unter Vorgabe der eigenen Urheberschaft bzw. ohne Quellenangabe übernommen worden sind.

Bei Verfehlungen aller Art treten Paragraph 39 und Paragraph 40 der Rahmenprüfungsordnung für die Bachelor- und Masterstudiengänge an der Zürcher Hochschule für Angewandte Wissenschaften vom 29. Januar 2008 sowie die Bestimmungen der Disziplinarmassnahmen der Hochschulordnung in Kraft.

Ort, Datum:	**Unterschrift:**
Münchenbuchsee, 05.02.2020	 Ariane Etter
Besenbüren, 05.02.2020	 Selina Fischer

Appendixes

Table of content

Appendix I:

Experimental design

bold dates = weekend, public holidays -> no measurements taken

Date	Total number of days	Number of days during treatment	D28F1	D28F2	D28F4
02.09.2019	1		*Colletion of sand and cobbles*		
03.09.2019	2				
04.09.2019	3		*Acclimatisation phase (2 weeks)*		
05.09.2019	4				
06.09.2019	5				
07.09.2019	6				
08.09.2019	7				
09.09.2019	8				
10.09.2019	9				
11.09.2019	10				
12.09.2019	11				
13.09.2019	12				
14.09.2019	13				
15.09.2019	14				
16.09.2019	15				
17.09.2019	16		xi	xi	xi
18.09.2019	17	1	*Treatment phase*		
19.09.2019	18	2			
20.09.2019	19	3			
21.09.2019	20	4			
22.09.2019	21	5			
23.09.2019	22	6			
24.09.2019	23	7			
25.09.2019	24	8			~~xp11~~
26.09.2019	25	9			*d*
27.09.2019	26	10			*d*
28.09.2019	27	11			
29.09.2019	28	12			
30.09.2019	29	13			*d*
01.10.2019	30	14			*d*
02.10.2019	31	15		~~xp11~~	~~xp18~~
03.10.2019	32	16		*d*	
04.10.2019	33	17		*d*	
05.10.2019	34	18			
06.10.2019	35	19			
07.10.2019	36	20		*d*	
08.10.2019	37	21		*d*	
09.10.2019	38	22		~~xp18~~	~~xp21~~
10.10.2019	39	23			*d*
11.10.2019	40	24			*d*
12.10.2019	41	25			
13.10.2019	42	26			
14.10.2019	43	27			*d*
15.10.2019	44	28			*d*
16.10.2019	45	29	~~x1~~		~~xp28~~
17.10.2019	46	30	*d*		
18.10.2019	47	31	*d*		
19.10.2019	48	32			
20.10.2019	49	33			
21.10.2019	50	34	*d*		
22.10.2019	51	35	*d*		
23.10.2019	52	36	x2	~~x1~~	~~xp31~~
24.10.2019	53	37		*d*	*d*
25.10.2019	54	38		*d*	*d*
26.10.2019	55	39			
27.10.2019	56	40			
28.10.2019	57	41			
29.10.2019	58	42			
30.10.2019	59	43		x2	~~xp38~~
31.10.2019	60	44			
01.11.2019	61	45			
02.11.2019	62	46			
03.11.2019	63	47			
04.11.2019	64	48			
05.11.2019	65	49			
06.11.2019	66	50			~~x1~~
07.11.2019	67	51			*d*
08.11.2019	68	52			*d*
09.11.2019	69	53			
10.11.2019	70	54			
11.11.2019	71	55			*d*
12.11.2019	72	56			*d*
13.11.2019	73	57	xf	xf	x2/xf

