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Universidad del País Vasco · Euskal Herriko Unibertsitatea

ZIENTZIA ETA TEKNOLOGIA FAKULTATEA

FACULTAD DE CIENCIA Y TECNOLOGÍA

EFFECTS OF WATER DIVERSION ON STREAM ECOSYSTEM FUNCTIONING:

INTERACTION WITH OTHER STRESSORS

ANA VICTORIA PEREZ CALPE

PhD Thesis - March 2023

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Supervised by Arturo Elosegi

“Si comparamos el río con la roca,

el río gana siempre, no gracias a su fuerza,

sino a su perseverancia.”

Buda

“El gran llibre,

sempre obert i que hem de fer un esforç per llegir,

és el de la Natura.”

Antoni Gaudí

A la meva mare,

Xabiri, nire bizikideari,

i al meu petit Pau.

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CONTENTS

SUMMARY

The intensive growth of human population and their activities has increased the global pressures on freshwater ecosystems. Especially, streams and rivers are among the most threatened systems with an intensive regulation for supply drinking-water, food, energy and goods demand. Hydropower is one of the main uses of water diversion schemes, and in the next years it is expected to increase since is considered a "green" alternative to fossil fuels. Most of diversion schemes are small dams or low weirs, which impact on river habitat, biological communities and ecosystem functioning. Unfortunately, most of streams and rivers are impacted simultaneously by multiple stressors. Hydromorphological alterations, as water abstraction or siltation, and nutrient pollution from urban wastewater are the more common stressors with known impacts on running waters. Multiple stressors can interact and generate complex effects on ecosystem structure and functioning, becoming a great concern for the ecological conservation of streams and rivers. This dissertation addresses the effects of water diversion and its interaction with urban pollution and fine sediments on stream ecosystem functioning, combining two observational field studies and one laboratory experiment.

In the first observational study, we addressed how hydropower affects the transport and retention of water, total suspended solids, suspended chlorophyll and nutrients across a river network affected by multiple diversion schemes. Our results showed a high re-routing of discharge and compounds through canals instead of river channels. Unexpectedly, diversion canals were biogeochemically active, retaining some particulate compounds but releasing dissolved nutrients. Impoundments retained strongly all compounds, and overall, the entire river network acted as a sink for most of particulate and dissolved compounds. Multiple diversion schemes could alter the natural pattern of retention and release of nutrients and particulate compounds in rivers, impacting on ecosystem functioning.

In the second observational study, we assessed the impact of water diversion and urban pollution on organic matter processing in wet channel and dry riverbeds. For this study, four rivers in a pollution gradient with a similar diversion scheme were selected and reaches upstream and downstream from the diversion weirs were compared. We measured leaf-litter decomposition and carbon dioxide (CO_2) fluxes. Water diversion and pollution in the wet channel did not affect CO_2 fluxes but reduced microbial decomposition, whereas in the dry riverbed, their interaction reduced total and microbial decomposition and CO_2 fluxes. Thus, both stressors affected organic matter processing stronger in dry riverbeds than in the wet channel,

emphasizing that dry riverbeds must be taken into account to assess impacts of human activities on river ecosystems.

Finally, in the laboratory experiment we evaluated the interactive effects of water discharge reduction and fine sediments deposition on biofilm metabolism in artificial indoor channels. After biofilm colonization of tiles in an unpolluted stream and the acclimation in artificial channels, we manipulated both stressors and measured biofilm biomass and metabolism. The interaction of water discharge reduction and fine sediment deposition increased biofilm biomass, but did not affect metabolism. Thus, our results indicated interactive effects of these stressors.

Overall, this dissertation shows that water diversion and the interaction with other stressors can affect key processes of stream ecosystem functioning, even in dry riverbeds which often are neglected in the assessment of multiple stressors impacts. Our results lead to propose to take into account the impact of other stressors when environmental flows are set in streams and rivers. Finally, we encourage managers to meet a less harmful water diversion exploitation with streams and rivers ecosystems.

RESUMEN

El intenso aumento de la población humana y de sus actividades ha incrementado las presiones globales en los ecosistemas de agua dulce. Entre los sistemas más amenazados se encuentran los ríos y arroyos, los cuales están sometidos a una intensa regulación para abastecer la demanda de agua potable, alimentos, energía y bienes. Uno de los principales usos de los esquemas de derivación de agua es la producción de energía hidroeléctrica, la cual está previsto que en los próximos años aumente debido a que se considera una alternativa "verde" a los combustibles fósiles. La mayoría de esquemas de derivación son pequeñas presas o azudes, las cuales impactan sobre el hábitat del río, las comunidades biológicas y el funcionamiento ecosistémico. Desafortunadamente, la mayoría de arroyos y ríos están afectados simultáneamente por múltiples estresores. Las alteraciones hidromorfológicas, como la abstracción de agua o la deposición de sedimento fino, y la contaminación por nutrientes de las aguas residuales urbanas son los estresores más comunes y conocidos en los ríos. Estos estresores múltiples pueden interactuar y generar efectos complejos en la estructura y el funcionamiento de los ecosistemas, llegando a ser preocupantes para la conservación de los arroyos y ríos. Esta tesis aborda los efectos de la derivación de agua y su interacción con la contaminación urbana y los sedimentos finos en el funcionamiento de los ecosistemas de ríos, combinando dos estudios de campo observacionales y un experimento de laboratorio.

En el primer estudio observacional, abordamos como afecta la derivación en el transporte y la retención del agua, los sólidos totales en suspensión, la clorofila en suspensión y los nutrientes a lo largo de una cuenca hidrográfica afectada por múltiples esquemas derivación. Nuestros resultados mostraron una alta recirculación de caudal y compuestos a través de los canales de derivación. Inesperadamente, los canales de derivación fueron biogeoquímicamente activos, reteniendo algunos compuestos particulados y liberando nutrientes disueltos. Los embalses claramente retuvieron los compuestos, y en general, toda la cuenca hidrográfica del río actuó como un sumidero para la mayoría de compuestos disueltos y particulados. Por lo tanto, los múltiples esquemas de derivación podrían alterar el patrón natural de retención y liberación de nutrientes y compuestos particulados en ríos, impactando así en el funcionamiento del ecosistema.

En el segundo estudio observacional, evaluamos el impacto de la derivación de agua y de la contaminación urbana en el procesado de materia orgánica tanto en el río como en el lecho seco de este. Para realizar el estudio, se seleccionaron cuatro ríos en un gradiente de contaminación

con sistemas de derivación similares y se compararon los tramos de aguas arriba y aguas abajo de las presas. Medimos la descomposición de hojarasca y los flujos de dióxido de carbono (CO_2). La interacción entre la derivación de agua y la contaminación urbana en el río no afectó a los flujos de CO_2, pero redujo la descomposición microbiana, mientras que en el lecho seco, la interacción redujo tanto la descomposición total como la microbiana, y los flujos de CO_2. Así, el procesamiento de materia orgánica fue afectado por la interacción de ambos estresores de forma más contundente en el lecho seco que en el río, remarcando de esta manera que los lechos secos también deben tenerse en cuenta a la hora de evaluar el impacto de las actividades humanas en los ecosistemas fluviales.

Finalmente, en el experimento de laboratorio, evaluamos los efectos de la interacción entre la reducción de caudal y la deposición de sedimentos finos en el metabolismo del biofilm en canales artificiales. Después de la colonización de los sustratos por parte del biofilm en un río no contaminado, y tras la aclimatación de este en los canales artificiales, manipulamos ambos estresores y medimos la biomasa y el metabolismo del biofilm. La reducción en el caudal y la deposición de finos aumentó la biomasa del biofilm, pero no afectó a su metabolismo. Así, nuestros resultados indicaron efectos interactivos de ambos estresores en este organismo.

En general, esta disertación muestra que la derivación de agua y su interacción con los estresores puede afectar a procesos clave del funcionamiento de los ecosistemas, incluso en los lechos secos, los cuales a menudo son menospreciados cuando se evalúan los impactos de estresores múltiples. A través de nuestros resultados, proponemos tener en cuenta el impacto de otros estresores cuando se estipulan los caudales ambientales en ríos y arroyos. Para acabar, animamos a las entidades gestoras a explotar las derivaciones de agua de forma menos dañina para los ecosistemas fluviales.

LABURPENA

Giza populazioaren eta bere jardueren hazkunde handiak presio globalak areagotu ditu ur gezako ekosistemetan. Sistema mehatxatuenen artean, ibaiak eta errekak daude, edateko uraren, elikagaien, energiaren eta ondasunen eskaria hornitzeko erregulazio bizia baitute. Ura deribatzeko eskemen erabilera nagusietako bat energia hidroelektrikoa sortzea da. Aurreikusita dago datozen urteetan energia horrek gora egingo duela, erregai fosilen alternatiba berdetzat jotzen baita. Deribazio-eskema gehienak presa txikiak dira, eta ibaiaren habitata, komunitate biologikoak eta funtzionamendu ekosistemikoa kaltetzen dituzte. Zoritxarrez, erreka eta ibai gehienek aldi berean hainbat estresore jasaten dituzte. Alterazio hidromorfologikoak, hala nola uraren deribazioa edo sedimentu finaren sedimentazioa, eta hiriko hondakin-uren mantenugaien bidezko kutsadura dira ibaietan ohikoenak eta ezagunenak. Estresagarri anitz horiek elkar eragin dezakete eta ondorio konplexuak sortu ekosistemen egituran eta funtzionamenduan, erreka eta ibaien kontserbaziorako kezkagarriak izatera iritsiz. Tesi honek ura deribatzeak eta horrek hiri-kutsadurarekin eta sedimentu finekin ibai-ekosistemen funtzionamenduan duen elkarreragina aztertzen du, behaketan oinarritutako bi ikerketa eta laborategiko esperimentu bat konbinatuz.

Behaketan oinarritutako lehen ikerketan, aztertzen dugu nola eragiten duen deribazioak uraren garraio eta atxikimenduan, bai eta mantenugai, solido zein klorofila esekiaenean deribazio-eskema ugari dituen arro hidrografiko batean zehar. Gure emaitzek erakutsi zuten ura zein konposatu ugariren birzirkulazio handia deribazio-kanaletan barrena. Ustekabean, deribazio-kanalak biogeokimikoki aktiboak izan ziren, konposatu partikulatu batzuk atxikiz eta mantenugai disolbatuak askatuz. Presek eratutako ur-geldoak, berriz, konposatuak atxiki zituzten, eta, oro har, ibaiaren arro hidrografiko osoa konposatu disolbatutako zein partikulatu gehienen hustubide izan zen. Beraz, deribazio-eskema anitzek ibaietan mantenuagai zein konposatu partikulatuak atxikitzeko eta askatzeko modu naturala alda dezakete, ekosistemaren funtzionamenduan eraginez.

Behaketan oinarritutako bigarren ikerketan, uraren deribazioak eta hiri-kutsadurak materia organikoaren prozesaketan duten eragina ebaluatu dugu, ibaiaren ibilgu heze zein lehorrean. Horretarako, antzeko deribazio-sistemak zituzten lau ibai hautatu ziren kutsadura-gradiente batean, eta presetatik gorako eta beherako ibai-tarteak alderatu ziren. Orbelaren deskonposizioa eta karbono dioxidoaren (CO_2) fluxuak neurtu ziren. Ur-deribazioaren eta kutsaduraren arteko elkarreraginak ez zuen eraginik izan CO_2 fluxuetan, baina deskonposizio mikrobiarra murriztu egin zen; ibilgu lehorrean, berriz, elkarrekintzak deskonposizio osoa eta mikrobiarra murriztu zituen, bai eta CO_2 fluxuak ere. Horrela, materia organikoaren prozesamendua bi estresoreen elkarreraginak eragin zuen modu sendoagoan ibilgu lehorrean hezea baino. Honek nabarmen uzten du ibilgu lehorrak ere kontuan hartu behar direla giza jarduerek ibai-ekosistemetan duten eragina ebaluatzerakoan.

Azkenik, laborategiko esperimentuan, kanal artifizialetan emaria murrizteak eta sedimentu finak metatzeak biofilmaren dituzten elkarreraginak aztertu ditugu. Sustratu artifizialak lehenik kutsatu gabeko ibai batean eduki ziren, biofilmak koloniza zitzan. Ondoren, kanal artifizialetan egokitu ziren, hirugarren urrats batean, bi estresoreen konbinazio desberdinen eragina aztertzeko. Emaria murrizteak eta sedimentu finaren metaketak biofilmaren biomasa handitu zuten, baina ez zioten metabolismoari eragin. Horrela, gure emaitzek bi estresagarriek organismo honetan zituzten ondorio interaktiboak adierazi zituzten.

Oro har, tesi honek erakusten du ura desbideratzeak eta estresatzaileekiko elkarreraginak ekosistemen funtzionamenduaren funtsezko prozesuei eragin diezaiekeela, baita ibilgu lehorretan ere, horiek askotan gutxietsi egiten badira ere estresatzaile anitzen inpaktuak ebaluatzen direnean. Gure emaitzen bidez, ibai eta erreketako ingurumen-emariak zehazten direnean estresatzaile gehiagoren inpaktua kontuan hartzea proposatzen dugu. Amaitzeko, erakunde kudeatzaileak animatzen ditugu ibai-ekosistemetarako kalte gutxien eragiten duten ur-deribazioak ustiatzera.

GENERAL INTRODUCTION

THE GLOBAL PRESSURE ON FRESHWATER ECOSYSTEMS

A changing planet

During the last 20th century, human population growth and industrial development has increased in an accelerated way (McNeill, 2000), together with a general increase in human well-being (UN, 2019a). Meanwhile, the natural systems have suffered from intense pressure: increased resource extraction and CO_2 emissions, global temperature change, reduced availability of freshwater resources, reduced forest surface, or decreasing abundance of vertebrate species, to give some examples (Fig. 1)(Ripple et al., 2017). This intense human activity, affecting all ecosystems of our planet, has entailed its degradation, endangering its status and resilience (Steffen et al., 2011), and consequently, threatening human health and well-being (Gupta et al., 2019).

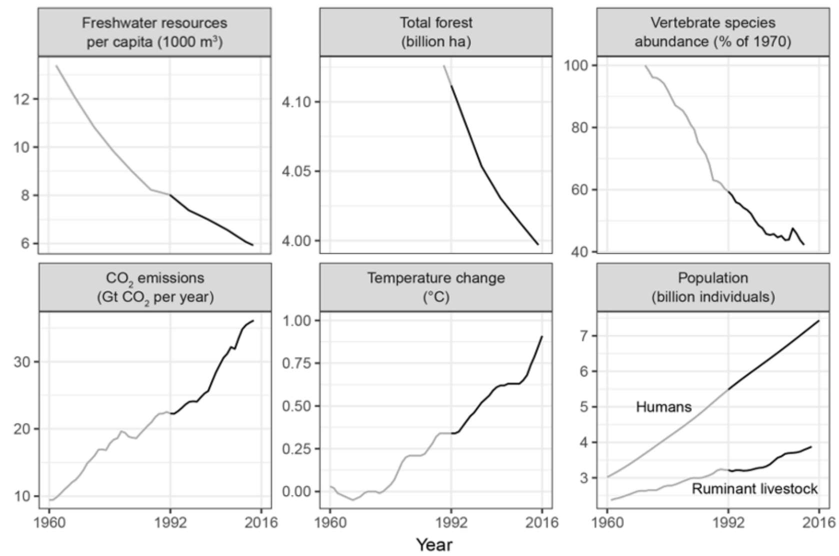

Figure 1. Evolution of freshwater resources per capita, total forest surface, vertebrate abundance, CO_2 emissions, temperature change and population between 1960 and 2016. Modified from Ripple et al., 2017.

Freshwater ecosystems, especially streams and rivers, are among the most threatened systems in the planet (Dudgeon, 2019), and the impact on them affects largely the health of people (Fig. 2) (Gupta et al., 2019).

Running waters are dynamic and complex ecosystems constituted by wet channels, flood plains, and riparian and hyporheic zones which host a large biodiversity (Sabater et al., 2009). They occupy the lowest-lying areas of the landscape and they are influenced by the upstream drainage network, surrounding land and downstream reaches (Sabater et al., 2009). Water is one of the most essential natural resources and it has been decisive for the human settlements along the history, occupying and altering floodplains and deltas (Grimm et al., 2008) to satisfy their demands of food, energy and goods production (Albert et al., 2021). The intensive use of water resources has been resulted in 65% of global freshwater ecosystems moderately or highly threatened, jeopardizing water security of 80% of the worldwide population (Vörösmarty et al., 2010). The main threats for streams and rivers include overexploitation, flow regulation, pollution, land-use change, invasive species and climate change (Dudgeon, 2019).

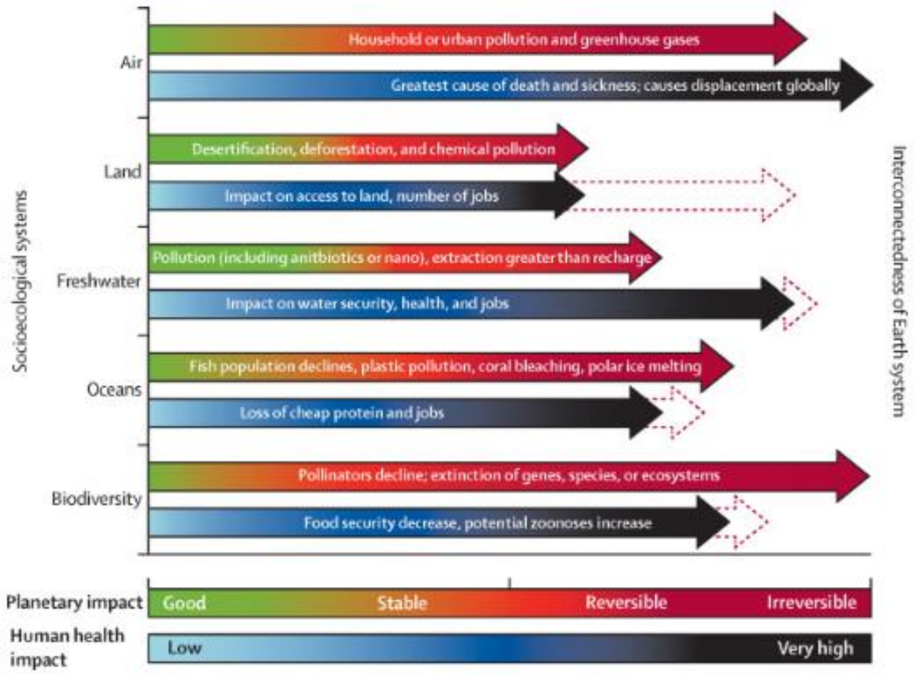

Figure 2. Global impacts on health of the planet and humans. From Gupta et al., 2019.

The rising water consumption

The global growth of human population and the rise of per capita water consumption resulted in freshwater overexploitation (Fig. 1) (Cafaro et al., 2022; Crist et al., 2017), which is projected to double by 2050 (UN, 2019b). From 1950 to 1998, per-capita water availability declined from 16,000 to 6,700 m^3 per year, and is projected to fall to 5,000 m^3 per year by 2025 (Dudgeon, 2019). In order to supply consumption demands for drinking-water, food, energy and goods, rivers and streams has been highly regulated (Belletti et al., 2020), with the construction of reservoirs or water transfer schemes between drainage basins. Vörösmarty and Sahagian (2000) calculated that around 600 large dams retain over 10,000 km^3 of water, five times the standing volume of the Earth's rivers. Irrigation and hydropower are the most frequent uses of reservoirs (Gleick, 2003). Irrigated land covers approximately 2.5 million km^2, with more than 50% rise between 1970 and 1995 (Gleick, 1998). Agriculture alone accounts for around 85% of global consumptive use of water, and in semiarid regions river flows have greatly decreased and even dry up (Gleick, 2003; Rosegrant et al., 2002). Future projections forecast a 70% raise in food demand by 2050 (Bruinsma, 2009), with the consequent environmental impacts. In the same way, hydropower is also expected to increase in the next years. The current energetic model based on fossil fuels is near to collapse, and countries are moving to renewable energy resources to meet their energy demand (UNEP, 2012). The European Renewable Energy Directive (2009/28/EC) aims to reach the 20% of the gross energy consumption through renewable resources, to reduce greenhouse gas emissions. Nowadays, hydropower represents 11.4% of total energy generation (Huđek et al., 2020) and its development is in a full swing since it has been considered an alternative to fossil fuels (Wagner et al., 2019) and to mitigate climate change (Berga, 2016).

Diversion schemes and hydropower

Currently, in Europe there are over 629,955 barriers fragmenting the river network, among which dams represent a 9.8% and weirs a 30.5% (Belletti et al., 2020). From these structures, an important number are hydropower facilities reaching to 21,387 developed plants and 8,507 planned to be built, especially small hydropower plants (Fig. 3) (Schwarz, 2019).

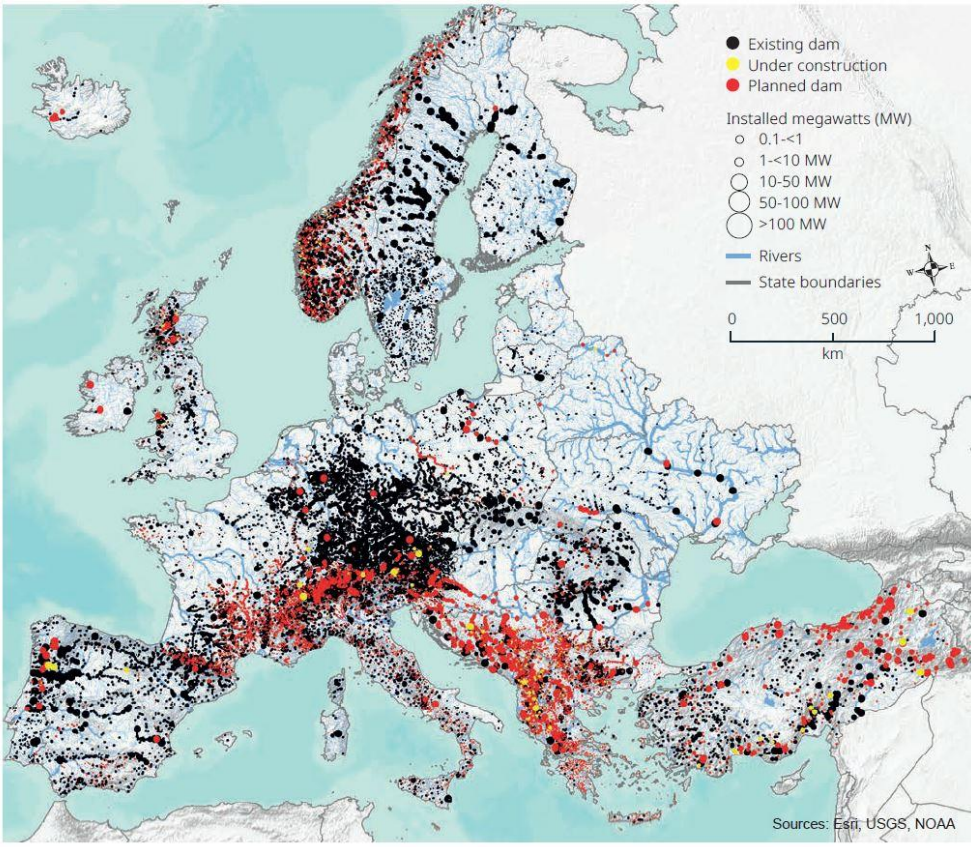

Figure 3. Distribution of existing, under construction and planned dams for hydropower in Europe. From Schwarz, 2019.

Most of these structures are diversion schemes called run-of-river schemes (Kuriqi et al., 2021), which consist of a low weir or dam with little storage capacity and a water diversion canal that diverts part of the water to a turbine some kilometers downstream before returning it to the river (Fig. 4) (Arroita et al., 2015). Although diversion schemes can potentially have effects on the bypassed river sections, these have received less attention than large dams whose effects are relatively well described (Aristi et al., 2014; Mor et al., 2018; Y. Li et al., 2020; Žganec, 2012).

Figure 4. Schematic picture of a diversion run-of-river scheme. Modified from Couto & Olden, 2018.

Effects of water diversion on river ecosystems

Anthropogenic effects can impact the structure and functioning of ecosystems. Ecosystem structure refers to biotic and abiotic characteristics such as channel morphology, water quality and the biological community (i.e. microbes, plants and animals) (Sabater & Elosegi, 2013); whereas functioning refers to ecosystem-level processes that regulate energy and matter fluxes due to the activity of organisms, including organic matter decomposition, nutrient cycling, biomass accrual, secondary production or ecosystem metabolism (von Schiller et al., 2017). These processes respond to specific environmental alterations (Young et al., 2008), they change at different spatial and temporal scales (Gessner & Chauvet, 2002) and they are key drivers for ecosystem services which are so necessary to human well-being (Millennium Ecosystem Assessment, 2005).

Water diversion schemes impact river habitats, biological communities (Benejam et al., 2016; Bunn & Arthington, 2002; Martínez et al., 2013) and ecosystem functioning (Elosegi & Sabater, 2013). The main consequence of water diversion is discharge reduction in the bypassed section, which results in a contraction of river ecosystems, decreasing the surface and quality of the wetted channel whereas it increases the surface of dry riverbeds (Arroita et al., 2017; Stanley et al., 1997). In fact, in extreme reductions and in spite of environmental flows set, the superficial flow can be disrupted and the wet channel become a series of isolated pools in the bypassed reach (Arroita et al., 2015). Water quality is also affected by water diversion, increasing temperature (Bae et al., 2016), pH (McIntosh et al., 2002) and conductivity (Rader & Belish, 1999), and decreasing oxygen concentration (James et al., 2008). The reduction of water discharge also affects stream organisms, such as biofilm (Matthaei et al., 2010; Mosisch, 2001), macroinvertebrates (Dewson et al., 2007; González & Elosegi, 2021). and fish communities (Benejam et al., 2016).

In the same way as the structure, ecosystem functioning is also affected by water diversion. The storage and decomposition of allochthonous organic matter are essential processes for river ecosystems, especially in forested streams where light limits primary production (Vannote et al., 1980; Wallace et al., 1999). Decomposition of organic matter represents a main pathway of energy across food webs and nutrient recycling in rivers (Gessner, 1999; Perkins et al., 2010; Tank et al., 2010). Most of the organic matter inputs consist of leaf litter (Pozo et al., 1997), which comes from vertical or lateral inputs (Webster & Meyer, 1997). Leaf litter decomposition is a complex process that involves leaching of soluble compounds, physical abrasion, degradation by microbial decomposers and fragmentation by invertebrate detritivores (Abelho, 2001; Graça, 2001; Tank et al., 2010). Retention, storage and decomposition of leaf litter can be affected by water diversion. Retention and storage of organic matter can show different responses, since reservoirs can act as traps and reducing inputs into downstream (Arroita et al., 2015; Flores et al., 2011), but also discharge reduction can promote retention and storage of organic matter in bypassed reaches (Brookshire & Dwire, 2003; Death et al., 2009). All stages of leaf litter decomposition can be affected by water diversion. Discharge reduction reduce velocity, and consequently, abrasion (Heard et al., 1999), and microbial decomposition and fragmentation also can be slower due to the detrimental effects on fungal and macroinvertebrate community (Bastias et al., 2020; Death et al., 2009; Schlief & Mutz, 2009).

