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# EFFECTS OF HYDROLOGICAL INTERMITTENCY ON THE FUNCTIONING OF LOTIC ECOSYSTEMS


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# Thesis abstract

Over the past thirty years, the frequency and duration of droughts has increased dramatically across Europe, also in temperate regions causing perennial streams to shift to intermittency, modifying their abiotic and biotic benthic environment and impacting ecosystem processes. The hypotheses underlying this work concern the marked impact of water level fluctuations and hydrological intermittence on lotic ecosystem metabolism, processes and services. The ecological effects of flow intermittence on aquatic biodiversity are relatively well understood, but the effects on ecosystem processes and, ultimately, on ecosystem services is unclear and warrants greater attention. The aim of this study is to analyse the effects of hydrological intermittency on river ecosystem processes and functioning.

In the first chapter of this thesis I studied the effects of stream drying on the organic matter decomposition and nutrient turnover, that are key ecosystem processes in lotic ecosystem.

A microcosm experiment was conducted to investigate the interactive effect of water intermittency, macrofauna and leaf size on nutrient mineralization and recycling. Leaf disks (1 or 5 cm diameter) were incubated for 40 days with or without the leaf-consumer, *Potamophylax cingulatus* larvae (Trichoptera, Limnephilidae) and with or without an intervening, 10-days simulation of stream drying and subsequent rewetting. Nutrient fluxes, residual leaf biomass and leaf elemental composition were measured to evaluate how intermittency, macrofauna and leaf size affect organic matter mineralization rates and stoichiometry. Results suggest that drying slows decomposition rates, impacting both the microbial and setting to zero macrofauna activities. The presence of macrofauna increases mineralization and nutrient (C, N and P) regeneration rates. Findings also suggest that leaf disks with higher diameter display higher microbial activity and $NH_4^+$ regeneration. During the experiment, the C:N:P ratios of residual litter changed, as the leaf material became enriched with N and P.

Findings improve the knowledge about how stream processes are influenced by the interaction between physical, chemical and biological factors and how stream drought affects these interacting factors.

The second chapter of this PhD thesis is aimed to investigate the effects of sediments desiccation on microbial activities in hyporheic zone. Here I wanted to focus on how nutrient regeneration and microbial activity change in response to the hyporheic sediment desiccation and the implication for downstream river ecosystems. In addition, I expected that intermittent

and perennial microbial communities differ in their response to desiccation and in their capacity to recover after drying due to the adaptation to intermittent conditions.

In order to respond to these questions a flow-through experiment was carried out using mesocosms packed with sediments collected from 10 perennial and 10 intermittent streams in Austria and incubated for a total of 11 weeks under controlled conditions.

All mesocosms were exposed to three hydrological conditions: flowing phase; drying phase and rewetting phase. Overall, five samplings were carried out: after the flowing period and after the rewetting phase (1, 3, 7, 14 days after rewetting).

Oxygen respiration, denitrification and nutrient fluxes were measured by reactors inlet and outlet samplings. Results suggested that intermittency significantly affects aerobic metabolism rates and produce a rapid nutrient release immediately upon rewetting. No significant differences were found for intermittent and inundated systems, suggesting high plasticity of hyporheic communities. Thereafter, metabolic rates and nutrient processing returned to pre-desiccation values. Nitrate tended to be consumed in the hyporheic zone with high rates but not via denitrification. The latter was measurable, but rates were orders of magnitude lower than nitrate uptake and contributed little to the overall hyporheic metabolism.

Furthermore, results showed no differences between processes occurring in intermittent and perennial watercourses. This suggest that the maintenance of a certain degree of humidity within hyporheic sediments may facilitate both perennial and intermittent community recovery.

In the last chapter I moved the attention from natural to artificial aquatic environments. Human-made aquatic ecosystems are omni-present and their importance in regulating the functioning of downstream ecosystems is widely known.