Samplings:

d
Physicochemical parameters
Yield of photochemistry (ES & EL)

xi
Water content (ES)
AFDM (ES & EL)
Yield of photochemistry (ES & EL)
Aerobic respiration (ES)
Metabolism (ES & EL)

xp
before end of non-flow period (11, 21, 31)
Water content (ES)
AFDM (ES)
Yield of photochemistry (ES & EL)
Aerobic respiration (ES)

before end of flow period (18, 28, 38)
Water content (ES)
AFDM (ES)
Yield of photochemistry (ES & EL)
Aerobic respiration (ES)

x1
Water content (ES)
AFDM (ES & EL)
Yield of photochemistry (ES & EL)
Aerobic respiration (ES)
Metabolism (ES & EL)

x2
Water content (ES)
AFDM (ES & EL)
Yield of photochemistry (ES & EL)
Aerobic respiration (ES)
Metabolism (ES & EL)

xf
Water content (ES)
AFDM (ES & EL)
Yield of photochemistry (ES & EL)
Aerobic respiration (ES)
Metabolism (ES & EL)

Appendix II:

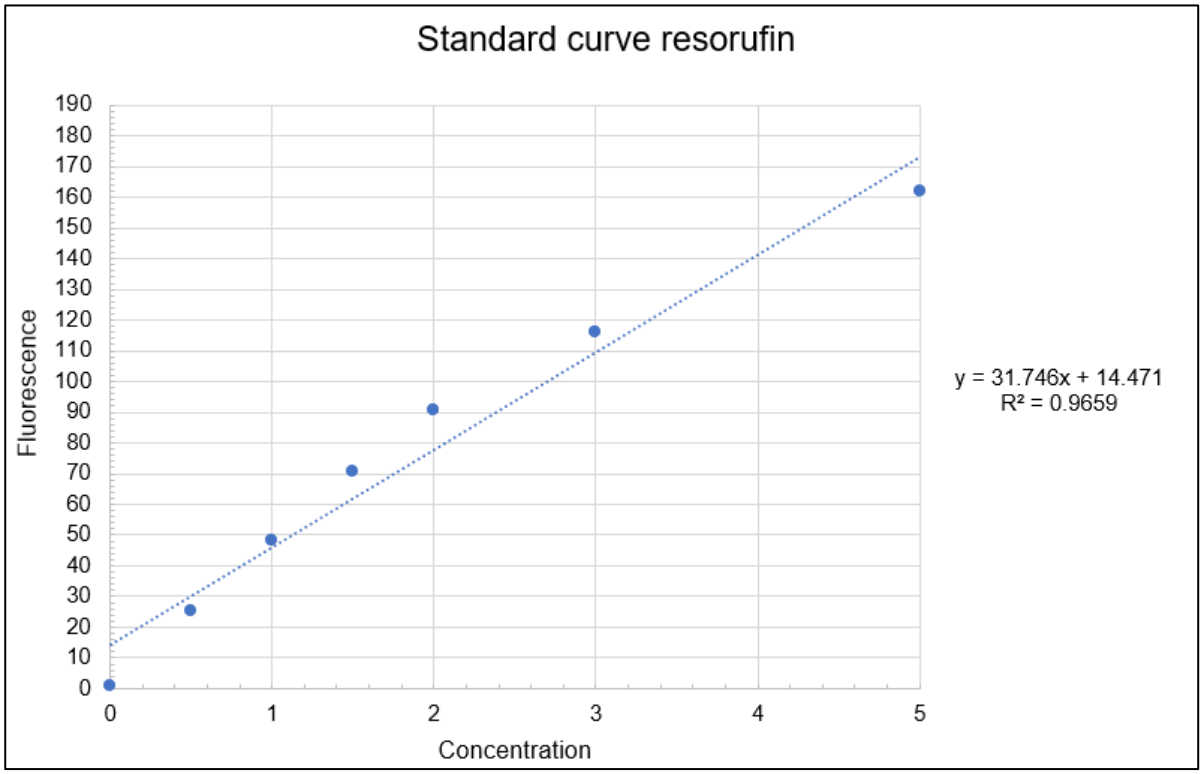
Resorufin standard curve

Preparation table for the seven samples for the resorufin standard curve (see below):