Another processes potentially sensitive to water diversion are biofilm activity and nutrient retention. Stream biofilms are complex biological communities formed by autotrophic and heterotrophic organisms, including algae, cyanobacteria, bacteria, fungi and microfauna, growing in a solid substrata and embedded in a matrix of polysaccharides and other polymers (Lock et al., 1984). These organisms produce extracellular enzymes for the degradation of organic matter into smaller molecules, which then become available for bacterial growth and microbial nutrient uptake (Romaní et al., 2012). Biofilms play a key role in river biogeochemistry, participating in primary production, CO_2 emissions, organic matter decomposition and nutrient cycles (Battin et al., 2023). They are relevant for carbon and nutrient dynamics in streams and rivers (Allan & Castillo, 2007), contributing to transform and retain up to 50-75% of the nitrogen and 30% of the phosphorus entering streams (Mulholland, 2004). Therefore, nutrient retention and biofilms are intimately linked, playing these a key role at the basis of food webs (Rowe & Richardson, 2001). In streams and rivers, nutrient retention reflects the process by which dissolved nutrients are removed from the water column and immobilized or transformed into gaseous from, leaving permanently the system (Newbold, 1996). This process can be controlled by physical mechanisms, such as turbulence or hyporheic flow, chemical mechanisms such as sorption processes, and biological mechanisms as biofilm uptake (Mulholland & Webster, 2010). Both biofilm activity and nutrient retention can also be affected by water diversion. On the one side, the reduction of water discharge can decrease the exchange of nutrients with biofilms, reducing its activity and nutrient retention (Arroita et al., 2015, 2017). On the other side, the reduction of discharge, can promote nutrient retention due to the increase of the residence time of water in bypassed reaches and enhancing contact with sediments (Hall et al., 2002; Wollheim et al., 2001).

MULTIPLE STRESSORS IN FRESHWATER ECOSYSTEMS

Unfortunately, streams and rivers are not only affected by a unique stressor, and almost half of European streams and rivers are simultaneously impacted by multiple stressors (Fig. 5) (Schinegger et al., 2012). Generally, the consequences of multiple stressor interactions are difficult to forecast since the mix of effects can result in additive, synergistic or antagonistic impacts on stream ecosystem structure and functioning (Sabater et al., 2018; Turunen et al., 2016). Moreover, the response also depends on the variability of environmental conditions and the biological or functional variable studied (Sabater et al., 2018). Traditionally, most studies address isolated impacts of individual stressors (O'Brien et al., 2019). In recent years, research of multiple interaction has focused in two or three stressors (Nõges et al., 2016), although these studies mostly have been run in mesocosm experiments, limiting the ability to scale up to the reach, ecosystem or catchment scale (Fig. 6a) (Craig et al., 2017). The increasing concern on the effects of multiple stressors have pushed an increasing number of studies (Fig. 6b); however, decision-makers and protection or restoration policies still mostly address anthropogenic impacts from a single stressor approach. Probably, managers know the presence of multiple stressors, but they may be poorly equipped to address the complex impacts of these interactions (Craig et al., 2017).

Figure 5. Examples of rivers affected by multiple stressors. (a) River affected by intensive forest activities and water diversion for hydropower, which promotes siltation (notice severe siltation in the upper right part of the picture). (b) River with a channel made of concrete and lack of riparian trees. (c) River highly channelized, with poor riparian vegetation and affected by urban pollution. (d) River affected by point-source pollution, with a degraded riparian vegetation and a low-weir. Pictures provided by Arturo Elosegi.

Hydromorphological alterations, as water abstraction or siltation, and nutrient pollution from urban wastewater or agricultural practices are the most common stressors in European rivers (EEA, 2012). Moreover, the intensive spread of small diversion schemes in Europe (Schwarz, 2019) transforms the impact of multiple diversion in rivers in another common stressor in running waters.

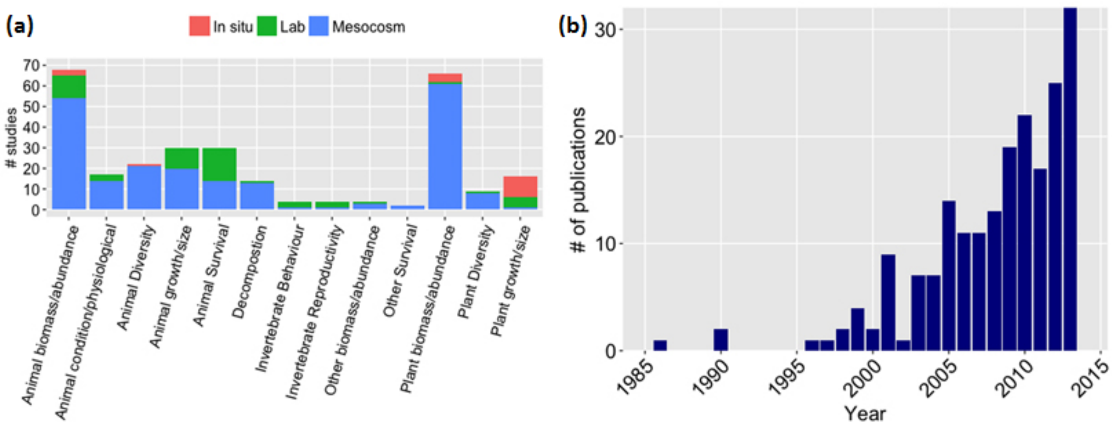

Figure 6. (a) Number of in situ, laboratory and mesocosm studies of multiple stressors for different response metrics. (b) Number of peer-reviewed multiple stressor studies (1986-2013). From Craig et al., 2017.

Multiple hydropower and river ecosystems

Many river catchments are affected by multiple diversion schemes, often located in series along the river continuum (Couto & Olden, 2018), which can cause downstream cumulative effects (Habets et al., 2018; Kibler & Tullos, 2013). A great number of studies have focused on the effect of large dams succession which is called dam cascade (Ouyang et al., 2011). Dam cascades affect the hydrologic regime (Kibler & Alipour, 2017), water quality (Silva et al., 2019), sediment transport and habitat (Smith et al., 2014), increase the abundance and biomass of phytoplankton (Li et al., 2013), affect macroinvertebrates (Callisto et al., 2005) and fish communities (Li et al., 2013; Ticiani et al., 2023). However, Kibler and Tullos (2013) suggested that small dams often generate greater cumulative biophysical effects than large dams. Despite the high number of low weirs, there are few studies regarding their cumulative effects, and even less than consider also the diversion canals.

Urban pollution and river ecosystems

In the last decades, urban pollution in freshwater ecosystems has increased as a direct consequence of the rapid growth of urban zones through the world (Jones & O'Neill, 2016). The implementation of waste water treatment plants (WWTP) is very different depending on countries (WHO & UNICEF, 2017). Moreover, WWTPs are unable to remove all contaminants from sewage and their effluents still contribute complex mixtures to freshwater ecosystems (Rodriguez-Mozaz et al., 2015). Therefore, the increase of nutrient concentration and other pollutants as heavy metals, pesticides, personal care products or drugs, deteriorates water quality and ecosystem status of streams and rivers (Hamdhani et al., 2020; Hering et al., 2015). Water physicochemical alterations include reduction of pH and dissolved oxygen (Englert et al., 2013), and increase of electrical conductivity and alkalinity (Hamdhani et al., 2020). These changes also affect structure of biological communities, affecting their growth, survival and reproduction, from microbes and algae (Corcoll et al., 2015; Drury et al., 2013) to invertebrates and fishes (Northington & Hershey, 2006; Ortiz & Puig, 2007). Moreover, the increase of nutrients, which generally refers to the rise concentration of different forms of nitrogen and phosphorus (Schweitzer and Noblet 2018), alters ecosystem metabolism (Aristi et al., 2015; Arroita et al., 2018), reduce biofilm SRP uptake(Pereda et al., 2019) and whole-reach nutrient uptake capacity (Martí & Sabater, 2009), and promotes primary production (Keck & Lepori, 2012) and organic matter processing (Ferreira et al., 2015; Halvorson et al., 2019). Therefore, this stressor together with water diversion are among the most pressures on streams, threatening its biodiversity and ecological quality (EEA, 2012; Nõges et al., 2016).

Fine sediments deposition and river ecosystems

Fine sediment (mineral and organic particles < 2 mm in size) occurs naturally in riverine benthic habitats (Wood & Armitage, 1997), however, its load and deposition is exacerbated by human activities such as agriculture or forestry (Syvitski et al., 2005). Suspended fine sediments affect river ecosystems, increasing turbulence and reducing light penetration to the bottom of stream bed (Davies-Colley et al., 1992). Moreover, they can abrade biofilms (Francoeur & Biggs, 2006), damage organisms gills (Kemp et al., 2011; McKenzie et al., 2019) and interact with dissolved nutrients and other pollutants damaging freshwater biota (Chase et al., 2017; Magbanua et al., 2016; Wagenhoff et al., 2013). Further, fine sediment is deposited in low water velocities causing siltation of streambeds (Graham, 1990), which can affect river hydraulics (Karna et al., 2015) and clog interstitial spaces (Rehg et al., 2005). This process also reduces the supply of oxygen and light to the bottom and impair river communities, damaging primary producers (Izagirre et al., 2009), macroinvertebrates (Jones et al., 2012; Kaller & Hartman, 2004) and fishes (Bilotta & Brazier, 2008; Kemp et al., 2011). Thus, fine sediment deposition is a pervasive stressor very present in running waters with a long-term ecological impact (Campbell & Doeg, 1989; Matthaei et al., 2006, 2010).

OBJECTIVES

This PhD dissertation studies the effects of water diversion and their interaction with common stressors in stream ecosystem functioning, by combining field and laboratory experiments. We try to answer the following questions:

1. What are the effects of multiple water diversion on nutrient transport and retention?

2. What are the interactive effects of water diversion and pollution on organic matter processing in wet channel and dry riverbeds?

3. What are the interactive effects of water diversion and fine sediment deposition on stream biofilm metabolism?

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Chapter 1

Re-routing of water and nutrients across a catchment as a consequence of multiple hydropower diversion schemes.

Ana Victoria Pérez-Calpe

ABSTRACT

European rivers are severely affected by human activities, by water regulation and abstraction among others. Weirs and small dams impact should not be underestimated, since represent over 91% of the barriers. Therefore, diversion canals and impoundments could have an important effect in the transport of water, nutrients and sediments across the landscape, especially in rivers affected by multiple hydropower schemes. In this study we addressed how hydropower affects the transport and retention of water, total suspended solids (TSS), suspended chlorophyll and nutrients across a river network affected by multiple diversion schemes. Our results showed a high re-routing of discharge through canals instead of river channels, which also resulted in particulate and dissolved compounds mostly running through diversion schemes. Unexpectedly, diversion canals were biogeochemically active, retaining chl-a and TSS but releasing dissolved nutrients. Impoundments retained strongly both, particulate and dissolved compounds. Overall, the entire river network acted as a sink for most of particulate and dissolved compounds. The close succession of canals and impoundments could alter the natural pattern of retention and release of nutrients and particulate compounds in multiple regulated rivers, concentrating these processes in specific points as impoundments and canals, avoiding the availability of these compounds in bypassed reaches and thus, impacting ecosystem functioning.

INTRODUCTION

The increase of human population and its demands for water and energy impact biodiversity and ecosystems worldwide (Crist et al., 2017), streams and rivers being among the most affected ecosystems (Vörösmarty et al., 2010). European rivers are severely affected by human activities (Tockner et al., 2022), by water regulation and abstraction among others. Overall, dams and weirs fragment the world river network (Grill et al., 2019), affecting transport of sediment, organic matter, nutrients and biodiversity (Friedl et al., 2004; Vörösmarty et al., 2003, 2010), as well as their contribution to global biogeochemical cycles (Syvitski et al., 2005). On the other hand, these are essential infrastructures, as 12 – 16% of global food production and 19 % of the world's electricity depend on river water (Albert et al., 2021).

Large dams impact strongly river ecosystems and cause special concern (Poff & Hart, 2002), but weirs and small dams impact should not be underestimated. Over 91% of the barriers in streams and rivers worldwide are weirs and small dams (Belletti et al., 2020). They reduce downstream biofilm biomass and activity (Arroita et al., 2017), affect the storage of organic matter (Arroita et al., 2015; Death et al., 2009; Riis et al., 2017), reduce leaf-litter decomposition (Martínez et al., 2017; Schlief & Mutz, 2009), and modify invertebrate (Dewson et al., 2007; González & Elosegi, 2021; González et al., 2018; Walters, 2011) and fish communities (Anderson et al., 2015; Benejam et al., 2016). Because of their extremely high numbers, the cumulative effects of diversion weirs and small dams might be serious in many streams and rivers.

Diversion schemes without significant water storage are among the most prevalent hydropower plants in the world (Couto & Olden, 2018). In these, a weir or small dam diverts water through a canal whose longitudinal slope is lower than that of the river, uses the difference in elevation so produced to gain potential energy, and converts that into electric energy in the power plant before returning the water to the river channel. Usually, diversion canals are straight and lined with concrete, what results in a fast flow with little turbulence, unlike that of the natural river channels. On the other hand, in the by-passed or de-watered river section, discharge is lower than natural, what results in slower flows and potentially higher interaction between water and the sediments. Therefore, diversion canals could have an important effect in the transport of water, nutrients and sediments across the landscape, especially in rivers affected by multiple hydropower schemes.

Among the elements transported by rivers, carbon, nitrogen and phosphorus, in dissolved or particulate forms, are of special biological significance (Cole et al., 2007; Mulholland & Webster, 2010). Also important are sediments and suspended algae, which affect water quality. Although there are some papers analysing the effects of water diversion on these variables (Izagirre et al., 2013; Oliveira et al., 2020), it is still unknown the overall effect of multiple diversion schemes on the transport and retention of nutrients, including river channels, diversion canals and zones impounded by dams.

Here we addressed this question in a river network affected by multiple diversion schemes, to describe how hydropower affects the transport and retention of water, total suspended solids (TSS), suspended chlorophyll and nutrients across the network. We aimed at performing mass-balances at the scale of the entire network, and at defining the environments (diversion canals, impounded areas or river reaches) where transport or retention processes dominate. Our hypotheses were: i) that diversion canals will be less active at retaining nutrients and suspended compounds than river reaches, which are more turbulent and physically complex; ii) impounded areas upstream from diversion dams will act as sinks for suspended compounds given their slow flow velocity; and iii) overall, diversion schemes will promote an increase in the export of dissolved nutrients, since a fraction of the water will be transported through biogeochemically inactive diversion canals.

MATERIALS AND METHODS

Study site

The Leitzaran River, at the N of the Iberian Peninsula (43.11° N, -1.94° W, Fig. 1), drains a catchment of 121.8 km^2, geologically dominated by limestone (mainly in the headwaters), slate and sandstones. A tributary of Oria River, the Leitzaran is 42 km long and runs along heavily incised meanders in a region with mountains higher than 1,000 m a.s.l. and a humid oceanic climate. The catchment land use is mainly dominated by forestry (56.3 % native forests and 40.7% plantations) and the rest of the surface includes agriculture and meadows (1.9%) and urban land uses (1.1%). Urban areas are located in the headwaters of Leitzaran River, in 2 towns (Areso and Leitza) of 3,200 inhabitants in total.

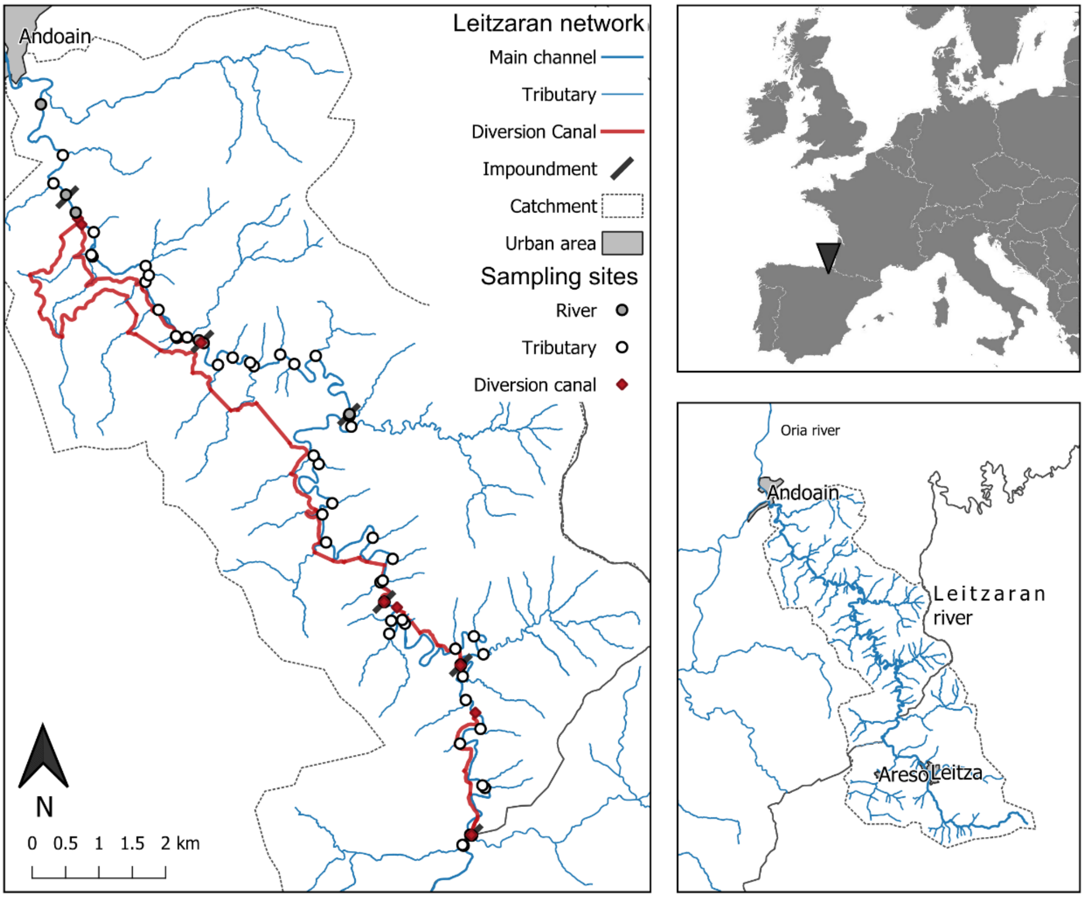

Figure 1. Leitzaran River location and network studied.

Downstream from this point, the Leitzaran runs for 30 km along a scarcely populated valley but with an intensive hydropower activity. Hydropower diversion schemes (5 in total, one of them returning the water into the diversion canal of another diversion scheme, instead of into the river) consist of low weirs (2-6 m of height) that divert water from river to hydropower plants through several km-long diversion canals (Table S1). Hydropower schemes are set in close succession, with diversion impoundments being located almost immediately below the outflow from the turbines (Izagirre et al., 2013).

Sampling design

We studied the mid- and lower parts of the Leitzaran River, which is the section affected by hydropower (Fig. 1). A total of 64 sampling points were sampled in the river network, including 13 points along the main stem of the river, all 43 tributaries joining the main channel, plus the inlets and outlets of all 4 diversion canals. The sampling lasted for 3 days (from June 5 to 7 of 2018) and was performed from upstream to downstream, trying to approach a Lagrangian strategy.

Water analyses

Physicochemical characteristics and discharge

Water temperature (T, °C), pH, electrical conductivity (EC, µS cm^{-1}) and dissolved oxygen (DO) concentration (mg L^{-1}) and saturation (%) were measured with hand-held probes (WTW Multi 350i and 340i SET, WTW, Weilheim, Germany; YSI ProODO; YSI, Yellow Springs, OH, USA). Water samples for dissolved carbon, nitrogen and phosphorus analyses were taken, filtered in-situ (0.7 µm pore size fibre glass filters, Whatman GF/F, Whatman International Ltd., Kent, UK) transported in a cooler box to the laboratory and frozen there until analyses.

Discharge (L s^{-1}) was measured from cross-section and water velocity measurements (ADV; Flow Tracker 2, SonTek Handheld-AD, USA or MiniAir 2; Schiltknecht Co, Gossau, Switzerland, depending on channel dimensions).

Network characteristics

We measured the length of each environment (diversion canal, impounded area and river reach) using geographic information systems from the Spanish Hydrology Database from the Spanish Government (https://centrodedescargas.cnig.es/) and QGIS v.3.10. The surface and volume of each environment were estimated from their length, width and depth. In the case of river reaches and canals, width and depth were measured with the discharge measurement probe (ADV; Flow Tracker 2, SonTek Handheld-AD, USA). In the case of impounded areas, width was estimated to equal the width of the dam and depth was estimated to equal one half of the height of the dam.

Suspended Chl-a

To determine Chl-a concentration (µg Chl-a L^{-1}), we filtered a known volume of water (0.7 µm, Whatman GF/F) from each sampled point and stored filters in a cooler box until the analysis. In the laboratory, we extracted the chlorophyll-a from the filters in 90% acetone (4 °C, 12 h in the dark) (Steinman et al., 2006). To ensure the complete extraction of Chl-a, we sonicated (Selecta sonication bath, operating at 360Wpower, 50/60 Hz frequency, JP Selecta S.A., Barcelona, Spain) and centrifuged (2000 rpm, G-value ¼ 657.4, PSelecta Mixtasel, JP Selecta S.A., Barcelona, Spain) the samples. Then, we determined Chl-a concentration spectrophotometrically (Shimadzu UV-1800 UV-Vis, Shimadzu Corporation, Kyoto, Japan) following the method described by Elosegi and Sabater (2009).

Total suspended solids

At each sampling point an additional sample was filtered through a pre-weighed Whatman GF/F filter and stored in a cooler box until the analysis. In the laboratory, filters were dried (72 h, 70 °C) and weighed to quantify the concentration of total suspended solids (TSS, mg L^{-1}).

Particulate and dissolved nutrients

From filters where we quantified TSS, we also analysed particulate organic carbon (POC), total particulate nitrogen (TPN) and total particulate phosphorus (TPP). From each filter we cut and weighed 2 discs of 1.2 cm of diameter. POC and TPN concentration (mg C L^{-1}; mg N L^{-1}) were analysed from one disc with a CHNSO elemental analyser (Euro EA3000, Eurovector, Pavia, Italy). From the second disc we analysed TPP concentration. For that purpose, the disc was acidified by air contact of HCl 12M in a closed chamber during 6 h to convert all organic forms of P to inorganic. Then, the disc was dried (70 °C, 1 h) and reweighed. We determined TPP concentration (mg P L^{-1}) on a Shimadzu UV-1800 UV–vis Spectrophotometer (Shimadzu Corporation, Kyoto, Japan) following the neutral digestion method adapted to our samples (Ma et al., 2017).

The concentration of dissolved organic carbon (DOC, mg C L^{-1}) and total dissolved nitrogen (TDN, mg N L^{-1}) were determined by catalytic oxidation on a Shimadzu TOC-L_{CSH} analyser coupled to a TNM-L unit (Shimadzu Corporation, Kyoto, Japan). To determine the total dissolved phosphorus (TDP) concentration (mg P L^{-1}) we also followed Ma et al., (2017).

To calculate total organic carbon (TOC), total nitrogen (TN) and total phosphorus (TP) we summed dissolved and particulate forms for each nutrient.

Mass balance calculations

Mass balance calculations were used to determine transport and retention of Chl-a, TSS and nutrients for the entire river network, for each river reach, canal and impoundment. We considered discharge to be conservative, i.e., outputs must equal inputs, as the study was performed in a period with low evapotranspiration. Therefore, when the water output of a reach was larger than the sum of inputs (discharge from upstream plus sum of discharge of tributaries), we considered that the difference corresponded to undetected discharge (Q_{Und}, L s^{-1}), coming from underground or subsuperficial inputs. When outputs were lower than inputs, what mainly occurred in diversion canals, Q_{Und} was assumed as loses from overflooding and cracks.

The load of the rest of variables (mg s^{-1}) was calculated by multiplying concentration (C, mg L^{-1}) by the discharge (Q, L s^{-1}). Retention (mg s^{-1}) was calculated as the difference between the expected and the measured load at downstream end. When retention was negative, we assumed a release of this element. For river network and reaches, the output load expected was calculated as the sum of inputs (upstream end and tributaries) plus undetected loads. For this last one, we considered the concentration of each variable in undetected fluxes to be equal to the average concentration in tributaries, which should be near the concentration of groundwater. To calculate retention in canals, output load expected was determined multiplying output discharge by concentration of variable in the input. Finally, in impoundments retention was calculated as the difference between output and input loads. To compare environments among each diversion canal, impoundment and river reach, we calculated retention rate (mg km^{-1} s^{-1}). Moreover, to estimate the global effect of each environment in the network we summed the retention or release (mg s^{-1}) of each group of environments.

We calculated confidence intervals of retention taking into account the uncertainty associated with concentration of undetected fluxes and by assuming that it could range from 0.5 to 2 times the average surface water concentration (Roberts & Mulholland, 2007).

Statistical analysis

Linear models with permutation tests (function lmp, in R package lmPerm (Wheeler, 2016)) were used to assess differences in retention among canals, river reaches and impoundments. Non-parametric test was chosen because data did not meet normality requirements. Linear models were used to test differences in physicochemical parameters among canals, river and tributaries. When needed, physicochemical parameters were log transformed to achieve homoscedasticity and normal distribution of residuals. The significance of source of variation was tested by means of ANOVA. All analyses were performed using R software, v. 3.4.0 (R Core Team., 2017).

RESULTS

Water physicochemical parameters

Tributaries had significantly higher temperature and oxygen saturation, but lower EC and pH, than river and canals (Table S2 and S3). Mean water temperature in tributaries was 13.1 ± 0.3 °C (mean ± SE), in river reaches it was 12.2 ± 0.3, and in canals it was 11.9 ± 0.3 °C (Table S2). The saturation of dissolved oxygen in water was higher in tributaries (95.3 ± 1.8%), and in reaches and canals it was lower (68.8 ± 4.4 and 67.3 ± 1.2%, respectively) (Table S2). Electrical conductivity was 75.0 ± 6.1 µS cm^{-1} in tributaries, reaches and canals showed higher values, 179.2 ± 13.2 and 179.3 ± 11.6 µS cm^{-1} respectively (Table S1). In tributaries pH was 7.4 ± 0.1, and in reaches and canals it was higher, 7.9 ± 0.1 and 7.8 ± 0.2 respectively (Table S2).

Table 1. Average retention km^{-1} s^{-1} (mean ± SE) of canals, impoundments and river reaches. Chl-a = Chlorophyll-a, TSS = Total Suspended Solids, POC = Particulate Organic Carbon, TPN = Total Particulate Nitrogen, TPP = Total Particulate Phosphorus, DOC = Dissolved Organic Carbon, TDN = Total Dissolved Nitrogen, TDP = Total Dissolved Phosphorus. Positive values indicate retention, negative values indicate release.