The aim of the fourth chapter of this thesis was to analyse how hydrological intermittency affects sediment biogeochemistry, carbon metabolism and the trends of $CO_2$ emissions along water saturation gradients in artificial canals and their regulation by environmental factors. Agricultural canals are artificial but widespread elements of irrigated floodplains, regularly subjected to artificial wet and dry annual cycles linked to agricultural practices.

The aim of this study is to quantify $CO_2$ emission along water saturation gradients in sediments of agricultural canals within the secondary irrigation and drainage network of the Po river basin and to understand which factors are involved in $CO_2$ emissions regulation.

Carbon dioxide measurements were performed in five canals within the Po River basin (Northern Italy). In each canal, three sampling zones were selected (permanently exposed sediments, saturated sediments and an intermediate zone). Besides dark $CO_2$ flux

measurements, sediments were collected for sediment characterization and determination of net potential nitrification, an aerobic process that should be stimulated by drying, and denitrification rates, that should be stimulated under water saturation and anoxic conditions. I hypothesized an inverse correlation between water content and $CO_2$ emission, due to increasing oxygen penetration under desiccation and stimulation of mineralization processes. Results suggest site-specific regulation, depending on water and organic matter content and on microbial N transformations. $CO_2$ emissions tended to increase along the considered water saturation gradients, almost tripling rates from saturated (158.16 ± 24.05 mmol $CO_2$ $m^{-2}$ $d^{-1}$) to dry sediments (416.54 ± 78.87 mmol $CO_2$ $m^{-2}$ $d^{-1}$). Results also suggest that net potential nitrification and denitrification allow tracing the effects of drying on N microbial communities involved in $CO_2$ fluxes. Net potential nitrification produces little effects on $CO_2$ fluxes, but is a god proxy of oxygen availability, whereas potential denitrification may be responsible for variable fractions (up to 100%) or $CO_2$ production. Further studies are necessary to evaluate the importance of these emissions for local carbon budgets, due to the capillary development of irrigation canals in the Po River basin.

# General Introduction

The importance of freshwater to our life support system is widely recognised, as can be seen clearly in the international context (e.g., Agenda 21, World Water Fora, the Millennium Ecosystem Assessment and the World Water Development Report). Freshwater is indispensable for all forms of life and is needed, in large quantities, in almost all human activities.

Water is the most important resource essential for sustenance of life on earth and drive the economic development of human society. Despite only 3% of the total water on earth is available as fresh water and only <1% is in the form of surface water, water resources are overexploited and rapid changes in human lifestyle coupled with urbanization and industrialization has created pressure on the limited freshwater resources.

Lotic ecosystems occupy a minor fraction of the Earth surface (0.8%) and represent a small fraction of freshwaters (0.01%). Nevertheless, they host a disproportional number of species (> 6%, Vörösmarty et al. 2010) and play a pivotal role in human life and development, providing essential ecosystem services. At the global scale, currently nearly the 65% of all riverine systems is subject to severe threats (Vörösmarty et al. 2010) due to the combined effect of several factors such as land use, watercourse alterations (damming, channelization, water abstraction) and climate change. The intensification of these pressures has caused an increase in frequency, intensity and duration of extreme events such as droughts (Meyer et al. 1999).

Over the past thirty years, the frequency and duration of droughts has increased dramatically across Europe, leading to an increase in the duration and extent of flow intermittency and converting permanent streams in temporaries with strong consequences for the structure and functioning of riverine communities, ecosystem processes and related services. (Huntington 2006; Palmer et al., 2008; Lehner et al., 2006; Datry et al., 2014).

Moreover, longer and extreme drought periods expected for natural intermittent streams also might have important consequences on the efficiency to recycling nutrients related with slower breakdown rates (Datry et al. 2011).