Initial concentration of standard solution resorufin [µM]	Initial volume of added standard solution resorufin [µL]	Final concentration of standard solution resorufin [µM]	Volume of added phosphate buffer [µL]	Final volume in tube [µL]
10	0	0.0	2000	2000
10	100	0.5	1900	2000
10	200	1.0	1800	2000
10	300	1.5	1700	2000
10	400	2.0	1600	2000
10	600	3.0	1400	2000
10	1000	5.0	1000	2000

Standard resorufin solution: 0.0118 g of resorufin salts were dissolved in 50 mL of phosphate buffer, creating the initial resorufin solution. This solution was diluted to a resorufin concentration of 0.01 mM (0.5 mL of initial resorufin solution and 49,5 mL of phosphate buffer).

With the following standard curve, the concentration of resorufin in µM was calculated in accordance with the detected fluorescence:



Appendix III:
Initial and final state per treatment

The average values per treatment of the initial state at *xi* and the final state at *xf*, including standard deviations:

			Initial and final state					
			F1		F2		F4	
			xi	xf	xi	xf	xi	xf
ES	Yeff	[f.u.]	3285 ± 1476	6667 ± 1566	4479 ± 3166	7138 ± 1343	3484 ± 1369	4806 ± 3719
	Ft	[f.u.]	25 ± 11	22 ± 12	29 ± 11	10 ± 4	19 ± 2	19 ± 6
	NEP	[$gO_2/m^3 \cdot d$]	18 ± 15	513 ± 55	15 ± 31	382 ± 54	23 ± 29	386 ± 151
	ER	[$gO_2/m^3 \cdot d$]	-33.4 ± 27.6	-100.4 ± 28.5	-34.1 ± 5.9	-88.0 ± 84.2	-35.2 ± 0.7	-114.7 ± 7.7
	GPP	[$gO_2/m^3 \cdot d$]	52 ± 36	451 ± 115	50 ± 33	470 ± 87	59 ± 40	501 ± 144
	AFDM	[mg/cm^3]	20.1 ± 4.1	32.4 ± 3.9	20.5 ± 3.1	34.3 ± 1.9	21.3 ± 5.9	32.1 ± 3.8
	AR	[$nmol/cm^3 \cdot h$]	3.2 ± 0.4	4.4 ± 0.4	3.6 ± 0.5	5.4 ± 1.2	3.4 ± 1.1	5.2 ± 1.5
EL	Yeff	[f.u.]	2857 ± 2106	2637 ± 1882	2037 ± 1573	4356 ± 1537	1467 ± 1597	3135 ± 3797
	Ft	[f.u.]	177 ± 327	19 ± 3	276 ± 37	13 ± 2	156 ± 10	16 ± 4
	NEP	[$gO_2/m^3 \cdot d$]	5 ± 2	11 ± 2	8 ± 2	8 ± 2	6 ± 3	9 ± 8
	ER	[$gO_2/m^3 \cdot d$]	-1.8 ± 0.2	-0.7 ± 0.3	-2.1 ± 0.1	-0.8 ± 0.5	-1.6 ± 0.6	-1.2 ± 0.5
	GPP	[$gO_2/m^3 \cdot d$]	7 ± 2	11 ± 2	10 ± 2	9 ± 2	8 ± 3	10 ± 8
	AFDM	[mg/cm^2]	1.6 ± 0.7	1.8 ± 0.7	1.7 ± 0.4	2.0 ± 0.4	1.0 ± 0.4	2.2 ± 0.8

Appendix IV:

Data per treatment (relative change)

The average values per treatment of the relative change (in %) of impact and recovery, including standard deviations. In addition, the sequence of the treatment going from highest to lowest impact and recovery value, respectively:

		Impact (%)			Recovery (%)			Sequence (hight to low)
		F1	F2	F4	F1	F2	F4	
ES	Yeff	11 ± 39	-54 ± 34	24 ± 73	40 ± 106	579 ± 354	39 ± 117	impact: F2-F1-F4 recovery: F2-F1-F4
	Ft	-90 ± 8	-68 ± 7	127 ± 31	1182 ± 820	241 ± 59	-53 ± 22	impact: F1-F2-F4 recovery: F1-F2-F4
	NEP	-954 ± 1470	726 ± 853	1467 ± 1698	5013 ± 2048	3278 ± 2895	94 ± 41	impact: F1-F2-F4 recovery: F1-F2-F4
	ER	-42 ± 28	57 ± 55	-3 ± 113	852 ± 886	3 ± 43	620 ± 876	impact: F1-F4-F2 recovery: F1-F4-F2
	GPP	*NA*	99 ± 176	433 ± 353	*NA*	803 ± 571	113 ± 53	impact: F4-F2 recovery: F2-F4
	AR	-97 ± 10	-82 ± 18	22 ± 44	984 ± 631	98 ± 98	36 ± 29	impact: F1-F2-F4 recovery: F1-F2-F4
EL	Yeff	-65 ± 59	-38 ± 70	-43 ± 53	12477 ± 10841	6113 ± 9915	44201 ± 75606	impact: F1-F4-F2 recovery: F4-F1-F2
	Ft	-80 ± 5	-92 ± 4	-84 ± 11	-1 ± 41	145 ± 84	-21 ± 26	impact: F2-F4-F1 recovery: F2-F1-F4
	NEP	-107 ± 5	-81 ± 11	-83 ± 10	2947 ± 3181	1353 ± 823	1513 ± 1570	impact: F1-F4-F2 recovery: F1-F4-F2
	ER	-79 ± 12	-58 ± 11	-59 ± 32	180 ± 177	-29 ± 4	143 ± 149	impact: F1-F4-F2 recovery: F1-F4-F2
	GPP	*NA*	-76 ± 12	-77 ± 10	*NA*	736 ± 312	761 ± 717	impact: F4-F2 recovery: F4-F2
	AFDM	-37 ± 19	-10 ± 37	134 ± 121	99 ± 90	35 ± 30	2 ± 23	impact: F1-F2-F4 recovery: F1-F2-F4

Stream biofilm response to an increasing number of non-flow periods

Zürcher Hochschule für Angewandte Wissenschaften
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Although intermittent waterways account for approximately 50% of the global river system and they provide various ecological, economic and societal services, they are vastly underestimated, lacking in legal, public and scientific recognition. In addition, research is needed because of their increasing number due to anthropogenic stressors and climate change. Therefore, the aim was to identify how the frequency of non-flow periods affects autotrophic and heterotrophic stream biofilm, as it plays a key role in stream processes such as the nutrient cycle or the food web. [2, 3, 4, 6, 7, 10]

Intermittent waterways

Their flow ceases at some point in time and space, often experiencing a periodic loss of some or all surface water (Figure 1). [1]

Figure 1: An intermittent river of Northeast Iberian Peninsula during dry and wet season. (received from Acuña, 2019)

Hypotheses

The hypotheses state that the increase of frequency of non-flow periods with the same total number of non-flow days …

- lessens the effect on autotrophic and heterotrophic biofilm, because the number of subsequent non-flow days is smaller and thus the biofilm is less stressed.
- leads to a slower recovery of autotrophs in epipsammic than in epilithic biofilm, because the amount of water retained in sand is higher.