Variables	Canals	Impoundments	River reaches
Chl-a (µg km^{-1} s^{-1})	16.8 ± 37.2	4786.9 ± 1643.3	-56.8 ± 46.2
TSS (mg km^{-1} s^{-1})	98.2 ± 453.6	11442.5 ± 4997.8	-402.7 ± 569.4
POC (mg km^{-1} s^{-1})	-41.0 ± 35.4	1011.8 ± 792.8	-54.0 ± 54.6
TPN (mg km^{-1} s^{-1})	-0.7 ± 4.6	62.6 ± 38.9	-7.1 ± 6.5
TPP (mg km^{-1} s^{-1})	0.2 ± 1.1	4.1 ± 13.2	0.2 ± 0.2
DOC (mg km^{-1} s^{-1})	-281.6 ± 381.7	1031.5 ± 3026.2	96.3 ± 43.8
TDN (mg km^{-1} s^{-1})	-43.2 ± 30.9	-106.6 ± 702.0	9.7 ± 11.0
TDP (mg km^{-1} s^{-1})	-0.8 ± 0.6	16.7 ± 42.6	-2.7 ± 2.9

Network characteristics

The river network studied measured a total length of 45.6 km, including a total of 20.4 km of canals, 24.2 km of river reaches and 1.1 km of impoundments. The total surface of each environment calculated was 0.04 km^2 for canals, 0.03 km^2 for impoundments and 0.45 km^2 for river reaches. The volume of each environment calculated was 0.07 hm^3 for canals, 0.04 hm^3 for impoundments and 0.12 hm^3 for river reaches.

Balance at the section scale

Contrary to our expectations, diversion canals were active at releasing or retaining particulate compounds, but they showed little consistency. Chl-a was released in canal 1 but retained in canals 2, 3 and 4 (Table S4). Overall, in canals the significant average retention rate of chl-a per linear km was 16.8 ± 37.2 mg $km^{-1} s^{-1}$ (Fig. 2A, Table 1 and S5). TSS were retained in canals 1 and 4 but released in canals 2 and 3 (Table S4). Their average retention rate was significantly of 98.2 ± 453.6 mg $km^{-1} s^{-1}$ (Fig. 2B, Table 1 and S5). POC was released in all canals except 4 (Table S4), the release rate averaging 41.0 ± 35.4 mg $km^{-1} s^{-1}$ (Fig. 3A, Table 1). TPN was released in 3 of 4 canals (Table S4), and the average released per linear km in canals was 0.7 ± 4.6 mg $km^{-1} s^{-1}$ (Fig. 4A, Table 1). Contrary, TPP was retained in 3 out of 4 canals (Table S4), being the retention rate 0.2 ± 1.1 mg $km^{-1} s^{-1}$ (Fig. 5A, Table 1).

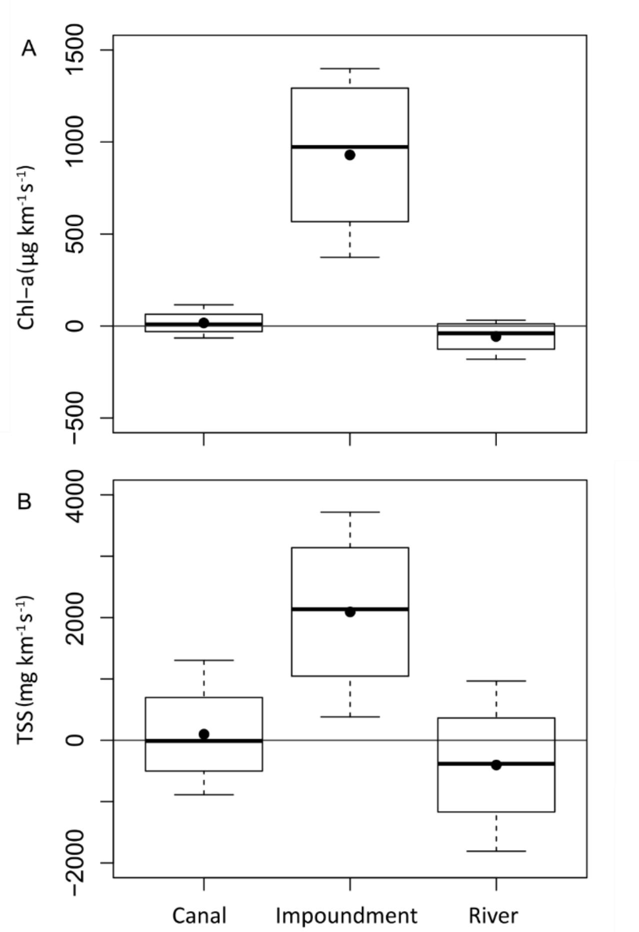

Figure 2. Chl-a retention km^{-1} (A) and TSS retention km^{-1} (B) in each environment. A positive value indicates retention and a negative value indicates release. The boxes display first and third quartiles, thick lines are medians, whiskers are range and solid points are means.

Canals were also active at releasing or retaining dissolved compounds. DOC mostly showed release, except in canal 2 where it was retained (Table S4). The release of DOC per linear km averaged 281.6 ± 381.7 mg km^{-1} s^{-1} (Fig. 3B, Table 1). TDN was released in canals 1 and 2, and small amounts were retained in canal 3 and 4 (Table S4). The TDN release average rate was 43.2 ± 30.9 mg km^{-1} s^{-1} (Fig. 4B, Table 1). TDP in canals showed similar pattern, since it was released in two canals and retained small amounts in the other two (Table S4), being the TDP average release of 0.8 ± 0.6 mg km^{-1} s^{-1} (Fig. 5B, Table 1).

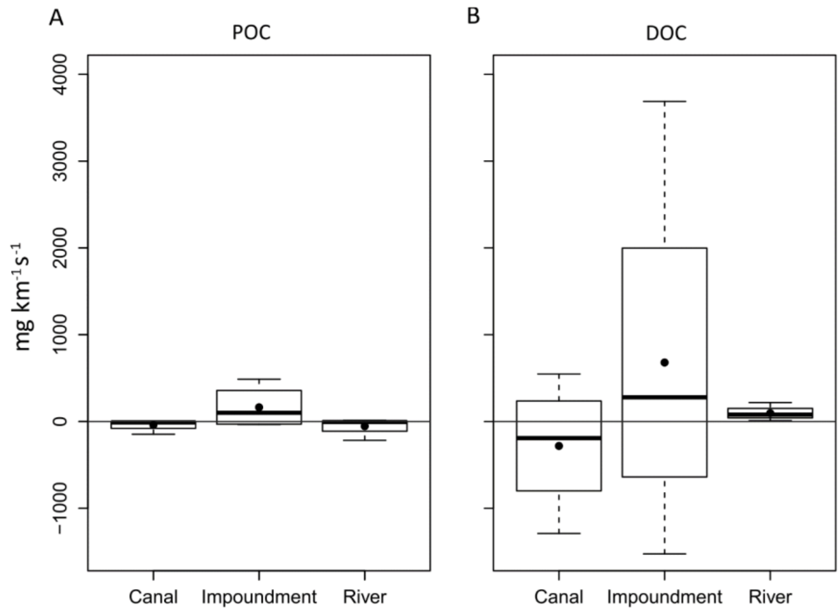

Figure 3. POC (A) and DOC (B) retention km^{-1} in each environment. A positive value indicates retention and a negative value indicates release. The boxes display first and third quartiles, thick lines are medians, whiskers are range and solid points are means.

Following our expectations, particulate compounds tended to be retained in impoundments. Chl-a was retained in all impoundments (Table S4), the average retention rate being 4786.9 ± 1643.3 µg km^{-1} s^{-1} (Fig. 2A, Table 1 and S5). TSS were also retained in all impoundments (Table S4 and S5), their average retention rate being 11442.5 ± 4997.8 mg km^{-1} s^{-1} (Fig. 2B, Table 1 and S5). The picture was more complex for POC, since small amounts were released in impoundments 1 and 4, larger amounts retained in impoundments 2 and 3 (Table S4). Consequently, the average retention rate of POC per linear km was 1011.8 ± 792.8 mg km^{-1} s^{-1} (Fig. 3A, Table 1). The TPN dynamics were also variable, since in impoundments 1 and 4 there was a little release and in impoundments 2 and 3 there was a larger retention (Table S4). As result, TPN retention rate was 62.6 ± 38.9 mg km^{-1} s^{-1} (Fig. 4A, Table 1). TPP was retained in 3 of 4 environments (Table S4) and the retention rate averaged was 4.1 ± 13.2 mg km^{-1} s^{-1} (Fig. 5A, Table 1).

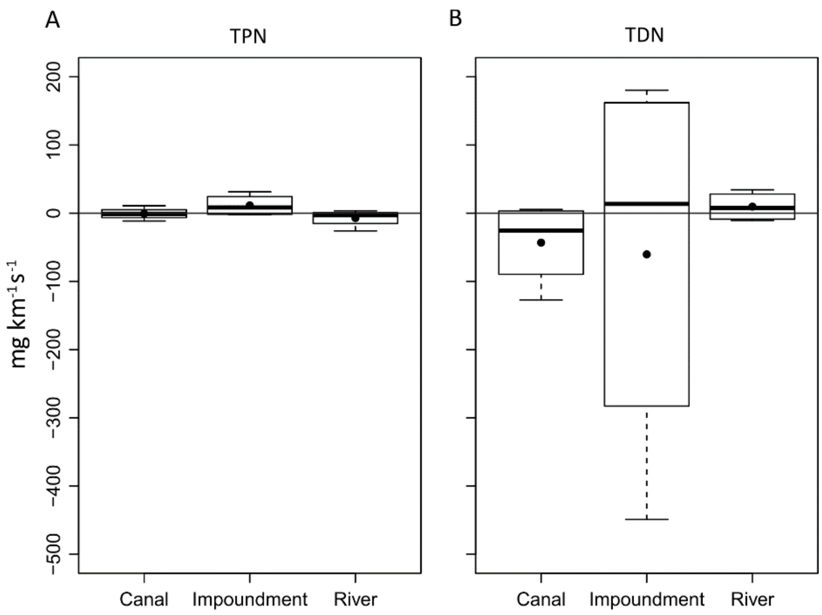

Figure 4. TPN (A) and TDN (B) retention km^{-1} in each environment. A positive value indicates retention and a negative value indicates release. The boxes display first and third quartiles, thick lines are medians, whiskers are range and solid points are means.

Regarding dissolved compounds, DOC showed retention in three impoundments but release in one (Table S4) and the average retention rate of DOC was 1031.5 ± 3026.2 mg km^{-1} s^{-1} (Fig. 3B, Table 1). TDN showed no clear pattern, being retained in two impoundments and released in the other two (Table S4). The average TDN release in impoundments was 106.6 ± 702.0 mg km^{-1} s^{-1} (Fig. 4B, Table 1). TDP was mainly retained, except in impoundment 1 (Table S4), being in consequence, the retention rate of TDP per linear km was 16.7 ± 42.6 mg km^{-1} s^{-1} (Fig. 5B, Table 1).

Regarding in river reaches, chl-a was released in 3 out of 4 river reaches (Table S4), with an average release of significantly 56.8 ± 46.2 µg km^{-1} s^{-1} (Fig. 2A, Table 1 and S5). TSS in river reaches showed mostly release except in reach 2 (Table S4), therefore, TSS release averaged significantly 402.7 ± 569.4 mg km^{-1} s^{-1} (Fig. 2B, Table 1 and S5). POC in river reaches was mainly released except in one reach (Table S4), thus release rate of POC averaged 54.0 ± 54.6 mg km^{-1} s^{-1} (Fig. 3A, Table 1). TPN also mostly showed release, except in reach 2 (Table S4) and consequently, the average release was 7.1 ± 6.5 mg km^{-1} s^{-1} (Fig. 4A, Table 1). Contrary, TPP was retained in most river reaches, except in reach 1 that showed release (Table S4) and resulting in a retention rate of TPP in river of 0.2 ± 0.2 mg km^{-1} s^{-1} (Fig. 5A, Table 1).

For dissolved compounds, DOC was retained in all river reaches (Table S4) and the retention rate averaged 96.3 ± 43.8 mg km^{-1} s^{-1} (Fig. 3B, Table 1). However, TDN did not show a clear pattern, since in reaches 1 and 3 were released and in reaches 2 and 4 were retained (Table S4). In consequence, in river reaches TDN retention rate was 9.7 ± 11.0 mg $km^{-1} s^{-1}$ (Fig. 4B, Table 1). In river reaches, TDP was mainly released except in reach 2 (Table S4), resulting in a release averaged 2.7 ± 2.9 mg $km^{-1} s^{-1}$ (Fig. 5B, Table 1).

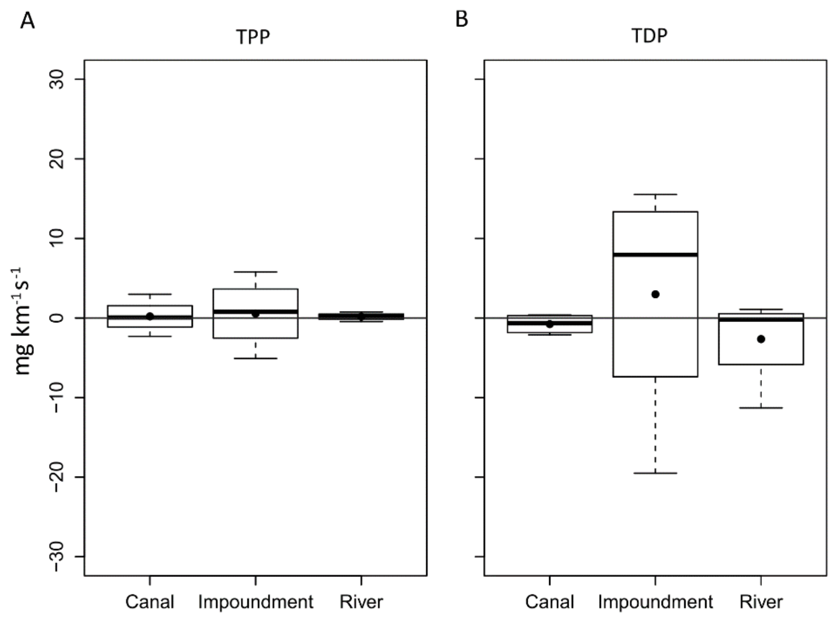

Figure 5. TPP (A) and TDP (B) retention km^{-1} in each environment. A positive value indicates retention and a negative value indicates release. The boxes display first and third quartiles, thick lines are medians, whiskers are range, and solid points are means.

In general, in the impoundments both particulate and dissolved compounds were retained, except TDN. In canals suspended chl-a and TSS were retained but in river reaches were released. On one hand, particulate nutrients showed the same pattern in canals and river reaches. But on the other hand, dissolved nutrients dynamic was different, whereas in river reaches DOC and TDN were retained and TDP released, in canals all of them were released.

When we analysed the global retention of each environment, in general this followed the same trend as retention rates. Canals retained chl-a, TSS and TPP, and released POC, TPN and all dissolved nutrients (Table 2). Impoundments retained all compounds except TDN (Table 2). River reaches released most of particulate compounds (Chl-a, TSS, POC, TPN and TDP) and released TPP, DOC and TDN (Table 2). However, TPP showed bigger retention in river reaches and TDN a bigger release in canals, instead in impoundments (Table 2). Impoundments showed a high rate of retention and release, but this environment is also the shortest one and therefore the effect of them in the global network can be lower than the other larger environments.

Table 2. Total retention in the environments of the entire river network studied. Chl-a = Chlorophyll-a, TSS = Total Suspended Solids, POC = Particulate Organic Carbon, TPN = Total Particulate Nitrogen, TPP = Total Particulate Phosphorus, DOC = Dissolved Organic Carbon, TDN = Total Dissolved Nitrogen, TDP = Total Dissolved Phosphorus. Positive values indicate, negative values indicate release.

Variables	Canals	Impoundments	River reaches
Chl-a (μg s^{-1})	107.7	3719.4	-1157.7
TSS (mg s^{-1})	604.7	8368.0	-8398.0
POC (mg s^{-1})	-365.8	652.9	-980.1
TPN (mg s^{-1})	-11.9	45.8	-135.2
TPP (mg s^{-1})	4.3	2.2	5.6
DOC (mg s^{-1})	-4317.1	2718.2	2588.4
TDN (mg s^{-1})	-264.1	-241.5	82.4
TDP (mg s^{-1})	-2.2	11.9	-48.4

Balance at the network scale

The upstream water input into the study network was 3048.9 L s^{-1}, the sum of the discharge from all tributaries 1567.5 L s^{-1} and the water output at the downstream end 5000 L s^{-1}. Therefore, we estimated that there were 383.6 L s^{-1} of undetected water inputs (subsuperficial and groundwater) (Figure 6). Moreover, 72.7 ± 6.9% of the water recirculated through canals instead than through river reaches.

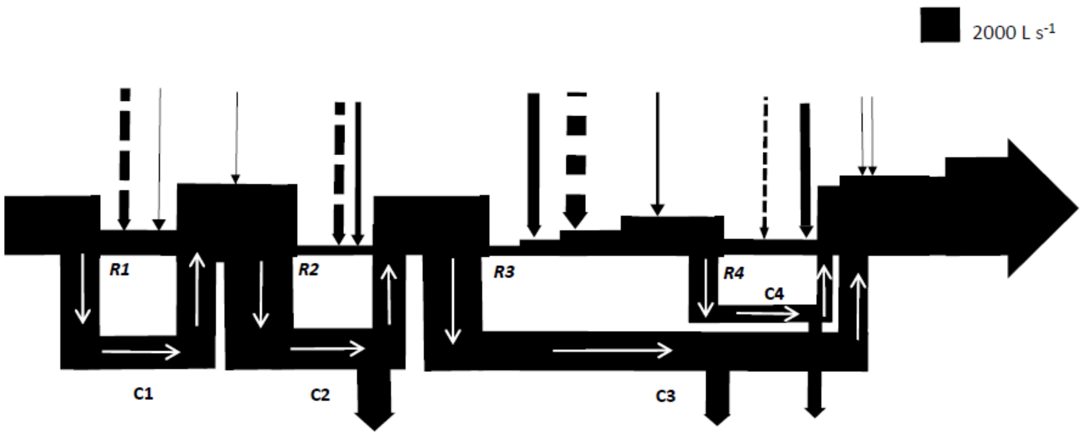

Figure 6. Mass balance of discharge along the Leitzaran River network. The width of the lines shows the flux. Intermittent arrows to the river show undetected fluxes, solid arrows tributary inputs. Solid arrows getting out from canals show canal loses. R means river reach studied in parallel of each canal. C correspond to each canal studied. White arrows show water flow on canals.

In general, particulate and dissolved compounds were retained in the network, except DOC that was released (Table 3). Suspended chl-a retention was 2.213 [2.206, 2.228] mg s^{-1} (mean [quartiles]) and TSS were 11.9 [11.7, 12.2] g s^{-1} (Table 3). Particulate nutrients also showed retention: the retention of POC was 1.22 [1.21, 1.23] g s^{-1}, that of TPN 0.159 [0.157, 0.161] g s^{-1}

and that of TPP 12.4 [12.1, 13.0] mg s^{-1} (Table 3). For dissolved nutrients, DOC was released 3.40 [3.61, 2.96] g s^{-1}, contrary to TDN and TDP, that were retained (0.39 [0.22, 0.73] g s^{-1} and 20.8 [18.5, 25.4] mg s^{-1}, respectively) (Table 3).

Table 3. Retention (mean and [quartiles]) in the river network studied. Chl-a = Chlorophyll-a, TSS = Total Suspended Solids, POC = Particulate Organic Carbon, TPN = Total Particulate Nitrogen, TPP = Total Particulate Phosphorus, DOC = Dissolved Organic Carbon, TDN = Total Dissolved Nitrogen, TDP = Total Dissolved Phosphorus. Positive values indicate, negative values indicate release.

Variables	Retention	Confidence Interval
Chl-a (mg s^{-1})	2.213	[2.206, 2.228]
TSS (g s^{-1})	11.9	[11.7, 12.2]
POC (g s^{-1})	1.22	[1.21, 1.23]
TPN (g s^{-1})	0.159	[0.157, 0.161]
TPP (mg s^{-1})	12.4	[12.1, 13.0]
DOC (g s^{-1})	-3.40	[-3.61, -2.96]
TDN (g s^{-1})	0.39	[0.22, 0.73]
TDP (mg s^{-1})	20.8	[18.5, 25.4]

DISCUSSION

This study tries to understand the transport and retention of particulate and dissolved compounds through a river network affected by multiple diversion schemes for hydropower. The Lagrangian approach used involved an important sampling effort but allowed following the transport and dynamics of studied compounds through the main environments (diversion canals, impoundments and river reaches) in a short time. Our results showed a high re-routing of discharge through canals instead of river channels, which also resulted in particulate and dissolved compounds mostly running through diversion schemes. Unexpectedly, canals were biogeochemically active, retaining chl-a and TSS but releasing dissolved nutrients. Impoundments retained strongly both, particulate and dissolved compounds. Overall, the entire river network acted as a sink for most of particulate and dissolved compounds.

We hypothesized diversion canals to be biogeochemically inactive, but our results showed retention and release of compounds. Mainly, suspended chl-a, TSS and TPP were retained, and POC, TPN and dissolved nutrients were released. This activity probably was caused by the sediments present in the bottom and the primary producers covering the wetted surface of canals. On one side, the presence of variable topography in the bottom of the canals could promote turbulent fluxes in the lower part of the water column and advective delivery due to local pressure variations facilitating deposition of heaviest particles (Karwan & Saiers, 2012; Packman et al., 2000; Ren & Packman, 2002). Furthermore, organisms as biofilms and bryophytes attached over these substrates could trap suspended particles transported in water (Battin et al., 2003; Drummond et al., 2014; Roche et al., 2017). On the other side, the release of POC, TPN and dissolved nutrients could be related with superficial inputs into the canals. Particulate and dissolved nutrients release could come from decomposition of allochthonous organic material from forest surrounding and trapped in the canals (Cowen & Lee, 1973; Hill et al., 2022; Meyer et al., 1998).

As expected, impoundments showed a clear retention of particulate compounds, and also dissolved nutrients except TDN. Impoundments are a lentic environment with a high residence time, and play a disproportionately large role in nutrient processing (Cheng & Basu, 2017). The total residence time of our impoundments (8.4 h) was similar to those of canals and reaches (7.8 h and 8.8 h, respectively), but for a much shorter distance. Impoundments promote the retention of particulate compounds basically by physical processes (Kufel, 1993; Maavara et al., 2020; Mulholland & Elwood, 1982; Vörösmarty et al., 2003), and dissolved nutrients retention by assimilation by organisms (Maavara et al., 2015; Sow et al., 2016). It is more difficult to explain the release of TDN in impoundments, since most of studies of nitrogen in reservoirs showed as sinks for this nutrient (Maavara et al., 2020; Powers et al., 2013; Wiatkowski, 2010).

We expected diversion schemes would promote an important export of dissolved nutrients because canals would act as pipelines and impoundments only would retain particulate compounds. However, our results showed that retention in impoundments was strong and included particulate and also dissolved compounds. Retention at network scale was also the main process, becoming river network as a sink of suspended compounds and particulate and dissolved nutrients, except DOC. The high re-routing of discharge through canals and the close succession between canals outputs and impoundments would promote the retention of nutrients and suspended compounds in impoundments and avoiding thus its availability in bypassed reaches.

Extrapolation of our results needs to be done with caution, as they derive from a single river network and a single sampling period. Even so, our main finding was that diversion canals were not inactive pipelines. Rivers affected by multiple diversion schemes would show reduced nutrient availability in river reaches since mostly of water would be diverted through canals, and strong retention at the impoundments would result in the entire network becoming a sink.

In conclusion, the close succession of canals and impoundments could alter the natural pattern of retention and release of nutrients and particulate compounds in multiple regulated rivers, concentrating the retention and release in specific points as impoundments and canals, and thus avoiding the availability of these compounds in bypassed reaches and ultimately in the river. Therefore, multiple diversion schemes in a river could alter transport, retention and release of compounds, thus impacting ecosystem functioning.

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Chapter 2

Organic matter processing on dry riverbeds is more reactive to water diversion and pollution than on wet channels

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ABSTRACT

Rivers are severely affected by human activities and many are simultaneously impacted by multiple stressors. Water diversion for hydropower generation affects ecosystem functioning of the bypassed reaches, which can alternate between periods with natural discharge and others with reduced flow that increase the surface of dry riverbeds. In parallel, urban pollution contributes a complex mixture of nutrients, organic matter, heavy metals, pesticides, and drugs, thus becoming an important stressor in rivers. However, there is little information on the interaction between both stressors on ecosystem functioning and, particularly, on organic matter processing, a key process linked to the input of energy to food webs. To assess the impact of water diversion and urban pollution on organic matter processing, we selected four rivers in a pollution gradient with a similar diversion scheme and compared reaches upstream and downstream from the diversion weirs. We measured leaf-litter decomposition and carbon dioxide (CO_2) fluxes in both the wet channel and the dry riverbed. Water diversion and pollution in the wet channel did not affect CO_2 fluxes but reduced microbial decomposition, whereas in the dry riverbed, their interaction reduced total and microbial decomposition and CO_2 fluxes. Thus, both stressors affected organic matter processing stronger in dry riverbeds than in the wet channel. These results show that dry riverbeds must be taken into account to assess and manage the impacts of human activities on river ecosystems.

INTRODUCTION

The increase of human population and its demands for water and energy impact biodiversity and ecosystems worldwide (Crist et al., 2017), streams and rivers being among the most affected ecosystems (Vörösmarty et al., 2010). European rivers are severely affected by human activities (Tockner et al., 2009), and almost half of them are simultaneously impacted by multiple stressors, such as hydromorphological alterations and pollution (Schinegger et al., 2012).

One of the most prevalent hydromorphological alterations is caused by water regulation and abstraction. Nowadays, 12%–16% of global food production and 19% of the world's electricity depend on river water (Albert et al., 2021), and the dams and weirs built for these purposes fragment the world river network (Grill et al., 2019) and impact their biodiversity (Vörösmarty et al., 2010), as well as their contribution to global biogeochemical cycles (Syvitski et al., 2005). Large reservoirs cause special concern, as they exert strong impacts on hydrology, channel form, water quality, and biodiversity (Poff & Hart, 2002). Although the environmental impact of individual weirs and small dams is likely smaller, their extremely high numbers probably result in a very significant cumulative impact, as they account for over 91% of the barriers in streams and rivers worldwide (Belletti et al., 2020).

One particular type of hydromorphological alteration is caused by diversion hydropower schemes, which divert from the river part of the discharge, usually in a weir or low dam, and transfer it through an artificial canal to a hydropower station where it goes through the turbines before being reverted to the river (Couto & Olden, 2018). The bypassed section of the river, the reach between the diversion dam and the reversion point below the hydropower station, is thus subject to artificially low discharge when the hydropower scheme is operating, which typically leads to an enhanced areal cover of the dry channel (Arroita et al., 2017). Hydropower schemes have a maximum operating capacity that puts a ceiling to the amount of water diverted. Therefore, the proportion of discharge diverted tends to be low in high-flow periods, increases as the hydrographs recede, and can swiftly fall to zero when hydropower schemes close to ensure environmental flows. Therefore, bypassed reaches can alternate between periods with natural discharge and others with various degrees of diversion. Water diversion affects river biota and processes. It reduces biofilm biomass and activity (Arroita et al., 2017), affects the storage of organic matter (OM) (Arroita et al., 2015; Death et al., 2009; Riis et al., 2017), reduces leaf-litter decomposition (Martínez et al., 2017; Schlief & Mutz, 2009), and modifies invertebrate (Dewson et al., 2007; González & Elosegi, 2021; González et al., 2018; Walters, 2011) and fish communities (Anderson et al., 2015; Benejam et al., 2016). These impacts probably are stronger during base flows, when a larger fraction of the water is diverted, but legacy effects from diversion periods can also affect the river during shutdown periods (Arroita et al., 2018). Therefore, it is important to study the effect of hydropower during both active and inactive periods.