Flow is a key driver of the structure and function of aquatic ecosystems, as it affects water quality, physical habitat, energy resources and biotic interactions (Allan and Castillo, 2007; Dewson et al., 2007; Poff et al., 1997). Therefore, hydrological intermittence and drought, as an extreme form of flow variation, have pervasive ecological effects on freshwater ecosystems (Larned et al., 2010). Hydrological intermittence reduces water velocity and

depth, reduces hydrological connectivity, promotes sedimentation (Dewson et al., 2007), alters water physicochemical conditions (von Schiller et al., 2011) and affects the inputs, storage and quality of organic matter (Sanpera-Calbet et al., 2016; Ylla et al., 2010). Shifts from perennial to intermittent flow regimes may lead to irreversible modifications of biotic communities and have important effects on the ecosystem's structure and functioning (Lake, 2003). Therefore, it can affect not only stream biological assemblages (Bonada et al., 2006; Filipe et al., 2013), but also ecosystem processes (Acuña et al., 2005; Martínez et al., 2015). These effects including changes in physico-chemical properties (flow, stream connectivity, water temperature, oxygen, and nutrient concentrations and availability; Stanley et al., 1997, Dahm et al., 2003, von Schiller et al., 2011), ecosystem metabolism (Acuña et al., 2004), food webs (Power et al., 2013), ecological processes (Sabater and Tockner, 2009) and ecosystem services (nutrient cycling, carbon sink, facilitation), deriving from benthic communities and multiple interactions between bacteria, meio and macrofauna, micro and macroalgae and macrophytes. All these processes can be affected during the transitions from wet to dry (drought) and from dry to wet (flood or rewetting events) conditions that occur under water intermittency. In order to have a clearer and more complete knowledge of the effects of hydrological intermittency on ecosystem processes and to predict consequences of oncoming climate change, it is important to understand the effects of drought on river ecosystem functioning. Stream ecosystems are variable over both time and space, but their function is dependent upon the presence of water. In a future where water is predicted to be limiting, is needed to better understand the basic functions of streams to put them in a context for the future.

## Thesis framework and aims

This PhD thesis focusses on the effects of hydrological intermittence on the functioning of lotic ecosystems, focusing on different river's compartments: the upstream reaches of mountainous stream, the hyporheic zone and the rivers drainage canals.

In each of these compartments the effects of drought on specific processes were tested.

In the upstream reaches (Figure 1, point 1) I tested the effects of drought-rewetting cycle on organic matter decomposition rate and nutrient regeneration and the role of macrofauna in driving these processes (chapter 1). In the hyporheic zone (Figure 1, point 2) I tested the effects of drought and rewetting on nutrient release, respiration and denitrification and the temporal dynamics of these processes after rewetting (chapter 2). Ultimately, I tested the effects of periodically exposure to air of drainage canals sediments (Figure 1, point 3), to test the effects on $CO_2$ release and its regulation.