Experimental Stream Facility (ESF)

The indoor facility of the Institute for Water Research (ICRA) consisting of artificial streams, which allows for controlled experiments concerning flow intermittency (Figure 2). [1]

Figure 2: One unit of the ESF, which shows the development of epipsammic and epilithic biofilm under different treatments. (Etter, 2019)

Experimental design and methods

The experiment using the ESF included three treatments involving one drought duration (28 days) and three frequencies:
- F1 with 1x 28 non-flow days
- F2 with 2x 14 non-flow days
- F4 with 4x 7 non-flow days

To determine the response to the treatments, different variables were measured at defined points in time in epipsammic (sand) and in epilithic (cobbles) biofilm (Figure 3). The variables represented biomass (structure) and function (activity) of the stream biofilm:

Biofilm	**autotrophic**	**heterotrophic**	**both**
Biomass	Ft		AFDM
Function	Yeff, GPP	AR, ER	NEP

To quantify the effects of frequency two parameters were considered: [8, 9]
- **impact:** indicator of the biofilm's resistence during the disturbance between x1-xi
- **recovery:** indicator of the biofilm's resilience after the disturbance between x2-x1

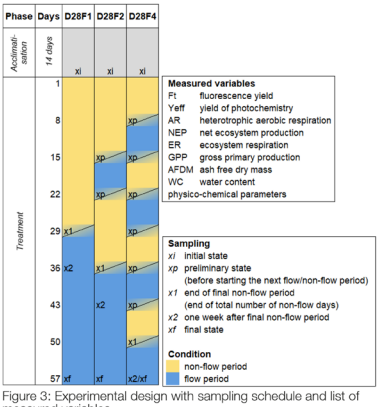

Figure 3: Experimental design with sampling schedule and list of measured variables.

Findings

The frequency of non-flow periods had no effect on epilithic biofilm but influenced epipsammic biofilm (Figure 4).

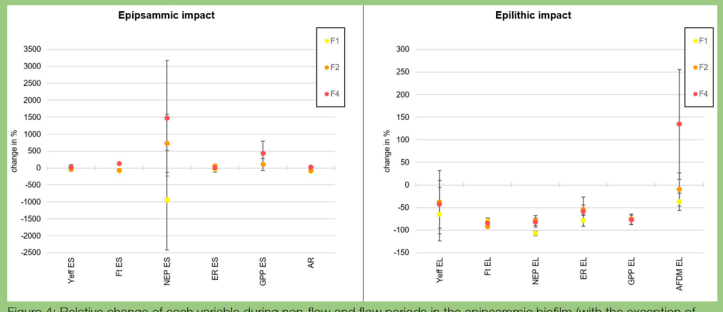

Figure 4: Relative change of each variable during non-flow and flow periods in the epipsammic biofilm (with the exception of AFDM).

Water content
The highest WC of approximately 6% was found in F4, whereas fewer frequencies led to complete desiccation between flow periods.

Autotrophic and heterotrophic biofilm
Significant differences were found in several epipsammic autotrophic and heterotrophic variables during non-flow and flow periods, showing that the biofilm responded differently in F4, compared to F1 and F2. Since all variables, except NEP and ER, followed this pattern a threshold between 7 and 14 subsequent non-flow days might exist.
-> First hypothesis is partially confirmed

Autotrophic function and biomass
Autotrophic biomass diminished equally (80-90%) in all three treatments in the epilithic biofilm, which is comparable to natural conditions. The frequency affected both function and biomass in the epipsammic biofilm, but only function recovered slowest at highest frequency.
-> Second hypothesis is partially confirmed

Impact and recovery
The majority of the variables experienced the most severe impact and the quickest recovery in the same treatment.

Conclusion

An increasing frequency lessened the effects only on epipsammic biofilm. Additionally, only autotrophic function recovered more slowly in sand than on cobbles. However, at the end of the experiment none of the differences persisted. Therefore, frequency only had an effect on a short-term, but not on a long-term scale. Thus, both hypotheses were discarded. A practical implementation of the findings could include the improvement of management strategies regarding the impact-recovery balance of stream communities to restore degraded intermittent waterways.

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Bachelor thesis / February 2020

The authors gratefully acknowledge the valuable support of the supervisors Prof. Dr. Michael Doering (ZHAW) & Dr. Vicenç Acuña (ICRA)