In parallel, human activities also increase the concentration of nutrients and other pollutants in the environment, thus degrading water quality and ecosystem status around the world (Hering et al., 2015). Especially worrying is the increase in urban pollution, a direct consequence of the rapid growth of urban areas through the world (Jones & O’Neill, 2016). Urban pollution usually consists of a complex mixture of pollutants that include nutrients and OM (Carey & Migliaccio, 2009), heavy metals (Deycard et al., 2014), pesticides, personal care products, and drugs (Kuzmanović et al., 2015; Mandaric et al., 2018; Osorio et al., 2016), among others. Depending on its composition, on the level of dilution in the receiving waters, and on the variable studied, urban pollution can have contrasting effects, from increases in biofilm biomass (Pereda et al., 2020; Ribot et al., 2015), ecosystem metabolism (Aristi et al., 2015; Gücker et al., 2006), and leaf litter decomposition (Englert et al., 2013; Ferreira et al., 2015), to reduced invertebrate diversity

(Mor et al., 2019) and nutrient uptake efficiency (Martí et al., 2010). Although urban pollution is likely to interact with water diversion, most experimental evidence has been gathered from mesocosm experiments (Arias-Font et al., 2021; Baekkelie et al., 2017; Matthaei et al., 2010), real river studies being scarce, especially those dealing with ecosystem functioning.

In forested rivers, litter breakdown and mineralization are important processes linked to the energy inputs to food webs and to the emission of carbon dioxide (CO_2) to the atmosphere (Marks, 2019; Marx et al., 2017; Wardle et al., 2004). Although most studies on river ecology focus on the wet channel (Attermeyer et al., 2021), dry riverbeds can have an important contribution to organic matter processing and CO_2 emissions (Boodoo et al., 2019; Datry et al., 2018; Keller et al., 2020). Because water diversion increases the proportion of dry riverbeds, it is important to study ecosystem processes in these as well. The aim of this study was to analyze the interactive effects of water diversion and pollution on river carbon processing, including the role of wet and dry channel. To this end, we studied reaches upstream and downstream from diversion schemes in four rivers across a pollution gradient, from clean water to moderate pollution. We predicted that:

1) Water diversion would reduce OM stock and decomposition in the wet channel because of degraded environmental conditions. In the dry channel, there would not be an effect on the OM stock, but decomposition would decrease because of reduced drying–rewetting cycles. The CO_2 efflux would respond as decomposition, as both processes are strongly linked to carbon mineralization.
2) Moderate pollution would promote OM decomposition and CO_2 efflux, especially in the wet channel, which is in continuous contact with the pollutants. This increase in decomposition would in turn reduce the OM stock in the wet channel. Conversely, in the dry channel, we would expect no changes in OM stock and enhancement of OM decomposition and CO_2 efflux.
3) Water diversion and pollution would interact in an antagonistic way in both wet and dry channel. Consequently, OM decomposition and CO_2 efflux rates in bypassed reaches of polluted rivers would be closer to that of control reaches in clean rivers. In addition, the OM stock would be reduced in the wet channel but not in the dry channel.

MATERIALS AND METHODS

Study sites and sampling design

We selected four rivers (Urumea, Leitzaran, Kadagua, and Deba) in the Basque Country (northern Iberian Peninsula) (Figure S1), a mountainous, industrial region with wet, temperate climate (Table 1). The catchments drained by these rivers differ in the intensity of human activities and in the proportion of urban land use (0.1%–4.6%; Table 1). As a result, water quality ranges from good to acceptable (Table 1 and Table S1). A complex cocktail of pollutants, including nutrients, metals, and organic contaminants is detected in the worst situations (URA, 2016). Although rainfall tends to be higher in the area from winter to spring, precipitation is highly fluctuating, which can result in both flooding and base flow discharge at any season of the year (Elosegi et al., 2006).

The four rivers are affected by diversion schemes of similar characteristics consisting of a low weir that diverts water through a canal to a hydropower plant, strongly reducing the discharge in the bypassed section. Each hydropower plant is regulated by a specific water allowance, but generally, environmental flows are set at 10% of the monthly mean discharge (BOE, 2016). Therefore, hydropower plants typically operate in periods of several months (active period), are punctuated by day-to-month-long periods of no diversion during base flows (inactive period), although these periods do not necessarily coincide for all rivers. In this study, we sampled the four systems when the diversion was active but also when it was inactive, to detect potential legacy effects. We sampled two 100-m-long reaches per river: one upstream from the stagnant area created by the weirs (Control), and another one below the weirs (Regulated) (Figure 1). We sampled both the wet and the dry channel in each reach and measured variables linked to the structure and functioning of the river ecosystem. Structural variables included the dimension of the wet and dry channels, water quality, and stocks of OM. Among functional variables, those that reflect biologically mediated processes occurring within the stream channel (von Schiller et al., 2017), we measured leaf litter decomposition and CO_2 fluxes.

Water quality

Water temperature (°C), pH, electrical conductivity (EC, µS cm^{-1}), dissolved oxygen (DO) concentration (mg L^{-1}), and saturation (%) were measured with hand-held probes (WTW Multi 350i and 340i SET, WTW, Weilheim, Germany; YSI ProODO; YSI Incorporated, Yellow Springs, OH, USA). Water samples were taken, filtered in situ (0.7 µm pore size fiber glass filters, Whatman GF/F; Whatman International Ltd., Kent, United Kingdom) and frozen until analysis. We determined the soluble reactive phosphorus (SRP) concentration (mg P L^{-1}) with the molybdate method (Murphy & Riley, 1962), and ammonium (N-NH_4^+, mg N L^{-1}) with the salicylate method (Reardon et al., 1966), on a Shimadzu UV-1800 UV–vis Spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The concentration of total dissolved nitrogen (TDN, mg N L^{-1}) and dissolved organic carbon (DOC, mg L^{-1}) were determined by catalytic oxidation on a Shimadzu TOC-LCSH analyzer coupled to a TNM-L unit (Shimadzu Corporation, Kyoto, Japan). The concentrations (mg L^{-1}) of nitrate (N-NO_3^-), sulfate (SO_4^{2-}) and chloride (Cl^-), were measured by capillary electrophoresis (Agilent CE; Agilent Technologies, Santa Clara, CA, USA) (USEPA, 2000).

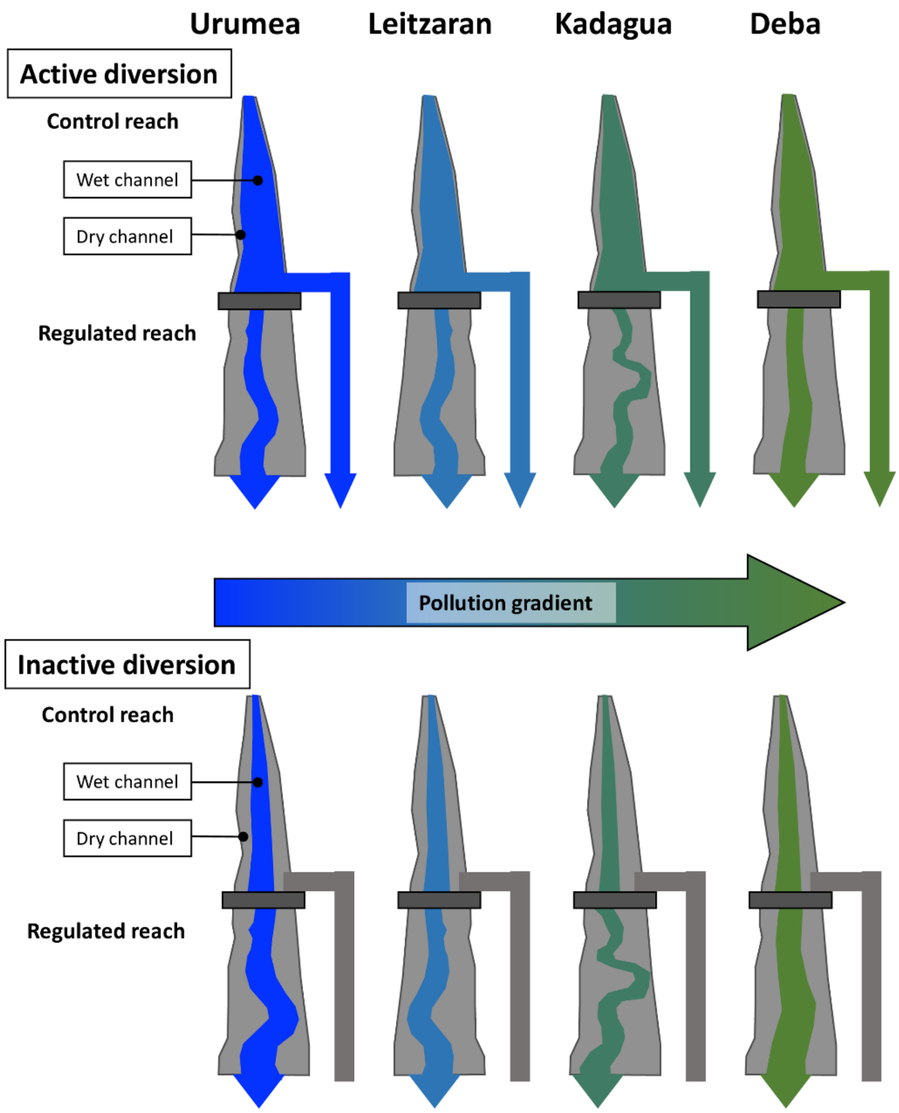

Figure 1. Schematic drawing of the experimental design.

Hydrology

Mean discharge (m^3 s^{-1}) and velocity (m s^{-1}) of whole-reach were estimated from time vs. conductivity curves obtained from pulse additions of NaCl (Martí & Sabater, 2009) at each reach (see below). Additionally, to obtain continuous discharge data, we placed absolute pressure loggers (Solinst Levelogger Edge 3001; Solinst Canada Ltd., Georgetown, ON, Canada) and atmospheric pressure loggers (Solinst Barologger Edge 3001; Solinst Canada Ltd.) at each of the eight reaches to record data every 15 min from June 2017 to September 2018. Absolute pressure was corrected by the atmospheric pressure, and water level regressed against discharge registered at nearby gauging stations to calculate continuous discharge at each reach. Additionally, we measured the width of wet and dry channel at 10 equidistant transects along each reach.

OM standing stocks

In each reach, nine samples of coarse benthic OM (CBOM) were randomly taken in the wet channel, and nine more in the dry channel. In the wet channel, CBOM was collected with a Surber sampler (0.09m^2, 0.5 mm mesh), it was sieved (8mm), and the retained material was stored in zip bags. In the dry riverbed, a Surber frame (0.09m^2) net was used to delimit the area, and all litter found on top of the sediments was collected and stored in zip bags. Then, in the laboratory, the samples were dried (72 h, 70°C), weighed, combusted (5 h, 500°C), and re-weighed to determine the ash-free dry mass (AFDM, g m^{-2}). To estimate fine benthic OM (FBOM, g m^{-2}), nine samples were randomly collected in the wet channel per reach with a plastic sampling corer (81.7 cm^2 surface). The corer was forced to the substratum, the volume of sediment inside was measured, benthos was stirred by hand, and a sample was taken and stored in a cool box. In the laboratory, samples were filtered through pre-weighed glass fiber filters (0.7 µm pore size), which were then treated as the CBOM samples. In the dry channel, five sediment samples per reach were collected and, in the laboratory, were dried to determine the water content (%) and then combusted to quantify the sediment OM content (SOM, %), as for the CBOM samples described previously.

Table 1. Main characteristics of the studied rivers and the water diversion schemes within. The total annual precipitation and mean annual air temperature are the average of the 2 years of the study (www.euskalmet.euskadi.eus). Weir height, concession volume and bypassed reach were obtained from the Cantabrian Hydrographic Confederation and the Institute of the Cultural Heritage of Spain (CHC, 2013; Pérez-Marrero, 2017). Population density was obtained from the National Statistics Institute (www.ine.es). The General Quality Index (GQI), based on water chemistry, was obtained from the Basque Water Agency (www.uragentzia.euskadi.eus).

River	Weir location coordinates Y, X	Weir height (m)	Concession volume (m^3 s^{-1})	Bypassed reach (km)	Stream length (km)	Annual rainfall (mm)	Mean air temperature (°C)	Upstream catchment area (km^2)	Population density (inhab. km^{-2})	Urban land use (%)	General Quality Index (GQI)
Urumea	43.214806, -1.904639	6.5	5.8	3.5	59.4	1838.6	13.5	186.1	4.3	0.1	89.0 – Good
Leitzaran	43.132667, -1.937056	4.0	3.0	4.1	42.0	2268.4	13.6	62.8	52.3	1.1	86.0 – Good
Kadagua	43.227194, -3.016333	3.0	4.0	2.2	65.0	1288.0	13.3	449.0	64.3	2.5	74.4 – Intermediate
Deba	43.160444, -2.402389	3.5	5.0	1.3	62.4	1316.2	12.7	355.1	178.0	4.6	69.9 – Acceptable

Leaf Litter Decomposition

We measured the total and microbial leaf litter decomposition of black alder [Alnus glutinosa (L.) Gaertn.], both in wet and dry channel. Fresh leaves were collected the previous autumn, dried at ambient temperature, and stored in a dark and dry place until the experiment. Then, leaves were enclosed in bags of coarse (5 mm, 4 ± 0.05 g) and fine mesh (100 µm, 3 ± 0.05 g) and five bags per mesh type were secured per habitat (wet vs. dry channel) and reach by means of metal bars randomly anchored along the reach. Leaching was estimated from five bags of each mesh size that were kept in tap water for 24 h in the laboratory and the mass loss measured as for the rest of the bags from both habitats. Water and air temperature were recorded by means of Smart Button temperature loggers (ACR Systems, Surrey, BC, Canada) deployed with the bags. After 3 weeks of incubation, bags were collected and transported to the laboratory in a cool box. Once there, litter was cleaned with tap water to remove invertebrates and mineral particles, and then dried (72 h, 70°C), weighed, combusted (5 h, 500°C), and weighed again to obtain AFDM. Decomposition rates were calculated following Petersen and Cummins (1974) and expressed per degree day (d d^{-1}). The breakdown rate measured in coarse mesh bags was considered total; that in fine mesh bags, microbial (Bärlocher et al., 2020). In addition, fragmentation by detritivorous macroinvertebrates was also calculated following Lecerf (2017).

CO_2 Fluxes

CO_2 fluxes were measured in both the wet and dry channel. In the wet channel, the partial pressure of CO_2 in the atmosphere and water ($pCO_{2,a}$ and $pCO_{2,w}$, respectively) was measured per triplicate with an infrared gas analyzer (EGM-5; PP-Systems, Amesbury, MA, USA). To measure pCO_2,w, a membrane contactor (MiniModule, 3M, Germany) was coupled to the gas analyzer. We took a 10-L sample of water in a container, the water was circulated through the contactor at 300 ml min^{-1} by gravity, and the equilibrated gas was continuously recirculated into the gas analyzer (Teodoru et al., 2011). According to the manufacturer, the accuracy of the infrared gas analyzer was within 1% over the calibrated range. Then, we estimated the CO_2 flux between surface water and atmosphere ($FCO_{2,w}$, mmol m^{-2} d^{-1}) applying Fick's First Law of gas diffusion (Eq. 1):

$$F_{CO_{2,w}} = k_{CO_2}\, K_h\, (pCO_{2,w} - pCO_{2,a}) \qquad \text{Eq. 1}$$

where kCO_2 is the specific gas transfer velocity for CO_2 (m d^{-1}), Kh is Henry's constant (mmol µatm^{-1} m^{-3}) adjusted for salinity and temperature (Millero, 1995; Weiss, 1974), and $pCO_{2,w}$ and $pCO_{2,a}$ are the partial pressures of CO_2 in the water and atmosphere (µatm). To estimate the

kCO_2, we used the night-time drop in dissolved oxygen concentration (Hornberger & Kelly, 1972), as measured from optical dissolved oxygen sensors (YSI 6150 connected to YSI 600 OMS; YSI Inc., Yellow Springs, OH, USA) deployed for 24 h in each reach. We standardized the oxygen reaeration coefficient for depth to calculate the mean gas transfer velocity of oxygen (k_{O2}, m d^{-1}). Finally, we determined kCO_2 by means of Eq. 2:

$$k_{CO_2} = k_{O_2}\left(\frac{Sc_{CO_2}}{Sc_{O_2}}\right)^{-n} \qquad \text{Eq. 2}$$

where k_{CO2} is the mean gas transfer velocity of CO_2 (m d^{-1}), Sc_{CO2} and Sc_{O2} are the Schmidt numbers of CO_2 and O_2 at a given water temperature (Wanninkhof, 1992), and n corresponds to the surface water motion, that was set to 1/2 according to the turbulence environment of running waters (Bade, 2009).

In the dry channel, we measured CO_2 fluxes from five randomly selected sites per reach with the closed dynamic chamber method (Livingston & Hutchinson, 1995). We used an opaque chamber (SRC-2, PP-Systems) connected to the infrared gas analyzer and measured the gas concentration every 4.8 s. CO_2 flux measurements lasted until a change of at least 10 µatm was reached, with a duration of 120–300 s. From the rate of change of CO_2 inside the chamber, we estimated the CO_2 flux between dry riverbed and atmosphere ($FCO_{2,d}$, mmol m^{-2} d^{-1}) by means of Eq. 3:

$$F_{CO_{2,d}} = \left(\frac{\mathrm{d}p_{CO_2}}{\mathrm{d}t}\right)\left(\frac{V}{RTS}\right) \qquad \text{Eq. 3}$$

where dp_{CO2}/dt is the slope of the gas accumulation in the chamber along time (µatm s^{-1}), V is the chamber volume (1.171 dm^3), R is the ideal gas constant (L atm K^{-1} mol^{-1}), T is the air temperature (Kelvin), and S is the chamber surface (0.78 dm^2). At each site, we also measured the substrate temperature with a portable soil probe and collected sediment samples (upper 5 cm), which were stored in a cool box and transported to the laboratory, where we determined water content and OM content as described above.

Data analysis

We statistically tested the variation of ecosystem structure and functioning variables using linear models. In these models, we firstly tested for differences between Control and Regulated reaches, which would result from the direct effect of the barrier on the response variables measured. Secondly, we also tested for the short-term effects of the diversion (when it was Active) against the long-term effects of it (when it was Inactive). The interaction of these two sources of variation would show, for instance, if the differences between Control and Regulated sites were only evident when the diversion was active, or, on the contrary, differences were apparent any time. Thirdly, we tested for the implications of the pollution levels of the water on the measured variables. Thus, linear models built included period (comparison between Active and Inactive periods; factor), reach (comparison between Control and Regulated reaches; factor), pollution (measured through the General Quality Index, GQI; covariate), and second-order interactions as sources of variation. We tested the triple interaction between reach, pollution, and period, but since it was not significant or marginally significant for most of the variables, we decided to remove it from all analyses. Season (Spring/ Autumn; factor) was included as a block factor. For water quality variables, linear models considering river, reach, and their interaction were built. As respiration and CO_2 fluxes of the dry channel can depend on SOM and water content (Keller et al., 2020; Marcé et al., 2019), we tested relationship among these variables by means of Pearson correlation tests. The significance of each source of variation was tested by means of ANOVA. The behavior of residuals was checked by means of diagnostic plots to assure linearity, normality, homoscedasticity, and absence of outliers. When necessary, log-transformation of the data was enough to meet these requirements for linear models. All analyses were performed using R software, v. 3.4.0 (R Core Team., 2017).

RESULTS

Water quality

Water quality did not change between Control and Regulated reaches (Table 2), and differences among rivers were not statistically significant for temperature, DO concentration, and saturation and NO_3^- concentration (12.2°C –21.3°C, 8.6–12.7 mg O_2 L^{-1}, 95%–122% and 0.1–2.0 mg N L^{-1}). In contrast, there were significant differences among rivers, but not among reaches, for pH (7.4–8.5), EC (64–640 µS cm^{-1}), NH_4^+ (0.0–0.1 mg N L^{-1}), TDN (0.8–2.6 mg N L^{-1}), SRP (0.01–0.08 mg P L^{-1}), DOC (1.5–8.4 mg L^{-1}), Cl^- (1.8–16.1 mg L^{-1}), and SO_4^{-2} (1.0–31.8 mg L^{-1}) (Table 2 and Table S2). The interaction between river and reach, i.e., within-river variation, was also non-significant for all variables measured (Table 2).

Table 2. Results of linear models testing for the effect of river, reach and their interaction on water quality variables (n = 24). P-values were obtained by two-way ANOVA test. EC = Electrical conductivity, DO = dissolved oxygen, NO_3^- = nitrates, NH_4^+ = ammonium, TDN = total dissolved nitrogen, SRP = soluble reactive phosphorus, DOC = dissolved organic carbon, Cl^- = chloride and SO_4^{-2} = sulfate. F-values are shown in the table and the degrees of freedom indicated at the top of the table are the same for all the tests. Significant p-values are shown in bold.

	River		Reach		River × Reach	
Variables	$F_{3,16}$	p-value	$F_{1,16}$	p-value	$F_{3,16}$	p-value
pH	16.41	**<0.001**	0.00	0.960	0.42	0.741
EC (µS cm^{-1})	63.69	**<0.001**	0.00	0.953	0.01	0.999
Temperature (°C)	1.42	0.273	0.02	0.897	0.03	0.993
DO conc. (mg L^{-1})	0.09	0.764	2.48	0.098	0.60	0.624
DO sat. (%)	2.45	0.101	0.10	0.755	1.21	0.340
NO_3^- (mg N L^{-1})	2.18	0.129	0.71	0.512	0.43	0.731
NH_4^+ (µg N L^{-1})	15.29	**<0.001**	0.14	0.711	0.08	0.969
TDN (mg N L^{-1})	18.70	**<0.001**	0.10	0.752	0.20	0.893
SRP (µg P L^{-1})	5.48	**0.009**	0.07	0.786	0.05	0.982
DOC (mg C L^{-1})	33.32	**<0.001**	3.92	0.065	0.40	0.756
Cl^- (mg L^{-1})	10.75	**<0.001**	0.84	0.371	0.37	0.774
SO_4^{-2} (mg L^{-1})	31.10	**<0.001**	0.73	0.404	0.27	0.846

Hydrology

Water discharge in control reaches was 61.0% ± 14.7% higher during the active than during inactive periods (Table S2). During the active period sampling, the percentage of water diverted ranged from 40% (Kadagua) to 90% (Leitzaran). Discharge and water velocity did not differ between reaches or periods (Table 3 and Tables S2, S5). Nevertheless, the percentage of width of wet channel was on average 17.9% smaller in the regulated reaches during the active period (Table 3 and Tables S2, S5).

OM Standing Stock

The stock of CBOM in the wet channel ranged from 0.0 to 236.9 g m^{-2} and was not significantly affected by pollution (Table 3; Tables S3, S5; Figure 2A).

Table 3. Results of linear models testing for the effects of reach, status, pollution and season on variables measured in the wet and the dry channel. CBOM = coarse benthic organic matter, FBOM = fine benthic organic matter, $pCO_{2,w}$ = CO_2 partial pressure in water, kCO_2 = CO_2 reaeration coefficient, $FCO_{2,w}$ = CO_2 flux between water and atmosphere, $FCO_{2,d}$ = CO_2 flux between dry riverbed and atmosphere, SOM = sediment organic matter. P-values were obtained by ANOVA. Significant p-values are shown in bold. Number of data used (N) is shown. Sign of the coefficients and comparisons between levels of main significant effects are given (C: control; R: Regulated; In: Inactive; A: active; + : increase; - : decrease; S: Spring; Au: Autumn). Interaction class corresponds to reach and pollution interaction and it was classified following Piggott et al 2015.
* Given the limited replication a simpler model was used for the statistical analysis.

	Variables	N	Reach		Period		Pollution		Season		Reach × Period	Reach × Pollution	Interaction class	Period × Pollution
Wet channel	Discharge*	16	0.099		0.144						0.102			
	Velocity*	16	0.212		0.650						0.658			
	Wet width *	157	**<0.001**	C > R	0.087						**0.001**			
	CBOM	142	**<0.001**	C > R	**0.034**	In < A	0.055		0.374		0.081	0.874		0.337
	FBOM	143	0.592		0.556		**0.007**	+	**0.038**	S > Au	0.628	**0.018**	+ Antagonistic	0.305
	Total decomposition	74	0.995		0.821		0.079		0.159		0.515	0.960		0.261
	Microbial decomposition	74	0.784		0.282		0.238		0.187		0.231	**0.027**	- Synergistic	0.591
	Fragmentation	58	0.142		0.743		**0.002**	-	0.592		0.203	0.209		**0.047**
	$pCO_{2,w}$	47	0.444		**<0.001**	In > A	**<0.001**	+	0.841		0.544	**0.004**	+ Synergistic	**<0.001**
	kCO_2 *	16	0.410				0.155					0.363		
	$FCO_{2,w}$	47	0.501		0.876		0.107		**0.019**	S > Au	0.585	0.514		**0.021**
Dry channel	CBOM	144	0.442		0.242		**<0.001**	-	**<0.001**	S < Au	**0.008**	0.592		**0.031**
	SOM content	79	0.617		0.659		**<0.001**	+	0.213		0.747	**0.014**	+ Antagonistic	**0.047**
	Sediment water content	80	**0.034**	C > R	0.678		0.166		0.207		0.174	**<0.001**	- Synergistic	0.393
	Total decomposition	77	**0.032**	C > R	**<0.001**	In < A	**<0.001**	+	0.593		0.478	**0.037**	+ Antagonistic	0.871
	Microbial decomposition	76	0.963		**0.002**	In < A	**0.003**	+	0.498		0.275	**0.007**	+ Antagonistic	0.405
	Fragmentation	53	**0.014**	C > R	**0.043**	In < A	**0.023**	+	0.135		0.103	0.220		0.938
	$FCO_{2,d}$	78	**0.041**	C > R	0.117		**0.001**	+	0.130		0.371	**<0.001**	+ Antagonistic	0.717

It was lower in regulated than in control reaches and higher in the period of active diversion. In dry channels, CBOM ranged from 0.0 to 7548.0 g m^{-2} and decreased with pollution (Table 3; Tables S4, S5). Although reach and period caused no significant differences, their interaction did, with CBOM being lower in regulated reaches when the diversion was active (Table 3; Tables S4, S5; Figure 2B). Moreover, the period × pollution interaction was also significant, indicating a reduction of CBOM with pollution when the diversion period was active (Table 3; Tables S4, S5; Figure 2B). The stock of FBOM in the wet channel ranged from 0.4 to 300.1 g m^{-2} and increased with pollution (Table 3; Tables S3, S5, Figure S2). Reach and period did not affect FBOM stocks. The interaction between reach and pollution was significant, with FBOM differences being higher in regulated reaches of the most polluted rivers. In the dry channel, the SOM content ranged from 1.8% to 10.8% and increased with pollution (Table 3; Tables S4 and S5; Figure S3A). Reach or period did not affect SOM content. The interaction between reach and pollution was significant, showing a lower SOM content only in the polluted and regulated reaches (Table 3; Tables S4 and S5; Figure S3A). The interaction between period and pollution was also significant; thus, differences in SOM content between periods became larger with increasing pollution, being the lowest OM content during the active period (Table 3; Tables S4, S5, Figure S3A). However, water content ranged from 2.5% to 44.8% and was not affected by pollution (Table 3; Tables S4 and S5; Figure S3A). Water content was significantly lower in regulated than in control reaches. The interaction of reach and pollution was also significant, with regulated reaches in polluted rivers showing the lowest water content (Table 3; Tables S4, S5; Figure S3B).