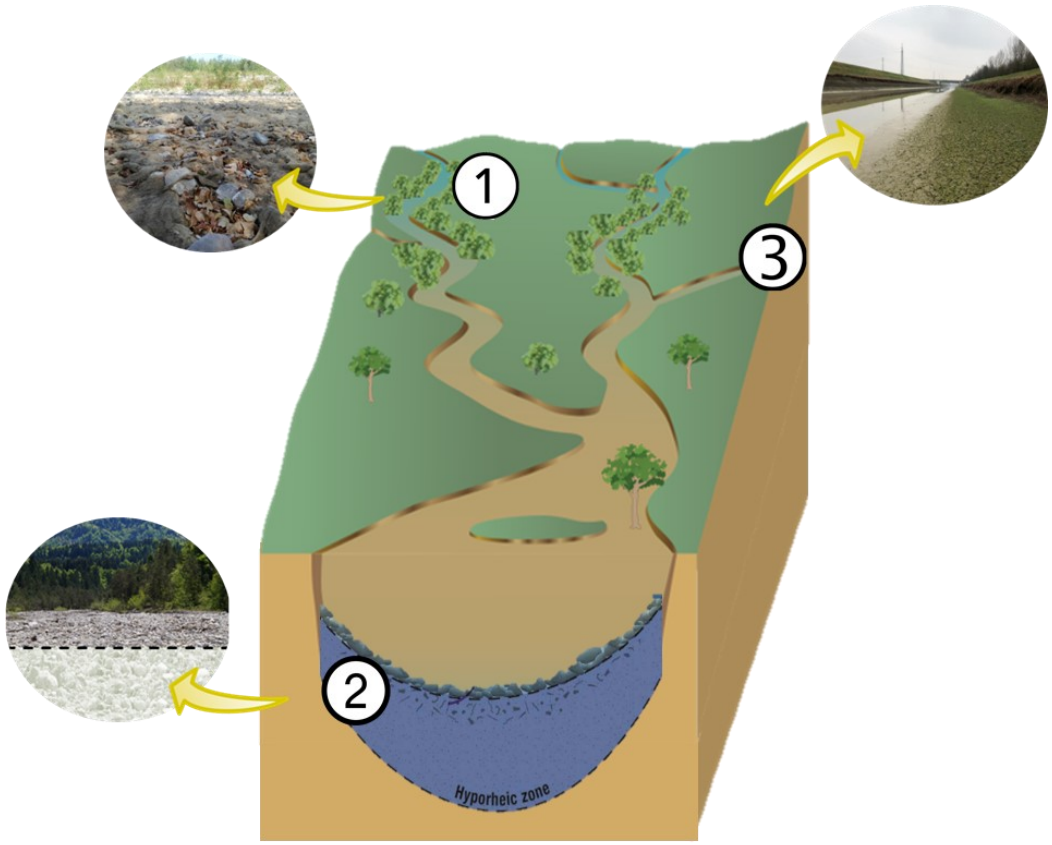


**Figure 1:** graphic representation of the three comparts of lotic ecosystems studied in this thesis.

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# Chapter 1: Leaf litter decomposition in stream

Decomposition is essential for sustaining life on Earth, as it is the only process enabling massive recycling of nutrients on a whole biosphere scale. Organic matter (OM) decomposition is a critical ecosystem process in streams and other aquatic environments since OM serves as substratum and food source for microorganisms and detritus-feeding animals, influencing food web composition and dynamics (Moore et al. 2004). In aquatic ecosystems, organic matter has traditionally been divided into three classes: 1) coarse particulate OM (CPOM, >1 mm), 2) fine particulate OM (FPOM, 0.5 µm-1 mm), and 3) dissolved OM (<0.5 µm, DOM), and it might come from both autochthonous and allochthonous sources (Allan and Castillo 2007). Autochthonous organic matter is originated within the aquatic ecosystems and includes dead macrophytes, animal faeces and dead biofilm material (Hanlon 1982), while allochthonous organic matter comes from terrestrial ecosystems and is mainly composed by leaves, stems and logs (Benfield 1997).

Decomposition is a complex process involving three main mechanisms, which are overlapping over time: leaching, microbial conditioning and fragmentation (Petersen and Cummins 1974, Webster and Benfield 1986, Gessner et al. 1999, Allan and Castillo 2007). Leaching is defined as the loss of soluble compounds from the leaf, such as phenolics, carbohydrates and amino acids (Bärlocher 2005). This mechanism dominates at the early stages of leaf litter decomposition and results in a rapid mass loss in the first week. During the conditioning phase, the chemical composition of plant tissue is modified mainly through four mechanisms: 1) conversion of plant tissue into microbial materials (microbial growth); 2) breaking of complex leaf molecules into simpler ones by means of microbial extracellular enzymes; 3) mechanical alteration of leaves mainly due to fungal hyphae growing; and 4) microbial nutrient incorporation, mainly inorganic nitrogen and phosphorus dissolved in the stream water (Bärlocher and Kendrick 1975, Gessner et al. 1999). Fragmentation results in the release of fine-particulate organic matter to the stream water either from shredding, consumption and production of faeces by invertebrates, or from physical fragmentation due to water abrasion (Gessner et al. 1999, Abelho 2001, Ferreira et al. 2006).