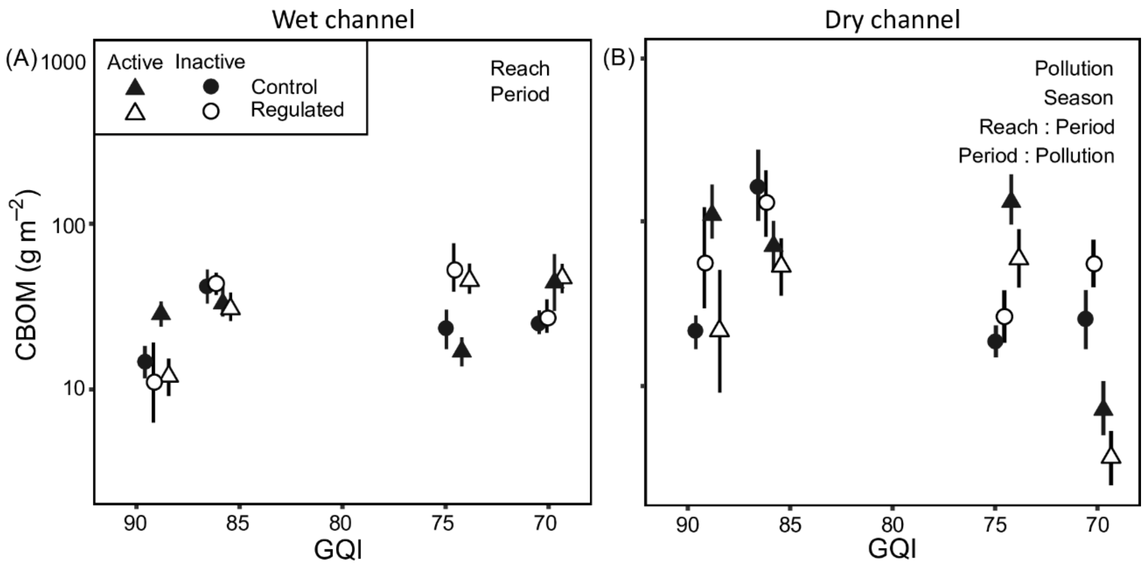

Figure 2. Stock of coarse benthic organic matter (CBOM) in (A) wet and (B) dry channel. Values are mean and error bars show standard error. The text in the background indicates significant single-factor effects or interactions. GQI is the General Quality Index and the scale is inverted to make easier the interpretation of the figure, ranging from good (low pollution) to acceptable quality level (moderate pollution). GQI values have been jittered to avoid overlapping points.

Leaf litter decomposition

In the wet channel, total decomposition ranged from 0.0005 to 0.0043 d d^{-1}, microbial decomposition from 0.0005 to 0.0024 d d^{-1}, and fragmentation from 0.0000 to 0.0021 d d^{-1}. Total decomposition was unaffected by the investigated factors (Table 3; Tables S3, S5; Figure 3A). Microbial decomposition was not affected by single stressors but was interactively affected by the reach × pollution interaction, being slower in regulated reaches of the most polluted rivers (Table 3; Tables S3 and S5; Figure 3C).Fragmentation decreased significantly with pollution (Table 3; Tables S3 and S5; Figure 3E). Moreover, the period × pollution interaction was significant, with fragmentation being lowest during inactive periods in the most polluted rivers (Table 3; Tables S3 and S5; Figure 3E).

In the dry channel, total decomposition ranged from 0.0005 to 0.003 d d^{-1}, microbial decomposition from 0.000 to 0.002 d d^{-1}, and fragmentation from 0.0000 to 0.0006 d d^{-1}. The three processing rates increased significantly with pollution. Total and microbial decomposition and fragmentation rates were also significantly lower when diversions were inactive compared to when they were active (Table 3; Tables S4 and S5; Figures 3B,D,F). However, total decomposition and fragmentation were significantly lower in regulated reaches (Table 3; Tables S4, S5; Figures 3B,F). The reach × pollution interaction was significant for total and microbial decomposition, with differences between reaches being higher in most polluted rivers (Table 3; Tables S4 and S5; Figures 3B,D). Total and microbial decomposition was lower in regulated reaches but the reduction was not below the control of the cleanest river.

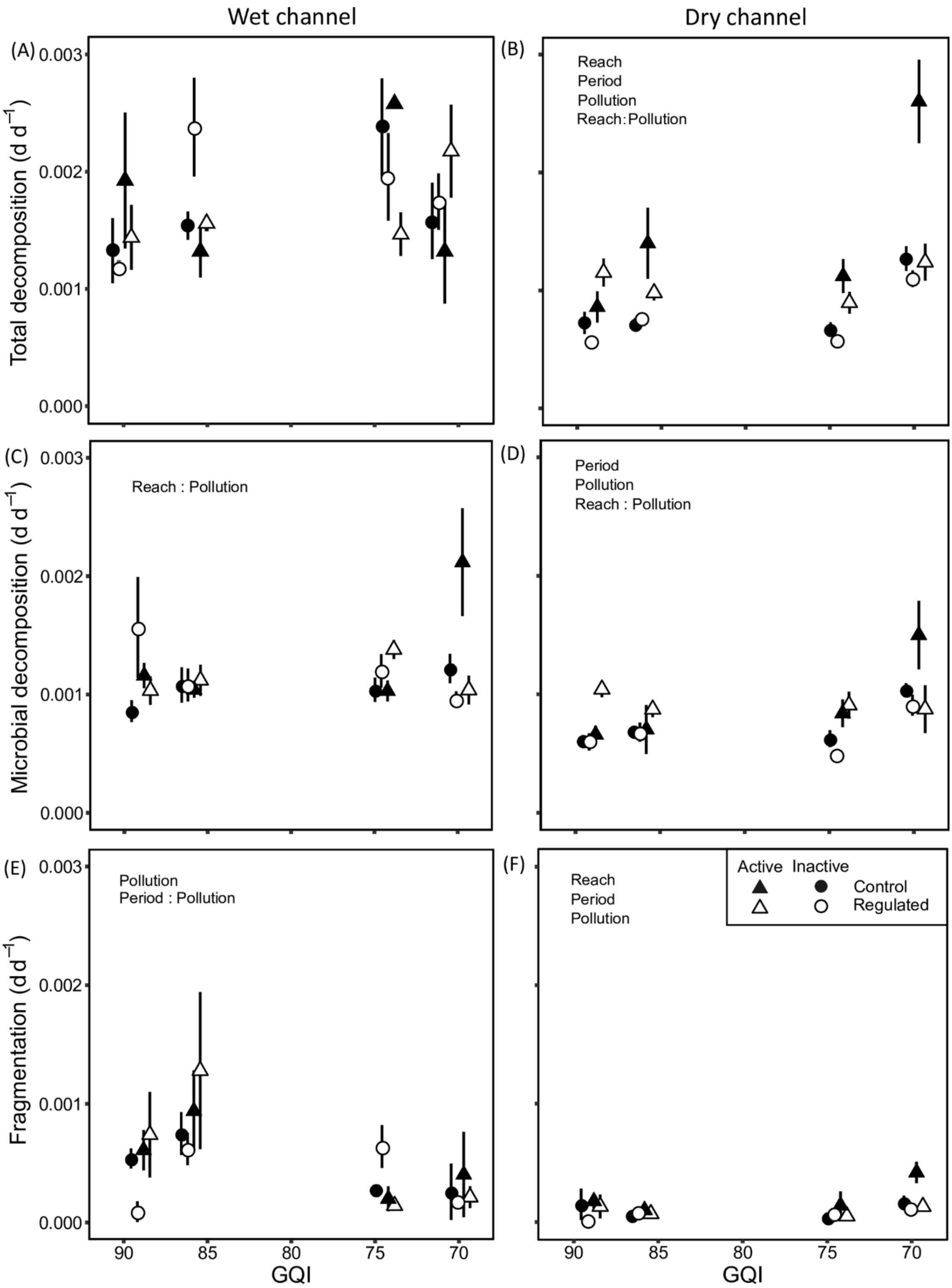

Figure 3. Leaf litter decomposition in wet (left side) and dry channel (right side): (A, B) total decomposition, (C, D) microbial decomposition and (E, F) fragmentation. Values are mean and error bars show standard error. The text in the background indicates significant single-factor effects or interactions. GQI is the General Quality Index and the scale is inverted to make easier the interpretation of the figure, ranging from good (low pollution) to acceptable quality level (moderate pollution). GQI values have been jittered to avoid overlapping points.

CO_2 Flux

In the wet channel, $pCO_{2,w}$ ranged from 369 to 1175 µatm and increased with pollution (Table 3; Tables S3 and S5; Figure S4). When the diversions were active, $pCO_{2,w}$ was significantly lower compared to when it was inactive; however, there were no differences between reaches. The reach × pollution interaction was significant, showing lower $pCO_{2,w}$ in regulated reaches of the most polluted rivers. The period × pollution interaction also was significant, with $pCO_{2,w}$ decreasing more clearly when the diversions were active in the most polluted sites. The reaeration coefficient kCO_2 ranged from 6.0 to 44.2 m d^{-1} and neither reach, pollution nor their interaction showed any significant effect (Table 3; Tables S3 and S5). In the wet channel, $FCO_{2,w}$ ranged from −6.7 to 430.3 mmol m^{-2} d^{-1} and was not affected by reach, period, or pollution (Table 3; Tables S3 and S5; Figure 4A). The reach × pollution interaction was not significant, but that between period and pollution was, with pollution increasing differences in $FCO_{2,w}$ between periods (Table 3; Tables S3 and S5; Figure 4A). In the dry channel, $FCO_{2,d}$ ranged from 28.4 to 986.6 mmol m^{-2} d^{-1} (Table 3; Tables S4 and S5; Figure 4B) and was significantly related to SOM and water content (log transformed data; Pearson $r = 0.363$, $p < 0.001$ and Pearson $r = 0.288$, $p = 0.010$, respectively). $FCO_{2,d}$ increased with pollution (Table 3; Tables S4 and S5; Figure 4B). Period did not affect it significantly, but in regulated reaches $FCO_{2,d}$ was significantly lower. The reach × pollution interaction was also significant, with $FCO_{2,d}$ decreasing only in regulated reaches of polluted rivers (Table 3; Tables S4 and S5; Figure 4B).

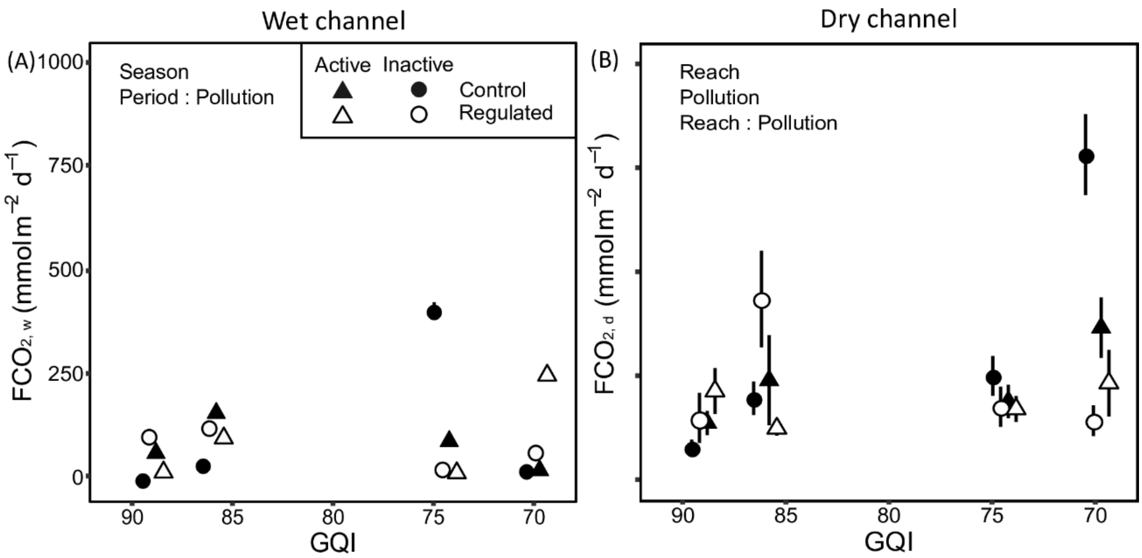

Figure 4. CO_2 efflux (FCO_2) in (A) wet and (B) dry channel. Values are mean and error bars show standard error. The text in the background indicates significant single-factor effects or interactions. GQI is the General Quality Index and the scale is inverted to make easier the interpretation of the figure, ranging from good (low pollution) to acceptable quality level (moderate pollution). GQI values have been jittered to avoid overlapping points.

DISCUSSION

This study adds to the existing knowledge on the effects of water diversion by small hydropower schemes. It does so by focusing mainly on river ecosystem functioning, a relatively little studied component of river integrity (von Schiller et al., 2017), and looking at the interaction between water diversion and pollution, one of the most common stressors in world rivers (Hering et al., 2015). Our approach is also seldom used, as we compared upstream control with downstream regulated reaches, both in periods of active and inactive diversion, thus yielding a comprehensive picture of the overall effects of hydropower schemes, which usually alternate between active and inactive periods. Our results show that water diversion and pollution have interactive effects on river OM processing, dry channels being more reactive to the interaction than wet channels. Overall, water diversion did not affect leaf litter decomposition and CO_2 fluxes in the wet channel, but in the dry channel, decomposition and CO_2 emissions were lower with water diversion. On the other hand, pollution reduced leaf litter fragmentation but did not affect the CO_2 flux in the wet channel, whereas in the dry channel it promoted both decomposition and CO_2 emissions. Both stressors interacted antagonistically for microbial decomposition in the wet channel, and for total and microbial decomposition and CO_2 fluxes in the dry channel.

Diversion

We hypothesized water diversion to reduce leaf litter decomposition and OM standing stocks in the wet channel, but results only confirmed this for CBOM. This reduction of CBOM might be a consequence of its retention in the dam (Schmutz & Moog, 2018), of its transport through the diversion canal (Arroita et al., 2015), or probably both, to counteract the effect of increased retention in the bypassed reach (Arroita et al., 2017). For the dry channel, we hypothesized a decrease of leaf litter decomposition and no effects on OM stocks with water diversion, but again, results only confirmed this partially. The content of CBOM and SOM were not affected by diversion, but total decomposition and fragmentation (but not microbial decomposition) were significantly reduced. In terrestrial habitats, leaf litter decomposition tends to be lower than in the moist sediments from dry beds, and much lower than in running waters (Abril et al., 2016; Lake, 2003). In our case, diversion caused the contraction of the wet channel by almost 20% in regulated reaches and reduced rewetting cycles and the water content of sediments in the dry channel, conditions that lead to slow decomposition in riparian and terrestrial areas (Tiegs et al., 2019). Microbial decomposition did not differ between reaches, maybe because the fine mesh used maintained a level of moisture in the bags high enough to keep an active microbial community (Romaní et al., 2017).

Regarding the CO_2 flux, we predicted a reduction by water diversion in both the wet and the dry channel, but results only confirmed our prediction for the dry channel. Water diversion caused a decrease in $FCO_{2,d}$ in regulated reaches, probably because of the reduction of water content in sediments, which restricts microbial activity and the subsequent release of CO_2 (Arce et al., 2019; Marcé et al., 2019). In this sense, dry sediments in regulated reaches would function in a way more similar to that of Mediterranean parafluvial areas (Almagro et al., 2009).

Pollution

Although we expected pollution to reduce the stock of OM in the wet channel and no effects in the dry channel, we found no effect in the wet channel and reduced CBOM stocks in the dry channel. The explanation for this unexpected result could be reduced riparian forests in the most urbanized basins (Pennington et al., 2010), which would limit OM inputs to dry bars in our most altered rivers, whereas urban wastewater would increase FBOM in the wet channel (Kelso & Baker, 2020). Unexpectedly, SOM content increased with pollution, thus suggesting this OM was at least in part derived from FBOM transported by flow and deposited on the dry channel during water level fluctuations. More detailed characterization of the SOM would be needed to elucidate its origin.

On the other hand, pollution did not affect total and microbial decomposition in the wet channel but reduced fragmentation. The response of decomposition to pollution is complex and often hump-shaped because of the differential sensitivity of microbes and detritivorous (Woodward et al., 2012). Detritivorous macroinvertebrates, the main responsible organisms for leaf litter fragmentation (Graça, 2001; Hieber & Gessner, 2002; Lecerf, 2017), tend to decrease at moderate levels of pollution, as occurs in our streams (de Guzman et al., in prep). Woodward and collaborators (2012) reported peak invertebrate-mediated breakdown (similar to our fragmentation rate) at 0.02 mg SRP L^{-1} and 5.6 mg DIN L^{-1}. Although in our rivers, the range of DIN concentration was below the maximum for invertebrate breakdown, the SRP concentration of most polluted rivers was above the maximum concentration promoting a lower fragmentation (Table S3). Therefore, probably SRP concentration in our rivers was high and damaging for fragmentation rates. Moreover, several studies have shown the detrimental effect of other pollutants also present in our rivers (e.g., heavy metals, biocides) on shredders and litter decomposition (Alonso & Camargo, 2006; Baldy et al., 2007; Brosed et al., 2016; Carlisle & Clements, 2005). In the dry channel, results confirmed our hypothesis and decomposition increased with pollution. Despite the temporal rewetting cycles of leaf litter on the dry channel, the moderate nutrient load of water promoted decomposition at all levels (i.e., total, microbial and fragmentation). Moderate nutrient enrichment stimulates fungal activity and, in consequence, palatability for invertebrate leaf consumption when litter is submerged (Dunck et al., 2015; Gulis & Suberkropp, 2003).

We also predicted an increase of CO_2 efflux with pollution in both habitats, but results only confirmed it partially. In the wet channel, $FCO_{2,w}$ was not affected by pollution. Higher nutrient and OM loads, as those detected across our pollution gradient, can increase river ecosystem respiration, thereby increasing $pCO_{2,w}$ (Borges et al., 2015; Hotchkiss et al., 2015; Prasad et al., 2013). Although there is a dependency between $pCO_{2,w}$ and $FCO_{2,w}$, the two processes have different drivers (Liu & Raymond, 2018), as the latter is also affected by the reaeration coefficient (kCO_2). There was a decrease in kCO_2 along the pollution gradient that compensated the increase in $pCO_{2,w}$, resulting in no effect on $FCO_{2,w}$. In the dry channel, as we expected, the CO_2 efflux increased with pollution. Microbial activity in sediment can be promoted by DOC and TN previously deposited from water (Gómez-Gener et al., 2016), and the water content on sediment during rewetting cycles would facilitate the microbial uptake of these compounds, thus increasing microbial respiration and CO_2 fluxes (Gómez-Gener et al., 2015; Luo & Zhou, 2010; Xu et al., 2004).

Diversion-Pollution Interaction

Although we expected the interaction between both stressors to reduce OM stock in the wet channel and to have no effect in the dry channel, results proved otherwise. The CBOM stock did not respond to the interaction in any habitat, but FBOM increased in the wet channel, whereas SOM decreased. The increase in FBOM seems to be a consequence of enhanced deposition under reduced flows (Riis et al., 2017), which would be more noticeable in the polluted rivers, where suspended solids are more abundant (URA, 2016) (Table S1). The decrease in SOM content is harder to explain but may be related to lower frequency of rewetting the dry sediments.

Regarding decomposition, in both habitats we expected an antagonistic effect between the reduction caused by diversion and the promotion caused by pollution, which would result in decomposition rates closer to those of control reaches in clean rivers. In the wet channel, results did not confirm our hypothesis since total decomposition and fragmentation did not respond, whereas microbial decomposition in the presence of both stressors was lower than control values. Because of wet channel contraction in regulated reaches, water depth also could be smaller resulting in an increase in the boundary layer thickness surrounding the microbial community growing on leaf litter (Bishop et al., 1997). In consequence, in spite of the presence of nutrients from moderate pollution of water, the exchange of nutrients and oxygen with the water column would be limited and microbial decomposition would be reduced (Bruder et al., 2016; Medeiros et al., 2009). In the dry channel, we also expected an antagonistic effect, partially confirmed by our results. Although the fragmentation rate did not respond, total and microbial decomposition were reduced, the interaction being closer to control reaches in cleaner rivers. Despite pollution stimulated decomposition, the contact with water is one of the most important factors that influences leaf litter decomposition (Northington & Webster, 2017).

For CO_2 fluxes, we also expected an antagonistic response to the interaction in wet and dry channels. Whereas $FCO_{2,w}$ in the wet channel did not respond to the interaction, $pCO_{2,w}$ was lower in polluted and regulated reaches than in control reaches. This reduction in $pCO_{2,w}$ might be a consequence of algal growth under moderate nutrient load, good solar irradiation, and low water velocity (Marx et al., 2017). Alternatively, it could be due to reduced hydrological connectivity between wet and dry channels, as soil respiration and weathering processes contribute to aquatic $pCO_{2,w}$ (Riveros-Iregui & McGlynn, 2009; Striegl & Michmerhuizen, 1998). In the dry channel, we also hypothesized an antagonistic response of $FCO_{2,d}$ to the interaction of both stressors, a prediction that was confirmed by our results. $FCO_{2,d}$ was lower in regulated than in control reaches in polluted rivers and closer to control reaches of cleaner rivers. The low water and OM content in sediment could lead to low microbial activity, which in turn causes low CO_2 efflux.

CONCLUSIONS AND IMPLICATIONS

Our results show that the interaction between water diversion and pollution can have important consequences on in-stream OM processing. These two stressors interacted antagonistically and their effects were more pronounced in the dry channel than in the wet channel. The observed changes in particulate OM decomposition both in the wet and the dry channel, as a result of water diversion and pollution, may cause profound shifts in the trophic structure and energetic balance of the ecosystem, as allochthonous OM is the main energy source supporting food webs in forested rivers (Marks, 2019). On the other hand, the two investigated stressors had significant effects on CO_2 emissions in the dry channel, emphasizing the role of dry riverbeds in gaseous carbon exchange along river networks (Marcé et al., 2019). Overall, our study reinforces the idea that not considering dry channels as an active part of rivers could lead to an underestimation of the effect of diversion and urban pollution on these ecosystems.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Chapter 3

Interactive effects of discharge reduction and fine sediments on stream biofilm metabolism.

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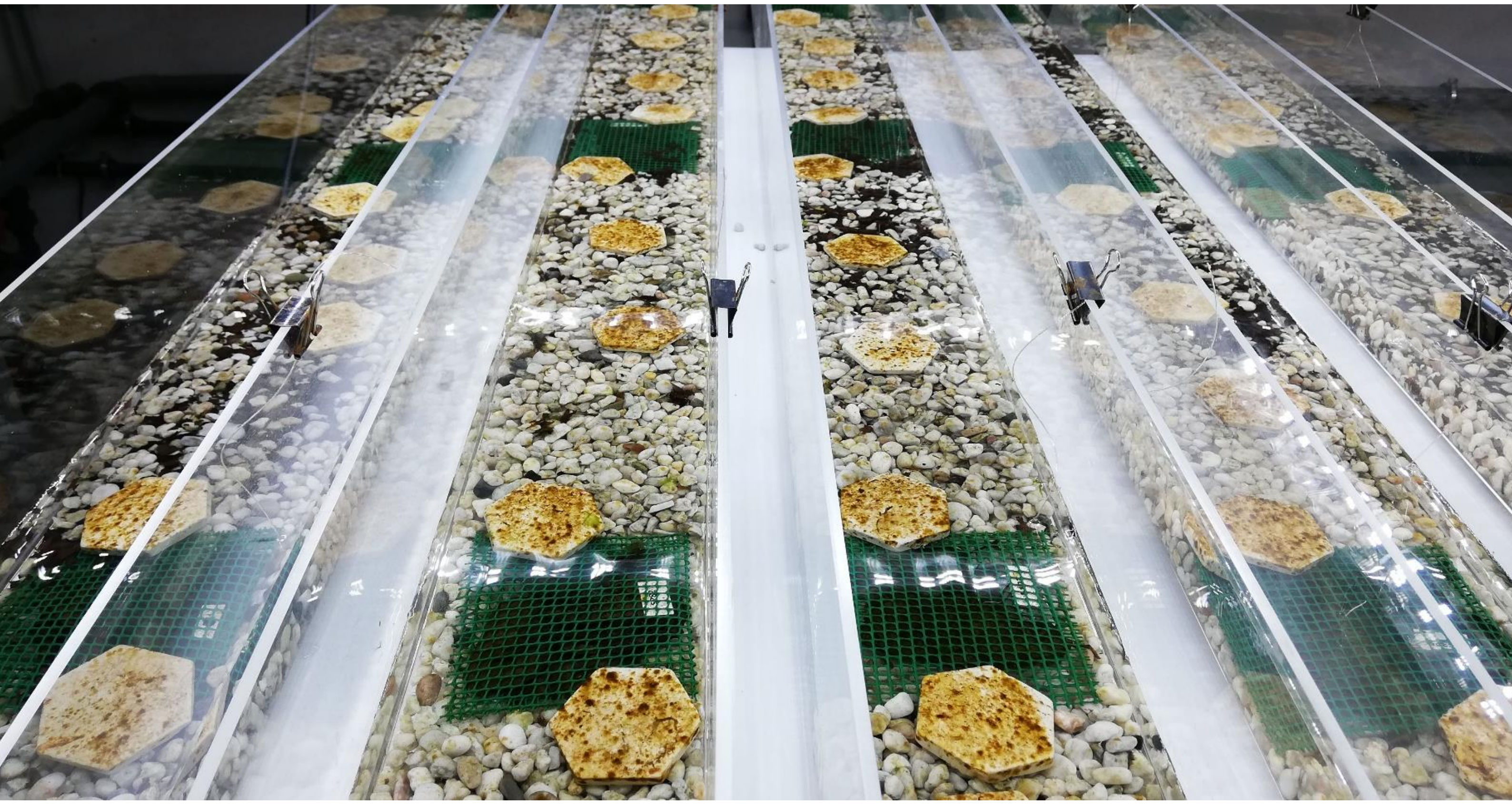

ABSTRACT

Discharge reduction, as caused by water diversion for hydropower, and fine sediments deposition, are prevalent stressors that may affect multiple ecosystem functions in streams. Periphytic biofilms play a key role in stream ecosystem functioning and are potentially affected by these stressors and their interaction. We experimentally assessed the interactive effects of discharge and fine sediments on biofilm metabolism in artificial indoor channels using a factorial split-plot design with two explanatory variables: water discharge (20, 39, 62, 141 and 174 $cm^3\ s^{-1}$) and fine sediments (no sediment or 1100 mg L^{-1} of sediments). We incubated artificial tiles for 25 days in an unpolluted stream to allow biofilm colonization, and then placed them into the indoor channels for acclimation for 18 days. Subsequently, we manipulated water discharge and fine sediments and, after 17 days, we measured biofilm chlorophyll-a concentration and metabolism. Water velocity (range, 0.5 to 3.0 cm s^{-1}) and sediment deposition (range, 6.1 to 16.6 mg cm^{-2}) increased with discharge, the latter showing that the effect of increased inputs prevailed over sloughing. In the no-sediment treatments, discharge did not affect biofilm metabolism, but reduced chlorophyll-a. Sediments, probably as a consequence of nutrients released, promoted metabolism of biofilm and chlorophyll-a, which became independent of water discharge. Our results indicate that pulses of fine sediments can promote biofilm algal biomass and metabolism, but show interactive effects with discharge. Although discharge reduction can affect the abundance of basal resources for food webs, its complex interactions with fine sediments make it difficult to forecast the extent and direction of the changes.

INTRODUCTION

Stream ecosystems are affected by multiple anthropogenic stressors (Sabater et al., 2018). Among these, damming and water diversion stand out as detrimental activities for stream biological communities (Bunn & Arthington, 2002; Martínez et al., 2013; Benejam et al., 2016) and ecosystem functioning (Elosegi & Sabater, 2013). The number of water diversion schemes is rising in response to escalating water demands (Vörösmarty et al., 2010; Reid et al., 2019). Stream discharge reduction caused by water diversion reduces the width of the wet channel (McKay & King, 2006), affects water chemistry (Dewson et al., 2007), alters transport and deposition of sediments (Poff et al., 1997), and impacts multiple ecosystem functions such as leaf litter breakdown (Mendoza–Lera et al., 2012; González et al., 2013; Arroita et al., 2015), nutrient retention (Arroita et al., 2017) and stream metabolism (Aristi et al., 2014).

Fine sediments are also considered an important stressor and often included among the most prevalent pollutants in streams (USEPA, 2000). High inputs of fine sediments can occur as a consequence of natural processes (Wood & Armitage, 1997; Buendia et al., 2016), but are often exacerbated by human activities such as forestry or agriculture (Syvitski et al., 2005). Suspended fine sediments reduce the light that reaches the stream bottom (Davies-Colley et al., 1992), can abrade biofilms (Francoeur & Biggs, 2006), damage organisms gills (Kemp et al., 2011; McKenzie et al., 2019) and interact with dissolved nutrients and other pollutants (Wagenhoff et al., 2013; Magbanua et al., 2016; Chase et al., 2017). Additionally, fine sediments tend to settle on stream beds, where they cause siltation (Rehg et al., 2005), reduce the supply of oxygen and light to the bottom and damage primary producers (Izagirre et al., 2009), macroinvertebrates (Kaller & Hartman, 2004) and fish (Bilotta & Brazier, 2008).

Periphytic biofilms (hereafter biofilms) consist of complex communities of microorganisms that include bacteria, algae, fungi and protozoa, and live attached to rocks or other surfaces (Romaní et al., 2016). They play a key role in stream ecosystem functioning (Battin et al., 2016) and are an important food resource for invertebrates and fish (Sabater et al., 2000). The abundance, composition and activity of biofilms is regulated by factors such as light, current, nutrients and grazing (Allan & Castillo, 2007). Therefore, biofilms are highly sensitive to environmental changes and can be potentially affected by multiple anthropogenic stressors (Sandin & Solimini, 2009; Sabater et al., 2016).

The response of biofilm to discharge reduction and fine sediments deposition is complex. In fast-flowing streams, water diversion reduces water velocity and shear stress, thus promoting biofilm growth and activity (Lau & Liu, 1993; Chester & Norris, 2006; Ponsatí et al., 2015). When natural discharge is low, further reductions can detrimentally affect biofilm by reducing nutrient exchange (Horner & Welch, 1981; Allan & Castillo, 2007). Besides, water diversion reduces the amount of fine sediments entering at reach, as most sediments are diverted with the water. At the same time, however, discharge reduction promotes the deposition rate of those sediments in the reach as a consequence of reduced water velocity, thus impacting benthic biota (Matthaei et al., 2010). The final outcome will depend on factors such as water velocity, the characteristics of fine sediments or the type of organisms. Biofilms can be damaged by sediments via abrasion or burial (Francoeur & Biggs, 2006), but can also benefit from fine sediments as a source of nutrients, especially phosphorus (Perry & Stanford, 1982). These complex interactions call for controlled experiments to examine how discharge and fine sediments affect biofilm structure and functioning.

Here, we experimentally assessed the interactive effects of discharge and fine sediments on biofilm algal biomass and metabolism. The experiment was carried out in artificial stream channels, which were subject to a gradient of water discharge in presence or absence of fine sediments. We tested the following three hypotheses:

1) Algal biomass and metabolism will be lower at low discharge because of limited nutrient exchange.
2) Addition of fine sediments will reduce algal biomass and metabolism because it hinders algal attachment and limits light availability.
3) Water discharge and fine sediments will interact, algal biomass and metabolism being lowest in the channels with sediments and lowest water discharge.

MATERIALS AND METHODS

Experimental design

The indoor artificial stream facility of the University of the Basque Country (Leioa, Spain) consists of 30 indoor methacrylate channels (length-width-depth: 200-15-20 cm) grouped in six blocks of five channels. From a primary tank, filtered (1 mm mesh) rainwater is fed to six 200-L tanks (hereafter 'block tanks') that supply water to each block of five channels. In each block, water was recirculated by a pump and run as a closed system (Fig 1). Discharge can be adjusted for each channel individually. In each channel, water depth was set at 3.4 ± 0.1 cm (mean ± SE) by means of a small dam at the lowermost end. LED lights (36 W 65000k, Aquael, Poland) with a 12/12 light/dark cycle and an intensity of 27.1 ± 1.0 µmol m^{-2} s^{-1} provided lighting. The bottom of the channels was covered by a 2-cm layer of commercial aquarium gravel of 8-16 mm average size (Karlie Flamingo, Germany). We used marble tiles (33.6 cm^2 of surface area) as standard biofilm substrata. To allow biofilm colonization, these tiles were incubated in an unpolluted and oligotrophic reach of the Urumea River (N Iberian Peninsula; 43°12'40.6" N, 1°54'06.2" W), attached on plastic trays with no protection from grazers and tied to the river bottom for 25 days.

After the incubation period, tiles were collected, transported to the artificial stream facility, and randomly distributed across the channels (12 tiles per channel). Additionally, to ensure biofilm development, we scraped several cobbles at the Urumea River, and the slush produced was split and uniformly distributed among the artificial channels. To allow biofilm acclimation, discharge was kept constant (discharge = 85.2 ± 2.48 cm^3 s^{-1}; water velocity = 1.7 ± 0.06 cm s^{-1}) in all the channels for 18 days. To avoid nutrient depletion, the water in each block tank was renewed every week during the acclimation and the experimental period.

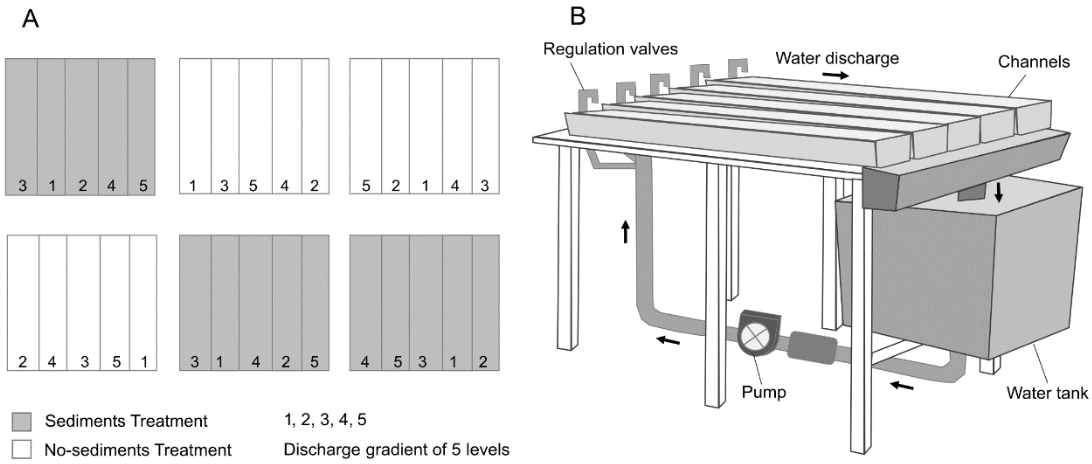

Figure 1. Experimental setup. (A) Schematic drawing of channel blocks setup and experimental levels of the factors discharge and fine sediments. (B) Detail of a channel block.

After the acclimation period, we started a factorial experiment with a split-plot design, which lasted for 17 days. Water discharge was set in five levels (19.8 ± 1.3, 39.0 ± 3.8, 62.4 ± 5.1, 141.3 ± 8.6 and 173.7 ± 7.1 $cm^3 s^{-1}$) and fine sediments in two levels: no fine sediments and 1100 mg L^{-1} of fine sediments, a concentration that is commonly found in the Urumea River during floods (Zabaleta et al., 2007), as well as during forestry operations. Water discharge levels were randomly assigned to each channel within each block. Water discharge was measured on days 1, 10 and 14, from the time to fill a container at the lower end of each channel; water velocity was estimated from the ratio between water discharge and average channel width and depth, measured with a ruler along each channel. Water discharge and velocity remained constant during the experiment. Sediments were added to three randomly selected block tanks in 2 pulses (days 1 and 10). Sediments were distributed through the water pump and circulated through the treatment channels, where they settled rapidly, turbidity returning to background values a few hours after the addition.

The fine sediments used in this experiment were obtained from the recently emptied Enobieta Reservoir (43°12'50.5" N 1°47'31.0" W), located in the Urumea basin upstream from the biofilm collection point. These sediments were dried, ground and sieved through 200 µm. Their organic matter content was 21.0 ± 0.11% and their C:N molar ratio 49:1. Sediment leachate was characterized in the laboratory by mixing 2.0 ± 0.01 gr (n=5) of dried sediments with 0.2 L of deionized water and kept at 20 °C with light (120 µmol cm^{-2}) and with a constant movement (70 rpm in an orbital shaker Multitron II, INFORS HT, Bottmingen, Switzerland) for 24 h to mimic channel conditions. This leachate had a content of 0.093 ± 0.003 mg g^{-1} of ammonium (N-NH_4^+), 0.012 ± 0.0002 mg g^{-1} of nitrate (N-NO_3^-), 0.36 ± 0.003 mg g^{-1} of total dissolved nitrogen (TDN), 3.96 ± 0.001 mg g^{-1} of dissolved organic carbon (DOC) and 0.008 ± 0.001 mg g^{-1} of soluble reactive phosphorus (SRP). Thus, sediment leachate contributed 20.9 ± 0.2 mg of N (sum of NH_4^+ and NO_3^-) and 1.7 ± 0.2 mg of P (from SRP) to each 5-channel block per sediment pulse. These quantities correspond to concentrations of 1.06 ± 0.03 mg L^{-1} of N and 0.08 ± 0.01 mg L^{-1} of P. See next section for analytical methods.

Water quality

Water quality was analysed six times: on the first day of the experimental period, before and after renewing water (days 7 and 14) and on the last day of the experiment. We measured temperature, pH, electrical conductivity and dissolved oxygen concentration and saturation in the block tanks with a hand-held probe (Multi 3630 IDS, WTW, Germany). Water samples were collected from the block tanks, filtered through 0.7-µm pore size glass fibre filter (Millipore GF/F, Ireland) and stored at -20 °C until analysis. The concentration of nitrate (N-NO_3^-), sulphate (SO_4^{2-}) and chloride (Cl^-), was measured by capillary electrophoresis (Agilent CE, Agilent Technologies, USA) (Environmental Protection Agency, 2007). The concentration of soluble reactive phosphorus (SRP) (molybdate method (Murphy & Riley, 1962)) and ammonium (N-NH_4^+) (salicylate method (Reardon et al., 1966)) were determined colorimetrically on a UV-1800 UV–vis Spectrophotometer (Shimadzu, Shimadzu Corporation, Kyoto, Japan). Total dissolved nitrogen (TDN) and total dissolved organic carbon (DOC) were determined by catalytic oxidation on a Shimadzu TOC-L_{CSH} analyser coupled to a TNM-L unit (Shimadzu, Shimadzu Corporation, Kyoto, Japan).

Response variables

At the end of the experiment biofilm variables were measured on the tiles. We measured chlorophyll-a (chl-a) concentration as a proxy of algal biomass by in vivo fluorimetry (BenthoTorch, bbe Moldaenke Gmbh, Germany) in six randomly selected tiles in each channel. BenthoTorch is a non-intrusive tool that quantifies the total algal biomass through the stimulation of cell pigments and the reading of red fluorescent light emitted (Harris & Graham, 2015). We summed the values of chlorophyll for green algae, cyanobacteria and diatoms, thus calculating total chl-a concentration (Echenique-Subiabre et al., 2016).

Biofilm metabolism was estimated in 0.21-L glass chambers hermetically closed without recirculation. We enclosed one tile per chamber (6 random replicates per channel, 3 incubated in light conditions, 3 in dark conditions), filled them with water from the corresponding tank and incubated them for 1 h submersed in the channel. After incubation, we measured dissolved oxygen using a portable fibre optic oxygen meter with a syringe-like probe (Microsensor NTH-PSt7 on Microx 4, PreSens, Germany) by inserting its needle through the hermetic membrane. Metabolism metrics (i.e., gross primary production GPP, community respiration CR and net community metabolism NCM) were calculated following Acuña et al. (Acuña et al., 2009). We also calculated gross primary production per unit of algal biomass (i.e., GPP/Chl-a) as a proxy of metabolic efficiency (Lamberti & Resh, 1983; Kendrick & Huryn, 2015).

Table 1. Water quality values for each sediment treatment during the experiment. T = temperature; DO = dissolved oxygen concentration and saturation; EC = electrical conductivity; SRP = soluble reactive phosphorus; NH_4^+ = ammonium; NO_3^- = nitrate; TDN = total dissolved nitrogen; DOC = dissolved organic carbon; Cl^- = chloride and SO_4^{2-} = sulphate. Values shown are mean ± SE. P-values and F-values were obtained by ANOVA. Degrees of freedom are 1, 29 for all variables. Significant P-values are shown in bold

Variable	(unit)	No-sediment	Sediment	F-value	P-value
T	(°C)	22.3 ± 0.2	22.3 ± 0.2	0.22	0.644
pH	-	7.5 ± 0.1	7.5 ± 0.1	0.70	0.410
DO	(%)	99.2 ± 1.3	100.9 ± 0.7	7.02	**0.013**
DO	(mg L^{-1})	8.6 ± 0.1	8.7 ± 0.1	8.60	**0.007**
EC	(µS cm^{-1})	105.8 ± 13.0	75.2 ± 2.8	20.30	**<0.001**
SRP	(µg P L^{-1})	18.1 ± 2.9	18.4 ± 2.9	0.08	0.783
NO_3^-	(mg N L^{-1})	0.6 ± 0.06	0.4 ± 0.08	8.61	**0.007**
NH_4^+	(µg N L^{-1})	22.2 ± 0.7	13.2 ± 0.2	2.82	0.104
TDN	(mg N L^{-1})	1.8 ± 0.1	1.6 ± 0.1	3.90	0.058
DOC	(mg C L^{-1})	3.2 ± 0.2	3.6 ± 0.3	3.38	0.076
Cl^-	(mg L^{-1})	5.7 ± 0.1	5.7 ± 0.1	0.07	0.796
SO_4^{2-}	(mg L^{-1})	5.6 ± 0.4	3.9 ± 0.1	17.30	**<0.001**

Finally, we quantified the total amount of sediments deposited in the channels throughout the experiment by washing all the substrate within a container and measuring the turbidity (NTU) of the homogenised solution with a hand-held turbidimeter (AQ4500 Aquafast IV, Thermo Scientific Orion, USA). Turbidity (NTU) was converted to sediment concentration (g L^{-1}) using an empirical equation ($sediment\ concentration = 0.0036 * turbidity + 0.0971$, $r^2 = 0.99$, $p < 0.001$) established in the laboratory by measuring of turbidity of several solutions with a known concentration of the fine sediments (0, 0.1, 0.2, 0.5, 1, 2 and 4 gr L^{-1}).

Data analysis

We analysed the differences among treatments in chlorophyll-a concentration (μg cm^{-2}) and biofilm metabolism metrics (GPP, CR, NCM; mg O_2 h^{-1} and GPP/Chl-a ; mg O_2 mg chl-a^{-1} h^{-1}) using Linear Mixed-Effects Models (LMEM) with REML (function lme, in R package nlme (Pinheiro et al., 2019)). Sediments (Yes vs. No) was set as fixed factor, Water discharge as a continuous explanatory variable, and blocks and channels nested within blocks, as random factors. We included a variance structure (varIdent in the nlme function) in the models to account for the variance heterogeneity between levels of the factor Sediments. The significance of each source of variation was tested by means of ANOVA. Chlorophyll-a concentration and GPP/Chl-a were log-transformed to meet homoscedasticity. All analyses were performed using R software, v. 3.4.0 (R Core Team., 2017).

RESULTS

Water quality

The values of water temperature (22.4 ± 0.2 °C) and pH (7.5 ± 0.1) were stable during the experiment, with no differences among levels of the treatments (Table 1). A small, but significant, increase in dissolved oxygen concentration and saturation was observed in the sediment treatment (8.7 ± 0.1 mg O_2 L^{-1} and 100.3 ± 0.8%) with respect to the no-sediment treatment (8.5 ± 0.1 mg O_2 L^{-1} and 98.4 ± 1.3%) (Table 1). Electrical conductivity was lower in the sediment treatment (73.9 ± 2.8 μS cm^{-2}) than in the no-sediment treatment (104.7 ± 14.3 μS cm^{-2}) (Table 1), suggesting potential sorption of dissolved ions by added sediments. Most measured solutes did not differ between sediment and no-sediment treatments (Table 1). However, NO_3^- and SO_4^{-2} were significantly lower in the sediment treatment than in the no-sediment treatment (1.6 ± 0.3 and 2.7 ± 0.3 mg L^{-1} vs. 4.0 ± 0.1 and 5.3 ± 0.4 mg L^{-1}, respectively), whereas DOC concentration was significantly higher in the sediment treatment (3.9 ± 0.2 vs. 3.2 ± 0.2 mg L^{-1}). Water renewal affected water quality: temperature, pH, dissolved oxygen, SO_4^{2-} and DOC decreased (an average of 0.5 °C, 0.5, 7.7% and 0.5 mg O_2 L^{-1}, 1.1 mg L^{-1}, 1.5 mg C L^{-1}, respectively) and, SRP and NO_3^- increased (an average of 17.5 μg P L^{-1} and 0.2 mg N L^{-1}, respectively) The rest of parameters showed no changes with water (S1 Dataset). Note that these changes were caused by water renewal, not by sediments, which were added to the corresponding treatments in days 1 and 10.

Table 2. Results of the Linear Mixed-Effects Models (LMEM) with water discharge as continuous explanatory variable, sediments as fixed factor and chlorophyll-a (Chl-a), gross primary production (GPP), community respiration (CR), net community metabolism (NCM), gross primary production per unit of chlorophyll-a (GPP/Chl-a), water velocity and deposited sediments as response variables. P-values, F-values and degrees of freedom (d.f.) were obtained by ANOVA. Significant P-values of main and interaction effects are shown in bold. The sign of the coefficient is indicated when the source of the variation is significant.

Variable		d.f.	F-value	P-value	Sign of coef.
Water velocity	Discharge	1, 22	254.22	**<0.001**	+
	Sediments	1, 4	4.08	0.113	
	Discharge × Sediments	1, 22	0.24	0.629	
Deposited sediments	Discharge	1, 4	401.11	**<0.001**	+
	Sediments	1, 22	8.15	**0.009**	+
	Discharge × Sediments	1, 22	60.39	**<0.001**	+
Chl-a	Discharge	1, 22	3.79	0.064	
	Sediments	1, 4	11.97	**0.026**	+
	Discharge × Sediments	1, 22	6.67	**0.017**	+
GPP	Discharge	1, 22	0.21	0.647	
	Sediments	1, 4	43.40	**0.003**	+
	Discharge × Sediments	1, 22	0.85	0.366	
CR	Discharge	1, 22	2.31	0.143	
	Sediments	1, 4	20.26	**0.011**	+
	Discharge × Sediments	1, 22	0.21	0.647	
NCM	Discharge	1, 22	1.09	0.306	
	Sediments	1, 4	40.93	**0.003**	+
	Discharge × Sediments	1, 22	0.47	0.499	
GPP/Chl-a	Discharge	1, 22	1.65	0.211	
	Sediments	1, 4	0.85	0.408	
	Discharge × Sediments	1, 22	5.90	**0.023**	-

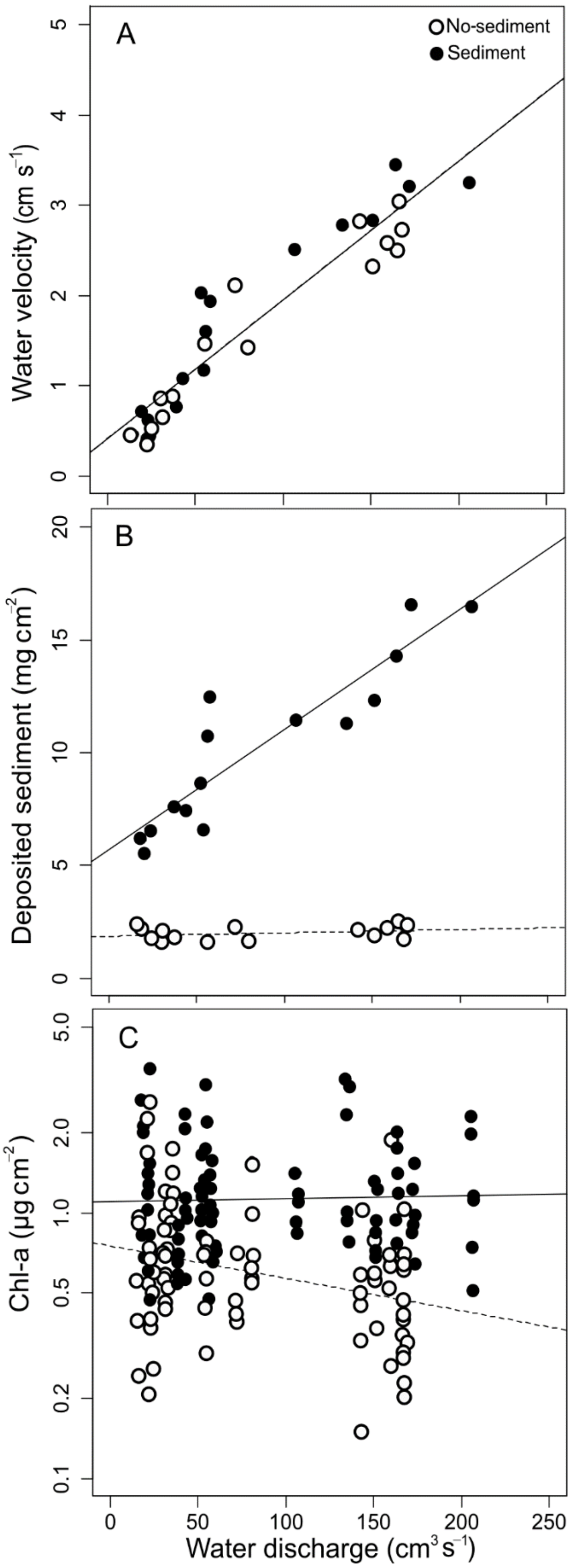

Figure 2. (A) Relationship between water discharge and velocity, (B) amount of deposited sediments in the channels and (C) chlorophyll-a concentration on tiles. Filled and empty dots correspond to channels with and without added sediments, respectively. Continuous and broken trend lines are built with the LMEM coefficients for channels with and without added sediments, respectively. When the interaction term is not significant a single line is shown. Note that in panels A and B each dot corresponds to a channel, and in panel C to a single tile.

Hydraulics and sediments

Water discharge correlated with water velocity (Table 2), which ranged from 0.5 to 3.0 cm s^{-1} (Fig 2A). Sediments and discharge had a significant effect on deposited sediments (Table 2, Fig 2B). The amount of sediments deposited in the sediment treatment (6.1 to 16.6 mg cm^{-2}) was higher than in the no-sediment treatment (1.7 to 2.3 mg cm^{-2}). Discharge affected sediment deposition only in the sediment treatment. Sediments deposited increased with discharge, as a consequence of higher mass of sediments entering the channels, since all channels in a block received the same concentration but different mass of sediments.

Biofilm

For the relationship between chl-a and discharge the LMEM showed a significant change of slope from the no-sediment to the sediment treatment (Table 2, p = 0.017). In the no-sediment treatment, chl-a concentration decreased significantly when water discharge increased, from 0.8 ± 0.3 µg cm^{-2} in the channels with lowest discharge to 0.5 ± 0.1 µg cm^{-2} in the channels with the highest discharge (Fig 2C). In the sediment treatment, on the other hand, chl-a concentration was higher (1.3 ± 0.1 µg cm^{-2}) and constant along the discharge range. These results indicate that the sediments promoted biofilm chl-a and counteracted the negative effects of high discharge (Fig 2, Table 2).

The biofilm metabolism metrics did not change with discharge but increased significantly with the addition of fine sediments (Table 2, Fig 3). GPP rose from 41.6 ± 6.3 mg O_2 $h^{-1}m^{-2}$ in the no-sediment treatments to 92.1 ± 11.9 mg O_2 h^{-1} m^{-2} in the sediment treatments, CR from 13.2 ± 2.0 to 19.2 ± 4.5 mg O_2 h^{-1} m^{-2}, and NCM from 28.4 ± 5.6 to 72.9 ± 12.2 mg O_2 h^{-1} m^{-2}. The interaction between water discharge and the addition of fine sediments was not statistically significant for any metabolism metric. The GPP/Chl-a ratio showed no significant main effects of discharge or sediments; however, the significant interaction between both factors indicated that in the absence of sediments, increasing discharge resulted in a higher metabolic efficiency (p = 0.023, Table 2, Fig 4).

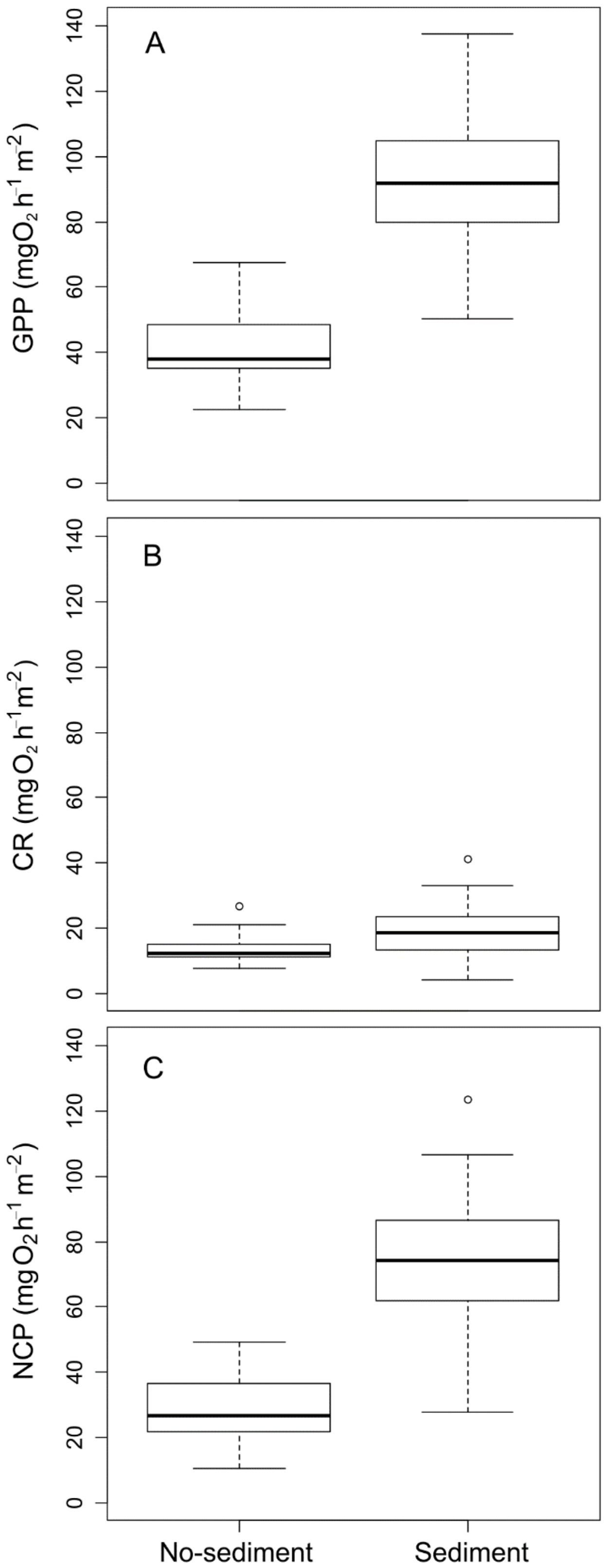

Figure 3. Differences in metabolism (A: Gross Primary Production, B: Community respiration and C: Net Community Metabolism) between channels with and without added sediments. The boxes display first and third quartiles, thick lines are medians, whiskers are range, and open circles are outliers.

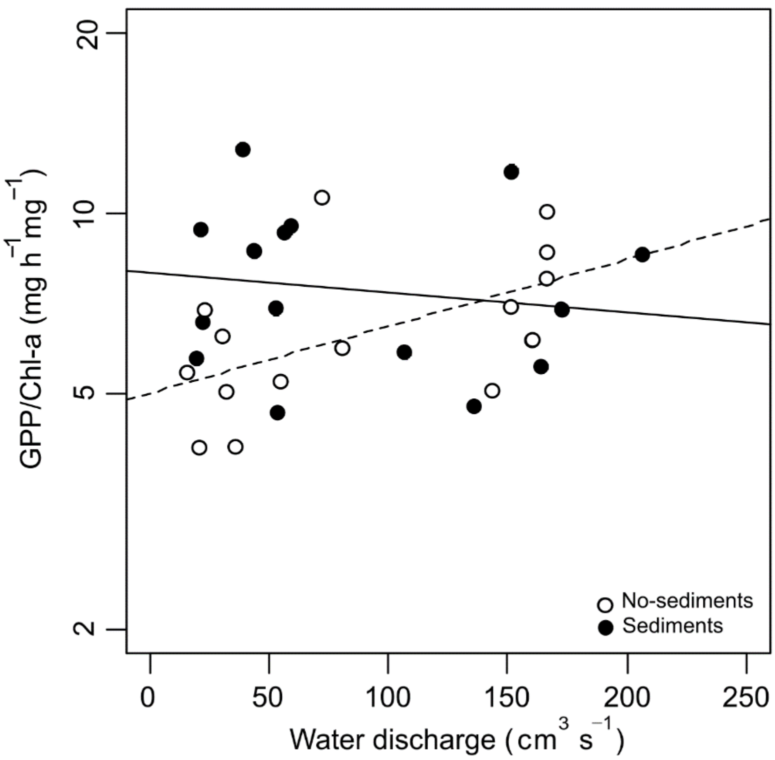

Figure 4. Gross primary production per unit biofilm biomass (GPP/Chl-a). Filled and empty dots correspond to channels with and without added sediments, respectively. The interaction between discharge and sediments is significant. Continuous and broken trend lines made with the LMEM coefficients for channels with and without added sediments, respectively.

DISCUSSION

Our experiment assessed the interactive effects of water discharge and fine sediments on biofilm metabolism. We expected both, discharge reduction and sediments, to exert negative individual effects, as well as an interaction effect of both stressors, but these predictions were not supported by our results. Contrary to our expectations, both discharge reduction and fine sediments promoted biofilm biomass, their interaction resulting in unchanged biomass across all discharge levels in the sediment treatments. On the other hand, metabolism was positively affected by fine sediments, but unaffected by discharge.

According to our first hypothesis, we expected discharge reduction to negatively affect biofilm biomass and metabolism because of limited nutrient exchange. On the contrary, we observed a weak increase in chl-a and no changes in metabolism metrics with varying water discharge. The literature shows contrasting effects of water discharge on biofilms. Some studies reported no response for algal biomass (Biggs & Hickey, 1994; Death et al., 2009; Baekkelie et al., 2017) as well as for metabolism (Biggs & Hickey, 1994; Arroita et al., 2017), whereas others showed that algal biomass decreased both above and below optimum velocities, a fact that would be explained by shear stress at high velocities, by nutrient limitation at low ones. This is the type of response reported by Biggs and Stokseth (Biggs & Stokseth, 1996) where the algal biomass peaked at a velocity of 30 cm s^{-1}. Similarly, in a flume experiment, Hondzo and Wang (Hondzo & Wang, 2002) reported that shear stress reduced biomass and photosynthetic activity above 15 cm s^{-1}, whereas Liu and Lau (Lau & Liu, 1993) reported optimum biofilm growth at 1.5 cm s^{-1}. The discrepancies among studies are large and probably caused by differences in experimental conditions. Our water velocities were in the lower range of those so far mentioned, with a range between 0.5 and 3 cm s^{-1}, but even so, we found an inverse relationship between chlorophyll and velocity. This effect could be explained by the fact that our biofilm was dominated by loose algal filaments, which are the growth forms dominant at low flow velocities (Biggs et al., 1998). The long and loose filaments in our experiment seemed especially prone to sloughing.

Our second hypothesis predicted that fine sediments would reduce biofilm biomass and metabolic activity, but we observed the opposite effect. The literature shows contrasting effects of fine sediments on biofilm. Several studies showed (Yamada & Nakamura, 2002; Matthaei et al., 2010; Aspray et al., 2017; Louhi et al., 2017) fine sediments to reduce biofilm biomass and metabolism, but some [65-66] reported increased biofilm, which was explained as a consequence of shifts in the dominant growth forms towards those (e.g., motile algae) more resistant to physical disturbance. We did not study algal composition of biofilms in our experimental channels, but unlike biomass, which showed clear differences between sediment and no-sediment treatments, by the end of the experiment we did not see any visual difference in the appearance of biofilm. Alternatively, the effects of sediments on biofilm could be caused by nutrients, as their leachates had high concentrations of N and P, important nutrients for algae (Perry & Stanford, 1982; Guasch et al., 1995; Aristi et al., 2016). This fertilisation effect would, nevertheless, not depend strictly on the amount of sediments deposited in each channel, since the sediments were added into the block tank and dissolved nutrients from leachates would be distributed across all the channels with the same concentration. The leaching of nutrients will of course depend on the type of sediments. The one used in our experiment, coming from a reservoir, could be unusually high in nutrients, but many other sediments also will act as fertilisers, as they are often linked to agricultural practices (Wood & Armitage, 1997; Syvitski et al., 2005).

Our third hypothesis predicted algal biomass and metabolism to be lowest in the sediment treatments with lowest discharge. However, although our results showed a significant interaction, it consisted of sediments eliminating the effect of discharge on biomass. This response is consistent with a fertilizing effect that is stronger than the sloughing effect, at least under the experimental conditions. The effects of nutrients and flow velocity on algal biomass tend to interact in complex ways, flow velocity promoting turbulence and the diffusion of nutrients into biofilms (Horner & Welch, 1981), until shear stress increases so as to produce algal sloughing. In a recent study, Baattrup-Pedersen et al. (Baattrup-Pedersen et al., 2020) measured the metabolic and biomass response of periphytic biofilm to fine sediments, nutrients and discharge reduction and, although they found negative effects of sediments on chl-a concentration and GPP, they concluded that after 1 week, nutrient enrichment to some extent mitigated these negative effects . The metabolic efficiency for GPP showed a significant response to the interaction of discharge and sediments. At high discharge, it was similar in sediment and no-sediment treatments, whereas at low discharge it was higher in the sediment treatment. This difference could be probably explained by a greater stimulatory effect of sediment on GPP than on chl-a. At low discharges and sediment treatment, the positive effect of sediment would compensated any sediment-shading effect, whereas, with no-sediment treatment, the low efficiency with a high chl-a would result from self-shading of algal biomass (Guasch et al., 1995; Mori et al., 2017). Then, higher discharges would reduce biomass, but the remaining algae would be more efficient in absence of sediments. However, with higher sediment deposition the stimulatory effect would be overtaken by the sediment-shading effect that would reduce the metabolic efficiency (Mori et al., 2017) matching to the no-sediment metabolic efficiency.

In conclusion, discharge reduction and sediment inputs can have interactive effects on stream biofilm biomass and metabolism. Nonetheless, the direction and magnitude of the responses may be strongly site-specific and difficult to forecast, as they likely depend on the range of water velocities, on the composition of the fine sediments, as well as on the composition and biomass of benthic biofilms.

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DATA AVAILABILITY STATEMENT

The original data that support the findings of this study are openly available in Figshare (https://doi.org/10.1371/journal.pone.0246719.s001).

CONFLICT OF INTEREST

The authors have declared that no competing interests exist.

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GENERAL DISCUSSION

OVERVIEW OF THE MAIN RESULTS

In this dissertation, two observational field studies and a laboratory experiment were combined to address the effects of the interaction of water diversion with common stressors on stream ecosystem functioning.

The field observational study (Chapter 1) addressed how multiple hydropower schemes affect the transport and retention of water and nutrients across a river network. Most water and nutrients studied were diverted from river channels and re-routed through canals. Impoundments systematically retained nutrients. On the other hand, diversion canals were not passive conduits, and showed retention and release of these compounds, although in a less coherent way. These results show that the natural pattern of retention and release are severely altered in rivers subject to multiple diversions, thus affecting ecosystem functioning. The second observational field study (Chapter 2) assessed the interaction between water diversion and urban pollution on organic matter processing in both wet channels and in dry riverbeds. Stressor interactions in the wet channel reduced microbial decomposition and did not affect CO_2 fluxes, whereas in the dry riverbed, the interaction reduced total and microbial decomposition and CO_2 fluxes. As result, organic matter processing was more affected by stressor interactions in dry riverbeds than in the wet channel. Therefore, the impacts on dry riverbeds should be taken into account to assess and manage the impacts of human activities on river ecosystems. Finally, the laboratory experiment (Chapter 3) analyzed the interactive effect between discharge reduction, a common consequence of water diversion, and fine sediment deposition on biofilm biomass and metabolism. Biofilm biomass was promoted by the interaction, contrary to metabolism. Nevertheless, others factors such as the type of fine sediment, the water velocity or the composition of sediments could affect the direction and magnitude of responses, thus making it difficult to generalize their effect on ecosystem functioning.

BEYOND THE RESULTS

Water diversion is a prevalent human activity (Nilsson et al., 2005) with an intensive plan of development in next years (Schwarz, 2019). Besides, hydromorphological alterations and pollution are the most common stressors in European rivers (EEA,2012). As almost half of European streams and rivers are simultaneously impacted by multiple stressors (Schinegger et al., 2012), the interaction of water diversion with other stressors is most probably globally widespread.

Nutrient cycling in streams is driven by hydraulics and biological activity (Battin et al., 2008), and consequently, also affected by water diversion (Arroita et al., 2017; von Schiller et al., 2016). Nutrient retention is the process by which dissolved nutrients are removed from the water column and immobilized by abiotic and biotic processes (Allan & Castillo, 2007; Newbold, 1992), reducing the load of nutrients transported downstream (Schlesinger & Bernhardt, 2013). Our results (Chapter 1) showed a high re-routing of nutrients through diversion canals, which were biogeochemically active, and a high retention in impoundments, thus reducing their availability in bypassed reaches. The fact that diversion canals showed retention and release of nutrients shows that they are geochemically active, rather than mere conduits. Nevertheless, canals also showed contrasting behavior, which makes it difficult to generalize the global effects of water diversion on the biogeochemistry of entire river basins. The reasons behind their contrasting behavior are unknown to us, but might be caused by differences in the amount of mosses and other primary producers, which can be locally abundant in diversion canals (Izagirre et al., 2013). From a biological perspective, even being biogeochemically active, canals cannot replace rivers, which have vastly more heterogeneous habitats, which results in complex processes and diverse biota. Apart from physically and biologically simple, diversion canals can act as mortal traps for fishes (Moyle & Israel, 2011), as well as for many other vertebrates, including the Pyrenean desman (MAGRAMA, 2013), and even terrestrial fauna such as wild ungulates and vultures (Elosegi, 2010).

In forested rivers allochthonous organic matter is the main source of energy, and litter decomposition and mineralization by microorganisms and macroinvertebrates play an important role in food webs and in the emission of carbon dioxide to the atmosphere (Marks, 2019; Marx et al., 2017; Wardle et al., 2004). Water diversion affects the storage organic matter and reduce its decomposition (Arroita et al., 2015; Death et al., 2009; Martínez et al., 2017). In our research regarding the interaction between water diversion and pollution (Chapter 2), the stock of organic matter increased in the wet channel but was reduced in dry riverbeds. Organic matter processing decreased with interaction, especially in dry riverbeds. Traditionally, dry riverbeds have been neglected from assessments of stream ecosystem functioning, as they are considered transitional habitats that differ from terrestrial or purely aquatic habitats in structure and biogeochemical dynamics (Larned et al., 2010; Steward et al., 2012). Nevertheless, dry riverbeds are biogeochemically active (Larned et al., 2007; McIntyre et al., 2009) and play a key role in carbon cycling (Datry et al., 2018; Keller et al., 2020). In our dry riverbeds the interaction between water diversion and urban pollution reduced OM processing, which could affect global carbon budgets. In the wet channels, changes in OM decomposition may cause profound shifts in the trophic structure as confirmed in a parallel study by de Guzman et al. (2021), who found that moderate pollution promotes food web complexity and water diversion amplifies this effect.

Biofilm is another important resource at the base of food webs (Peterson et al., 2001; Rowe & Richardson, 2001) and plays a key role in ecosystem processes in primary production, nutrient dynamics, organic matter decomposition and CO_2 emissions (Battin et al., 2016). The reduction of water discharge and consequently water velocity, reduces biofilm biomass (Arroita et al., 2017; Matthaei et al., 2010), enzymatic activity (Arroita et al., 2017) and affects metabolism (Hondzo & Wang, 2002; Munn & Brusven, 2004; Ponsatí et al., 2015). In our experiment (Chapter 3) where we tested the interaction between flow reduction and fine sediment deposition, the interaction of both stressors promoted chl-a but not metabolism. In other similar studies, GPP and CR decreased (Baattrup-Pedersen et al., 2020), biofilm chl-a increased (Neif et al., 2017) and the community composition changed (Baattrup-Pedersen et al., 2020; Neif et al., 2017). Doubtlessly, discharge reduction and fine sediment deposition affect biofilm, and even if the direction and magnitude of this effect is difficult to forecast, ecosystem processes could be threatened by their interaction.

ADDITIONAL CONSIDERATIONS

This dissertation combined field studies and a laboratory experiment to assess the interactions of water diversion with other stressors. Although the interactions are widespread, our results are necessarily limited in extent, as all rivers studied belong to a small geographical area and are under a specific climate. Therefore it is necessary to consider this caveat when trying to speculate about the global effects of water diversion and the future perspectives of the topic.

In the study of multiple diversion schemes (Chapter 1), two main limitations must be considered. First, the lack of replicate catchments makes ours a case study and makes it difficult to extrapolate our conclusions. Second, ours is just a snapshot of a situation that changes through time as a consequence of changes in river discharge and in the proportion of water diverted. However, in spite of these limitations, our study is among the few ones that tries to follow the transport and retention of compounds through diversion canals, impoundments and bypassed reaches of river network in a short time, trying to characterize what happens in a river affected by multiple diversion schemes.

In the observational study of the interaction between water diversion and pollution (Chapter 2), the gradient of pollution was characterized by means of a quality index based on chemical and biological variables (view appendix S1). Even so, urban pollution, can show large differences in composition, as there is a long list of potentially contaminant substances in urban wastewater, including nutrients, organic matter (Carey & Migliaccio, 2009), heavy metals (Deycard et al., 2014), pesticides, personal care products and drugs (Kuzmanović et al., 2015; Mandaric et al., 2018; Osorio et al., 2016). The composition and proportion of pollutants can differ depending on the catchment because of population density, presence of WWTP, the kind and the intensity of industrial activities, among others factors. In our research, the ranking based in the quality index used agrees with the percentage of urban land use in the catchment (Table 1, Chapter 2), but in other studies it is possible that changes in the mixture of pollutants affect the response of stream ecosystems.

In addition, the effect of urban pollution also depends on the dilution capacity of the receiving waters (Rice & Westerhoff, 2017), which changes with hydrological regime, depending of seasons (Petrovic et al., 2011) and climate (Martí et al., 2009). Here the location of the inputs of pollutants can be key, as if they enter the river in bypassed reaches their effects would be worse during low-flow periods. Besides, some diversion schemes also divert the whole discharge of tributaries (Izagirre et al., 2013), thus further reducing the dilution capacity, which would increase naturally along the bypassed reaches. This potential effect was not important in our study, as our bypassed reaches were immediately below the weirs. We wanted to study the downstream recovery of rivers, as tributaries join the main channel, but the bypassed reaches were not long enough for most of our rivers, thus making it impossible to check the recovery rate under a range of pollution.

Finally, in the laboratory experiment where we assessed the interactive effects of discharge reduction and fine sediment deposition (Chapter 3), the type of sediment tested might have played its role. Fine sediments in rivers can differ dramatically in quality, as they can be associated with intensive agriculture (Allan, 2004), deforestation (Naymik & Pan, 2005), urbanization (Taylor et al., 2004) and mining (Bruns, 2005). Consequently, sediments can be composed mostly of clay (Wood & Armitage, 1997), can be rich in organic matter (Aspray et al., 2017), or even in heavy metals (Tiwary, 2001), what could cause contrasting effects on biofilm biomass and functioning.

MANAGEMENT IMPLICATIONS AND FUTURE PERSPECTIVES

Our results showed pervasive effects of water diversion on river ecosystem functioning, as well as important interactions with others stressors. Moreover, it showed that the impacts also reached dry sediments, important riverine habitats.

Although local water agencies set a minimum environmental flow based on monthly mean discharge (URA & CHC, 2022), our results suggest that other stressors and parameters should also be considered to set environmental flows. First, we propose to adapt the estimation of environmental flows not only to each river or catchment, but also to their different segments, which can require a severe flow control in some sensitive reaches to protect their ecological status. Secondly, we would recommend to take into account the presence of other diversion schemes and their distance upstream and downstream from the river segments evaluated to set environmental flows. In a multiple regulated stream, an appropriate step would be to ensure a functional flow in the last diversion scheme in the lower stretch of the river and, depending on this flow, to determine the environmental flow of upstream segments. Lastly, the presence of other stressors, such as urban pollution or fine sediment deposition, should be taken into account to estimate environmental flows. The environmental flows calculated by local agencies, usually do not consider other impacts, and consequently, this flows can stress the ecosystem and reduce functionality of a segment that already is impacted. The ecological status index sums up information about hydromorphological, physicochemical and biological quality indexes for river segments, and can be an appropriate parameter to include in this estimation. Likely, rivers with a worst ecological status should set a greater environmental flow to help river ecosystem to mitigate and hold out better the impacts of the other stressors. Managers should start to address rivers conservation and restoration from the multiple stressors approach to be more efficient with their actions to protect running waters.

In this sense, more studies about the effects of multiple diversion schemes in small rivers and streams are necessary. There are studies of dam cascades in large rivers (Machado dos Santos et al., 2020; Rapin et al., 2020; Wang et al., 2018), but there is a gap of knowledge regarding small multiple diversion schemes. Low weirs are the most number of barriers in European rivers (Belletti et al., 2020), and consequently, in catchments is usual to find a high density of them. Our results concluded that transport and retention are affected by multiple diversion schemes, therefore, although there is research about impacts of low weirs from an isolated point of view, we see the need to address the impact of multiple diversions to understand better their cumulative effects on ecosystem functioning and biota.

Furthermore, during the last decades there has been increased a movement to remove dams in Europe and the US (Atristain et al., 2022; Foley et al., 2017; Habel et al., 2020) to eliminate obsolete or economically non-viable dams (Pohl, 2002) and thus, restoring river ecosystems. Most of the dams decommissioned are small dams (< 10 m height) (Foley et al., 2017) and the ecological impacts of removal have been minor, whereas the potential gains are very large (Tullos et al., 2014). Therefore, obsolete low weirs should be remove preferentially in rivers with a high conservation status to keep their conditions, against rivers with other significant pressures, where the prospects for improvement would be more limited.

In spite of this effort to restoring rivers connectivity with dam removal, population growth has led to increasing demand for electricity. In Europe, there are plans for intensive development of small hydropower schemes, especially in regions such as the Balkans, the Eastern Mediterranean, Norway and the Alpine Arc (Schwarz, 2019). Out of Europe, Brazil is an example of country that have enacted policies to promote small hydropower plants with a high proliferation in the last decade (Oliveira et al., 2020), and China, which accounts for almost 54% of small hydropower facilities in the world, is developing an important program promoting rural electrification to achieve in 2030 the 77% of its potential small hydropower development (Liu et al., 2019).

Wrapping up, human society depends on water provided by river ecosystems, and electricity demands in the coming years it will increase. Therefore, the trend of this intensive small hydropower development and their impacts will rise on running waters. Water diversion and its interaction with others stressors impairs streams ecosystem functioning. Thus, it is key to understand these impacts to implement a suitable exploitation activity that meet energy demands and enhance environment conservation.

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GENERAL CONCLUSIONS

1. Multiple diversion schemes affect transport of water and suspended compounds, recirculating mostly through canals instead of river channels, thus affecting ecosystem processes in by-passed reaches.

2. Impoundments above diversion dams are key points for the retention of particulate and dissolved nutrients in this multiple regulated river.

3. The interaction between water diversion and pollution affects OM stock and decomposition in the wet channel, whereas in dry riverbeds OM stock, decomposition and CO_2 fluxes show antagonistic responses. These results may alter the trophic structure and energetic balance of the stream ecosystems.

4. Dry riverbeds are an active part of rivers affected by diversion and urban pollution interaction, which emphasizes the importance of including them when assessing river ecosystems to avoid underestimate the effect of multiple stressors in river habitats.

5. Water diversion and fine sediment interaction have interactive effects on biofilm biomass and metabolism. Nonetheless, the magnitude and direction of the response may be strongly site-specific and dependent of other factors, such as water velocity, sediment composition or biofilm community.

6. These results add to the existing knowledge on the effects on ecosystem functioning of water diversion by small hydropower schemes and its interaction with other common stressors. The difficulties to forecast the effects of interactions among stressors remain, but the importance to take into account dry riverbeds has been made clear.

CONCLUSIONES GENERALES

1. Los múltiples esquemas de derivación afectan al transporte del agua y de los compuestos suspendidos en ella, siendo recirculados mayormente a través de los canales en lugar de circular por el cauce del río, y por lo tanto afectando así al funcionamiento del ecosistema.

2. Los embalses de las presas de derivación son puntos clave en la retención de nutrientes disueltos y particulados en los ríos afectados por múltiples derivaciones.

3. La interacción entre la derivación de agua y la contaminación afecta el stock de materia orgánica y su descomposición en el cauce del río, mientras que en el lecho seco el stock de materia orgánica, su descomposición y los flujos de CO2 muestran respuestas antagonistas. Estos resultados pueden alterar la estructura y el balance energético de los ecosistemas fluviales.

4. Los lechos secos son una parte active de los ríos afectados por la interacción entre la derivación de agua y la contaminación urbana, lo cual remarca la importancia de incluirlos cuando se evalúen los ecosistemas fluviales para evitar subestimar el efecto de los estresores múltiples.

5. La interacción entre la derivación de agua y los sedimentos finos tienen efectos interactivos en la biomasa del biofilm y su metabolismo. No obstante, la magnitud y la dirección de la respuesta puede ser muy específica del lugar y dependiente de otros factores, como la velocidad del agua, la composición del sedimento y la comunidad de biofilm.

6. Estos resultados se suman a los conocimientos existentes sobre los efectos en el funcionamiento ecosistémico de la derivación de agua por pequeñas centrales hidroeléctricas y su interacción con otros estresores habituales. Las dificultades para prever los efectos de las interacciones entre estresores siguen estando presentes, pero ha quedado patente la importancia de tener en cuenta el lecho seco de los ríos.

ONDORIO OROKORRAK

1. Deribazio-eskema anitzek uraren eta bertan esekitako konposatuen garraioari eragiten diote; hauek batez ere kanalen bidez birzirkulatzen dira, ibaiaren ibilgutik zirkulatu beharrean, eta, beraz, ekosistemaren funtzionamenduari eragiten diote.

2. . Deribazio-presetako urtegiak funtsezko puntuak dira desbideratze anitzen eraginpeko ibaietan disolbatutako eta partikulatutako mantenugaiak atxikitzeko.

3. Ur-deribazioaren eta kutsaduraren arteko elkarrekintzak materia organikoaren metaketari eta deskonposizioari eragiten die; ibilgu lehorrean, berriz, materia organikoaren metaketak, haren deskonposizioak eta CO2 fluxuek erantzun antagonistak erakusten dituzte. Emaitza horiek ibai-ekosistemen egitura eta energia-balantzea alda dezakete.
4. Ur-deribazioen eta hiri-kutsaduraren arteko elkarrekintzak eragiten dien ibaien zati aktiboa dira ibilgu lehorrak, eta horrek nabarmentzen du oso garrantzitsua dela ibai-ekosistemak ebaluatzen direnean zatiok sartzea, estresatzaile anizkoitzen eragina gutxiestea saihesteko.

5. Uraren deribazioak eta sedimentu finen deposizioak eragin elkarreragileak dituzte biofilmaren biomasan eta metabolismoan. Hala ere, erantzunaren tamaina eta norabidea lekuaren oso espezifikoa izan daiteke, eta beste faktore batzuen mende egon daiteke, hala nola uraren abiadura, sedimentuaren konposizioa eta biofilm komunitatea.

6. Emaitza horiei gehitu behar zaizkie zentral hidroelektriko txikien bidezko ur-deribazioak funtzionamendu ekosistemikoan dituen ondorioei eta ohiko beste estresatzaile batzuekiko elkarreraginari buruzko ezagutzari. Estresatzaileen arteko interakzioen ondorioak aurreikusteko zailtasunak daude oraindik, baina ibaien ibilgu lehorra kontuan hartzearen garrantzia agerian geratu da.

SUPPLEMENTARY MATERIAL

Chapter 1: Re-routing of water and nutrients across a catchment as a consequence of multiple hydropower diversion schemes

Tables

Table S1. Characteristics of diversion schemes studied in the Leitzaran river network.

Diversion Scheme	Impoundment coordinates (X, Y)	Weir height (m)	Weir width (m)	Canal length (m)	Canal width (m)	Bypassed reach (m)	Impoundment length (m)	Turbine power (kW)
Plazaola	43.109374, -1.938013	3.1	45	2250	2.0	4200	200	736
Mustar	43.132528, -1.939278	4.0	40	1400	2.0	4700	150	1100
Iberdrola	43.139336, -1.952148	2.1	32	13000	2.0	15300	220	3600
Bertxin	43.176583, -1.986361	6.0	45	3700	1.5	3200	475	808

Table S2. Physicochemical parameters (mean ± SE) for canals, river reaches and tributaries.

Parameters	Canals	River Reaches	Tributaries
T (°C)	11.9 ± 0.3 [a]	12.2 ± 0.3 [a]	13.1 ± 0.1[b]
EC (µS cm^{-1})	179.3 ± 13.2 [a]	179.2 ± 11.6 [a]	75.0 ± 6.1 [b]
pH	7.8 ± 0.2 [a]	7.9 ± 0.1 [a]	7.4 ± 0.1 [b]
DO (%)	67.3 ± 1.2 [a]	68.8 ± 4.4 [a]	95.3 ± 1.8 [b]

Table S3. Linear model results for physicochemical parameters. P-value shown corresponds to ANOVA to test differences among canals, reaches and tributaries.

Parameters	N	F-val	P-val
T	64	8.2	<0.05
EC	64	1.2	<0.05
pH	64	1.3	<0.05
DO	64	33.2	<0.05

Table S4. Retention km^{-1} s^{-1} in each canal (C), impoundment (I) and river reach (R). Chl-a = Chlorophyll-a, TSS = Total Suspended Solids, POC = Particulate Organic Carbon, TPN = Total Particulate Nitrogen, TPP = Total Particulate Phosphorus, DOC = Dissolved Organic Carbon, TDN = Total Dissolved Nitrogen, TDP = Total Dissolved Phosphorus. Positive values indicate retention, negative values indicate release.

Variables	C1	C2	C3	C4	I1	I2	I3	I4	R1	R2	R3	R4
Chl-a (µg km^{-1} s^{-1})	-65.0	115.3	3.6	13.4	6993.4	7911.5	3455.8	786.8	-71.4	-180.1	-7.3	31.5
TSS (mg km^{-1} s^{-1})	1302.7	-888.0	-114.7	92.7	8545.6	24788.0	11633.9	802.5	-1808.9	964.9	-238.2	-528.4
POC (mg km^{-1} s^{-1})	-8.0	-146.8	-11.6	2.4	-175.5	3247.3	1029.4	-53.8	-217.2	13.5	-7.7	-4.5
TPN (mg km^{-1} s^{-1})	10.9	-11.5	-1.4	-0.7	-3.0	116.0	142.7	-5.1	-26.0	3.5	-1.9	-4.2
TPP (mg km^{-1} s^{-1})	2.98	-2.30	0.02	0.12	-25.5	38.6	0.2	3.2	-0.44	0.75	0.22	0.15
DOC (mg km^{-1} s^{-1})	-1290.4	547.2	-75.0	-308.2	1238.5	2060.4	-6936.0	7762.9	217.8	73.4	84.9	9.2
TDN (mg km^{-1} s^{-1})	-51.9	-127.2	1.0	5.4	901.7	958.5	-2041.3	-245.3	-10.7	34.3	-6.8	21.8
TDP (mg km^{-1} s^{-1})	-1.55	-2.11	0.23	0.37	-97.5	103.4	50.8	10.0	-11.30	1.09	-0.39	-0.002

Table S5. Linear model with permutation test results for retention $km^{-1}\ s^{-1}$. Chl-a = Chlorophyll-a, TSS = Total Suspended Solids, POC = Particulate Organic Carbon, TPN = Total Particulate Nitrogen, TPP = Total Particulate Phosphorus, DOC = Dissolved Organic Carbon, TDN = Total Dissolved Nitrogen, TDP = Total Dissolved Phosphorus. P-values were obtained by ANOVA. Significant p-values are shown with an asterisk (*). N is given as the number of data used.

Variables	N	p-value
Chl-a	12	0.01*
TSS	12	0.03*
POC	12	0.14
TPN	12	0.17
TPP	12	0.97
DOC	12	0.68
TDN	12	0.91
TDP	12	0.72

Chapter 2: Organic matter processing on dry riverbeds is more reactive to water diversion and pollution than wet channel.

Tables

Table S1. Detail of variables (mean ± SE) measured during 2016 in each river to calculate the General Quality Index (GQI). EC = Electrical conductivity, DO sat. = dissolved oxygen saturation, SS = suspended solids, BOD_5 = biological oxygen demand, COD = chemical oxygen demand, NO_3^- = nitrates, PO_4^{3-} = phosphates, Ca^{2+} = calcium, Cl^- = chloride, Mg^{2+} = magnesium, Na^+ = sodium, SO_4^{2-} = sulfate, Total CN^- = total cyanide, Cd^{2+} = cadmium, Cu^+ = copper, Hg^+ = mercury, Pb^{2+} = lead and Zn^{2+} = zinc. Data extracted from URA visor UBEGI (www.uragentzia.euskadi.eus).

Variables	Urumea	Leitzaran	Kadagua	Deba
EC (µS cm^{-1})	84.7 ± 5.1	153.6 ± 6.1	509.9 ± 73.7	402.8 ± 39.3
pH	7.6 ± 0.1	8.1 ± 0.1	8.4 ± 0.1	8.6 ± 0.1
OD sat. (%)	102.6 ± 0.5	102.4 ± 0.7	108.9 ± 1.7	108.5 ± 2.0
SS (mg L^{-1})	2.4 ± 0.3	3.9 ± 0.5	8.1 ± 1.8	77.6 ± 74.6
BOD_5 (mg L^{-1})	2.0 ± 0.0	2.0 ± 0.0	2.3 ± 0.3	3.5 ± 1.2
COD (mg L^{-1})	5.1 ± 0.1	5.3 ± 0.2	8.6 ± 0.7	20.9 ± 12.9
Total coliforms (UFC 100 mL^{-1})	755.0 ± 242.5	542.5 ± 125.5	5387.5 ± 1234.1	16900.0 ± 11914.0
Phenols (mg L^{-1})	0.0 ±0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NO_3^- (mg N L^{-1})	0.44 ± 0.06	0.77 ± 0.12	1.14 ± 0.16	1.3 ± 0.2
PO_4^{3-} (mg P L^{-1})	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.06 ± 0.02
Ca^{2+} (mg L^{-1})	9.9 ± 1.3	32.8 ± 3.7	114.3 ± 8.6	73.1 ± 5.8
Cl^- (mg L^{-1})	10.0 ± 0.0	10.0 ± 0.0	34.2 ± 5.6	33.3 ± 7.6
Mg^{2+} (mg L^{-1})	1.5 ± 0.1	2.9 ± 0.4	12.2 ± 1.6	6.1 ± 0.4
Na^+ (mg L^{-1})	4.9 ± 0.3	6.1 ± 0.7	26.8 ± 4.8	26.0 ± 5.6
SO_4^{2-} (mg S L^{-1})	3.3 ± 0.0	3.3 ± 0.00	32.9 ± 5.7	17.4 ± 1.5
Total CN^- (µg L^{-1})	5.0 ± 0.0	1.5 ± 0.0	5.0 ± 0.0	5.0 ± 0.0
Cd^{2+} (µg L^{-1})	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0
Cr VI (µg L^{-1})	1.0 ± 0.0	5.0 ± 0.0	1.0 ± 0.0	5.8 ± 4.4
Cu^+ (µg L^{-1})	1.5 ± 0.0	1.0 ± 0.0	1.5 ± 0.0	1.5 ± 0.0
Hg^+ (µg L^{-1})	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.0	0.0 ± 0.0
Pb^{2+} (µg L^{-1})	1.0 ± 0.0	1.0 ± 0.0	0.0 ± 0.0	4.2 ± 3.2
Zn^{2+} (µg L^{-1})	16.0 ± 1.5	6.6 ± 0.8	6.7 ± 1.4	55.5 ± 42.1

S1. Appendix: General Quality Index (GQI) description. The GQI (Mingo, 1981; URA, 2004) is computed with values of 23 variables: dissolved oxygen, suspended solids, pH, electrical conductivity, OBD_5, OCD, total coliforms, phosphate, nitrate, calcium, magnesium, sodium, chloride, sulfate, total cyanide, phenols, cadmium, copper, chrome (VI), mercury, lead and zinc. Specifically, the value of the index is calculated as follows: $GQI = \sum(Q_j \times P_j)$, where Q_j is a dimensionless transformation for each variable (*j*) measured and P_j is the relative weight of the variable *(j)* in the index. The relative weight coefficients for each variables are as follows:

Weight coefficients	*Variables*
1	Dissolved oxygen, suspended solids, pH, electrical conductivity, OBD_5, total coliforms, total cyanide, phenols, cadmium, chrome (VI) and mercury
2	Chloride, sulfate, copper, lead and zinc
3	OCD, phosphate, nitrate and calcium
4	Magnesium and sodium

Depending on the QGI value, freshwaters are classified as follows:

Numeric value GQI	*Classification*
100-90	Excellent
90-80	Good
80-70	Intermediate
70-60	Acceptable
60-0	Unacceptable

References

Mingo, J. (1981). *La Vigilancia de la Contaminación Fluvial. Dirección General de Obras Hidráulicas (MOPU).*

URA. (2004). *Red de seguimiento del estado ecológico de los ríos de la CAPV. Metodologías utilizadas. Tomo 1.* (p. 84). Departamento de Ordenación del Territorio y Medio Ambiente, Gobierno Vasco.

Table S2. Water characteristics for the studied periods, rivers and reaches. EC = electrical conductivity, T = temperature, DO = dissolved oxygen, NO_3^- = nitrate, NH_4^+ = ammonium, TDN = total dissolved nitrogen, SRP = soluble reactive phosphorus, DOC = dissolved organic carbon, Cl = chloride, SO_4^{-2} = sulfate. Wet width values shown are mean ± SE.

Period	Inactive								Active							
River	Urumea		Leitzaran		Kadagua		Deba		Urumea		Leitzaran		Kadagua		Deba	
Reach	Control	Regulated	Control	Regulated	Control	Regulated	Control	Regulated	Control	Regulated	Control	Regulated	Control	Regulated	Control	Regulated
pH	7.8	7.5	8.2	8.1	8.2	8.2	7.8	8.1	7.5	7.4	8.2	8.2	7.9	8.2	8.4	8.2
EC (µS cm^{-1})	85.4	85.9	281	281	613	632	640	625	75	75	228	229	634	635	506	485
T (°C)	17.2	16.9	19.3	19.8	21.3	21.2	18.9	18.6	13.9	15.0	12.2	12.2	13.2	13.3	18.4	17.8
DO (mg L^{-1})	10.0	9.6	9.0	9.3	8.6	8.9	10.4	9.5	10.5	10.6	10.3	10.4	9.5	10.5	10.9	9.4
DO (%)	103.6	99.9	98.4	102.9	97.1	100.4	113.6	103.6	102.7	103.8	95.1	100.6	96.9	107.1	116.3	99.1
NO_3^- (mg N L^{-1})	0.12	0.14	0.58	0.44	0.22	0.20	0.34	0.51	0.32	0.30	0.52	1.06	0.17	0.17	0.64	0.56
NH_4^+ (mg N L^{-1})	0.01	0.02	0.01	0.01	0.03	0.03	0.03	0.03	0.01	0.01	0.01	0.01	0.05	0.04	0.02	0.01
TDN (mg N L^{-1})	0.8	0.8	1.3	1.4	1.3	1.6	2.5	2.6	1.0	1.0	1.0	1.5	1.7	1.6	1.5	1.5
SRP (mg P L^{-1})	0.01	0.01	0.02	0.02	0.04	0.04	0.07	0.08	0.01	0.01	0.06	0.05	0.05	0.04	0.03	0.02
DOC (mg C L^{-1})	2.1	2.6	3.7	4.7	6.7	8.4	7.2	6.6	2.0	1.5	3.3	3.8	7.1	6.3	3.9	6.1
Cl^- (mg L^{-1})	1.8	2.2	5.5	4.3	11.3	9.6	9.7	15.0	3.7	3.3	6.2	12.4	6.8	6.0	16.1	12.9
SO_4^{-2} (mg L^{-1})	1.0	1.2	5.3	4.0	31.3	20.9	13.2	14.8	2.5	2.4	8.5	13.5	19.6	18.3	31.8	31.4
Discharge (m^3 s^{-1})	2.0	2.3	0.5	0.2	1.2	0.9	0.4	0.7	5.9	1.5	2.0	0.2	1.5	0.9	2.6	0.9
Velocity (m s^{-1})	0.4	0.3	0.4	0.1	0.1	0.3	0.3	0.2	0.6	0.4	0.2	0.1	0.3	0.3	0.3	0.2
Wet width (%)	82.3 ± 3.7	85.6 ± 3.3	77.6 ± 3.2	74.1 ± 4.5	88.7 ± 4.7	59.6 ± 3.4	50.1 ± 1.4	73.0 ± 4.7	92.3 ± 1.9	72.5 ± 7.2	91.0 ± 2.3	75.1 ± 4.8	66.8 ± 4.8	46.8 ± 3.1	67.9 ± 0.1	50.0 ± 0.0

Table S3. Wet channel variables for each reach along pollution gradient during active and inactive periods. CBOM = coarse benthic organic matter, FBOM = fine benthic organic matter, $pCO_{2,w}$ = CO_2 partial pressure in water, kCO_2 = CO_2 reaeration coefficient and $FCO_{2,w}$ = CO_2 flux between water and atmosphere. Values shown are mean ± SE.

Period	Inactive								Active							
River	Urumea		Leitzaran		Kadagua		Deba		Urumea		Leitzaran		Kadagua		Deba	
Reach	Control	Regulated	Control	Regulated	Control	Regulated	Control	Regulated	Control	Regulated	Control	Regulated	Control	Regulated	Control	Regulated
CBOM ($g\ m^{-2}$)	8.2 ± 3.3	41.0 ± 27.5	18.6 ± 4.8	13.4 ± 3.7	0.8 ± 0.4	0.5 ± 0.1	19.4 ± 7.9	8.6 ± 4.3	16.6 ± 2.5	2.7 ± 0.9	26.4 ± 4.2	10.1 ± 3.1	2.9 ± 0.9	1.2 ± 0.4	30.0 ± 4.7	9.3 ± 2.6
FBOM ($g\ m^{-2}$)	17.1 ± 3.4	32.9 ± 4.1	51.7 ± 7.7	50.0 ± 7.6	29.9 ± 6.6	88.5 ± 31.7	25.8 ± 4.5	34.3 ± 7.0	30.7 ± 3.9	13.1 ± 2.2	40.2 ± 10.2	35.2 ± 7.1	19.9 ± 3.6	58.9 ± 17.3	83.9 ± 30.8	63.6 ± 15.3
Total dec. ($d\ d^{-1}$)	0.0016 ± 0.0003	0.0017 ± 0.0002	0.0024 ± 0.0004	0.0020 ± 0.0004	0.0015 ± 0.0001	0.0024 ± 0.0004	0.0013 ± 0.0003	0.0012 ± 0.0001	0.0013 ± 0.0004	0.0022 ± 0.0004	0.0026 ± 0.0005	0.0015 ± 0.0002	0.0013 ± 0.0002	0.0016 ± 0.0001	0.0019 ± 0.0006	0.0014 ± 0.0003
Microb. dec. ($d\ d^{-1}$)	0.0009 ± 0.0001	0.0016 ± 0.0004	0.0011 ± 0.0001	0.0011 ± 0.0001	0.0010 ± 0.0001	0.0012 ± 0.0001	0.0012 ± 0.0001	0.0010 ± 0.0001	0.0012 ± 0.0001	0.0010 ± 0.0001	0.0010 ± 0.0001	0.0011 ± 0.0001	0.0010 ± 0.0001	0.0014 ± 0.0001	0.0018 ± 0.0004	0.0010 ± 0.0001
Frag. ($d\ d^{-1}$)	0.0005 ± 0.0001	0.0001 ± 0.0001	0.0008 ± 0.0002	0.0006 ± 0.0001	0.0003 ± 0.0000	0.0006 ± 0.0002	0.0003 ± 0.0002	0.0002 ± 0.0000	0.0006 ± 0.0002	0.0007 ± 0.0004	0.0009 ± 0.0003	0.0002 ± 0.0001	0.0002 ± 0.0001	0.0001 ± 0.0000	0.0004 ± 0.0004	0.0002 ± 0.0001
$pCO_{2,w}$ (μatm)	392 ± 15	684 ± 7	758 ± 4	770 ± 20	1118 ± 29	970 ± 15	1102 ± 16	912 ± 8	519 ± 2	477 ± 3	549 ± 6	573 ± 4	768 ± 5	459 ± 4	670 ± 12	873 ± 4
kCO_2 ($m\ d^{-1}$)	24.2	19.2	15.7	44.2	23.0	11.0	8.7	16.5	25.4	14.2	18.5	29.3	25.9	12.9	6.0	38.8
$FCO_{2,w}$ ($mmol\ m^{-2}\ d^{-1}$)	-2.0 ± 3.0	94.4 ± 2.3	27.2 ± 0.4	115.5 ± 7.4	398.3 ± 16.2	20.3 ± 0.6	22.9 ± 0.6	65.7 ± 1.1	54.7 ± 0.8	13.2 ± 0.6	151.6 ± 6.0	88.5 ± 2.1	98.1 ± 1.5	6.2 ± 0.5	24.6 ± 1.2	246.3 ± 2.2

Figures

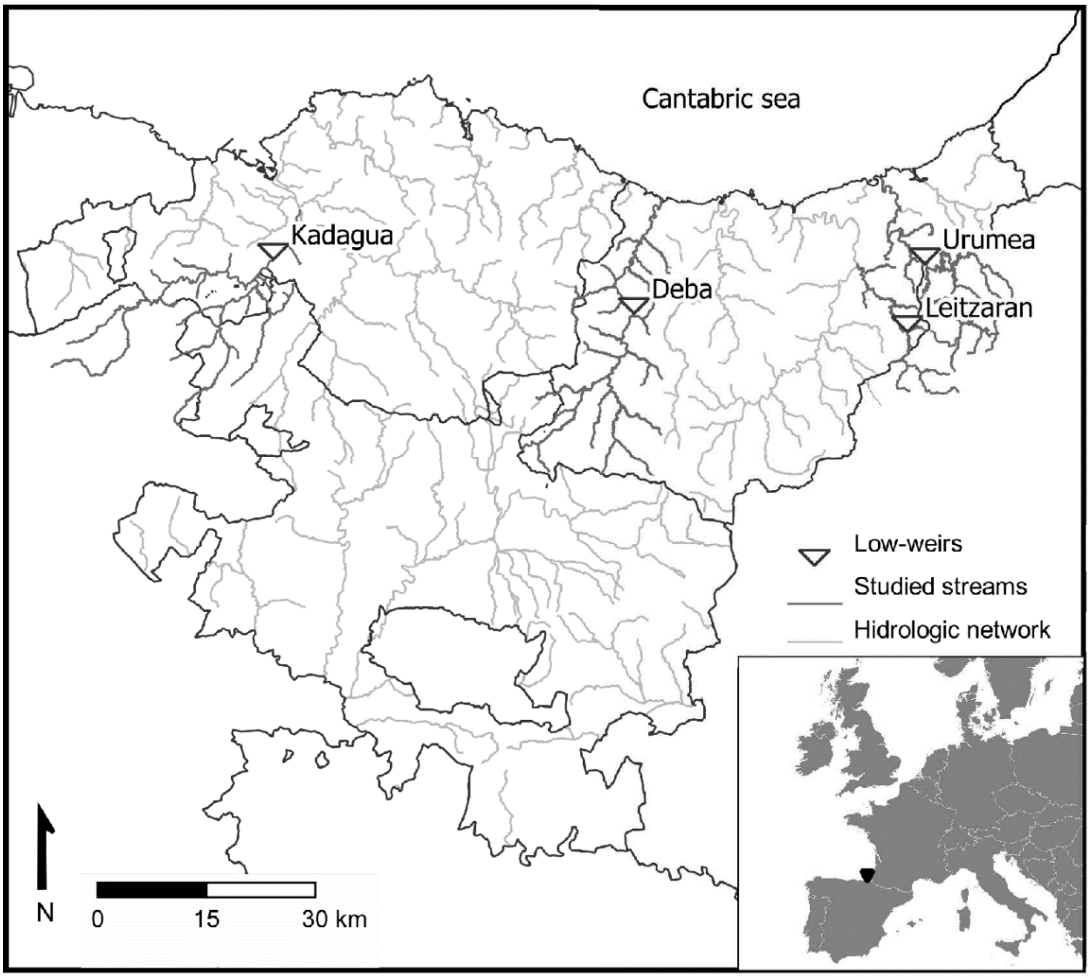

Figure S1. Low-weirs and rivers location. Inverted triangles point the location of low-weirs in each river.

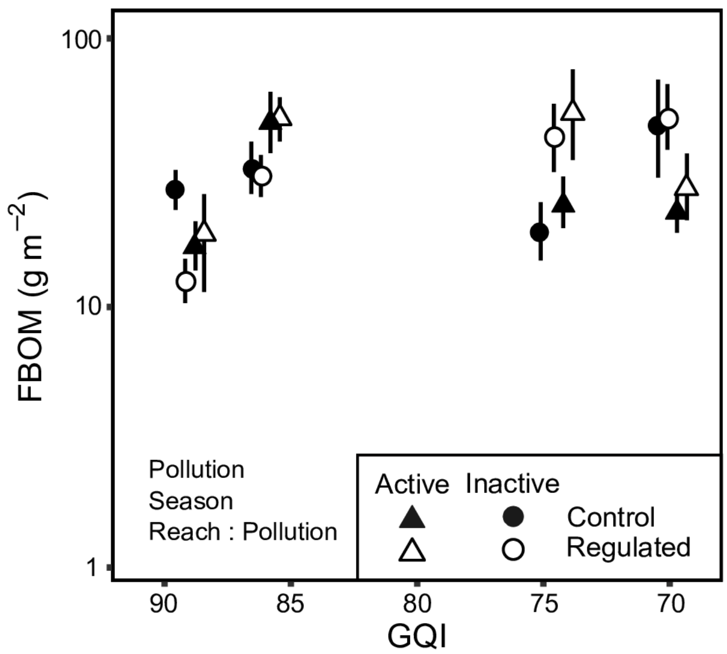

Figure S2. Stock of fine benthic organic matter (FBOM) in wet channel. Black and white colors distinguish between control and regulated reaches, respectively. Dots represent inactive and triangles active periods. Values are mean and error bars show standard error. Variable is $\log_{10}$ transformed. GQI values have been jittered to avoid overlapping among points. Text in background indicates significant single-factor effects or interactions. GQI scale is inverted to make easier the interpretation of the figure, ranging from good (low pollution) to acceptable quality level (moderate pollution).

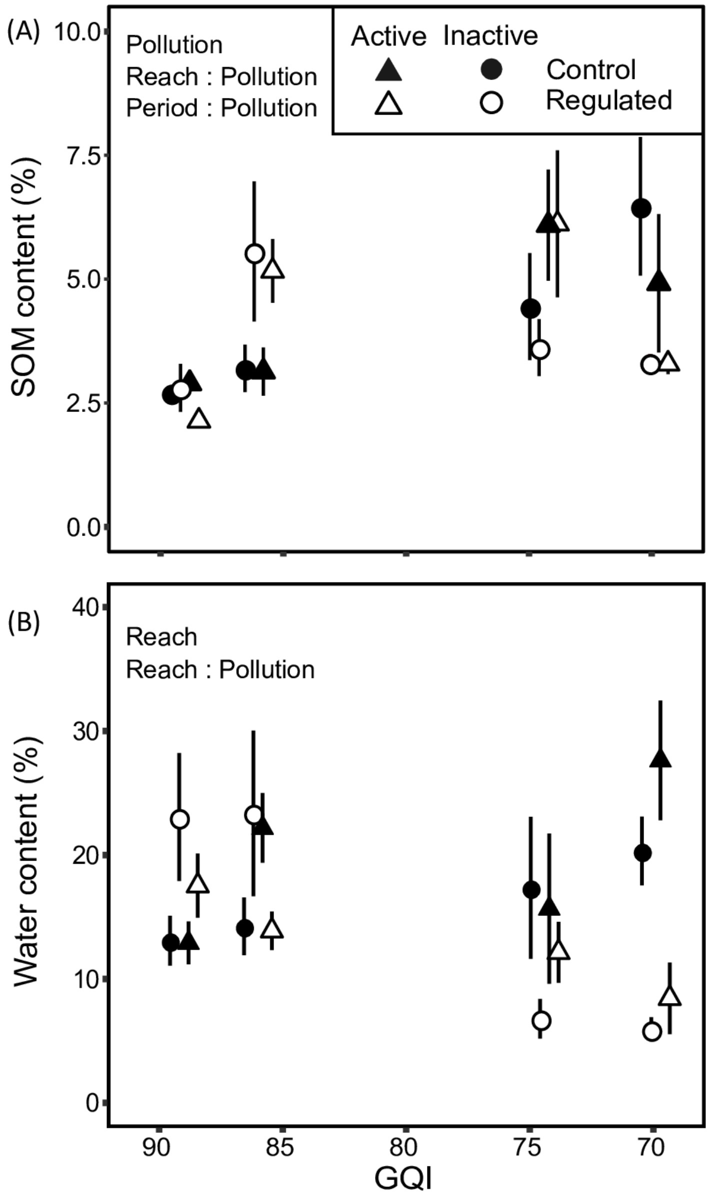

Figure S3. Sediment organic matter (SOM) content (A) and water content (B) in dry channel. Black and white colors distinguish between control and regulated reaches, respectively. Dots represent inactive and triangles active periods. Values are mean and error bars show standard error. Variable is $\log_{10}$ transformed. GQI values have been jittered to avoid overlapping among points. Text in background indicates significant single-factor effects or interactions. GQI scale is inverted to make easier the interpretation of the figure, ranging from good (low pollution) to acceptable quality level (moderate pollution).

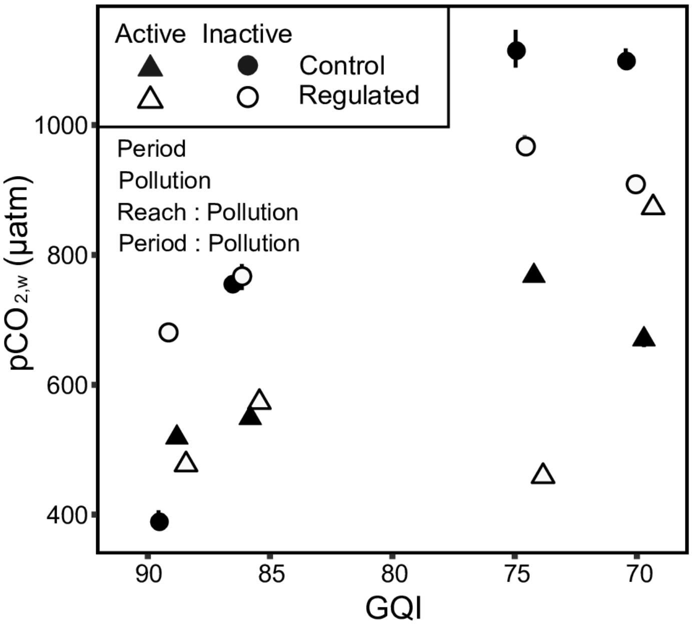

Figure S4. CO_2 partial pressure ($pCO_{2,w}$) in wet channel. Black and white colors distinguish between control and regulated reaches, respectively. Dots represent inactive and triangles active periods. Values are mean and error bars show standard error. Variable is log_{10} transformed. GQI values have been jittered to avoid overlapping among points. Text in background indicates significant single-factor effects or interactions. GQI scale is inverted to make easier the interpretation of the figure, ranging from good (low pollution) to acceptable quality level (moderate pollution).