Litter breakdown is thus an integrative process that includes physical, microbial and invertebrate mechanisms. The process recycles nutrients and results in products such as

FPOM and DOM, soluble inorganic compounds, $CO_2$, microbial and invertebrate biomass (Baldy and Gessner 1997, Gessner et al. 1999, Hieber and Gessner 2002).

Breakdown rates and the mechanisms involved in litter processing are highly dependent on the stream environment and are modulated by microbial and invertebrate responses to environmental factors, like temperature, pH, nutrient concentrations, oxygen level, and hydrological regime (Webster and Benfield 1986).

The first chapter of this thesis is aimed to explore the effects of drought in the upstream reaches of low-order forested streams, where the relevance of allochthonous organic matter is especially high and leaf litter breakdown is the main processes of carbon transformation (Vannote et al. 1980, Wallace et al. 1999).

# Effects of drying and re-wetting on litter decomposition and nutrient recycling: a manipulative experiment

## INTRODUCTION

In many areas of the world, climate change and water abstraction exceeding water availability are expected to change the timing, extent and frequency of drying that will alter the hydrology of many freshwater environments (Poff et al., 2010). A rapid decline in flow, with increasing intensity of dry episodes, has been already observed along many rivers (Milliman et al., 2008) and permanent streams are becoming or expected to become intermittent. Shifts from perennial to intermittent flow regimes modify the abiotic characteristics of benthic systems, plant, animal and microbial communities, nutrient concentrations and availability (Dahm et al., 2003; von Schiller et al., 2011), food webs (Power et al., 2013) and ecological processes (Sabater et al., 2009), resulting in changes of benthic ecosystem functioning. Headwater stream ecosystems, especially in forested regions, strongly depend on leaf litter inputs as sources of nutrients and energy for downstream sectors (Vannote et al., 1980). In these systems, upstream organic matter processing is a key ecosystem process (Webster et al., 1999). In low order creeks, the efficiency of organic matter breakdown, microbial use, and biogeochemical transformations are highly dependent on hydrology (Acuña et al., 2005). Drying profoundly affects leaf-litter decomposition, alters breakdown rates and affects benthic communities and processes (Corti et al., 2011; Mora-Gómez et al., 2018). Leaf material breakdown has been shown to be much slower in dry than under aquatic conditions, primarily because the absence of water limits the activity of decomposers (Corti et al., 2011; Riedl et al., 2013). During dry periods, the organic matter accumulated on riverbed depression or low-energy pools undergoes slow decomposition as the ability of microbes to colonize and initiate the process of litter breakdown is limited by water availability (Kaushik et al., 1971). Another critical aspect of drying concerns the marked changes in community structure and activity of microbial decomposers and invertebrate detritivores. Dry events usually reduce the functional and taxonomic richness of invertebrate communities (Datry et al., 2012). This reduction can include the loss of macroinvertebrate shredders, which through their feeding activity, play a substantial role in leaf breakdown and in nutrient cycling within and between ecosystem compartments (Vanni et al., 2002). Nutrient cycling by shredders may be especially important for the microbial-detritus compartment, stimulating microbial growth on the leaves and organic matter

decomposition (Villanueva et al., 2012) and can strongly regulate productivity within ecosystems. Organic matter breakdown may also be influenced by leaf size (de Souza Rezende et al., 2018; Gessner et al., 1999). Leaf size and geometry (e.g. flat, large surfaces) may facilitate the conditioning action that macrofauna produces on leaves and result in higher colonization by biofilm of bacteria, fungi and algae (de Souza Rezende et al., 2018; Arsuffi et al., 1985). During skeletonization of large leaves the lignin to cellulose ratio may increase significantly and influence the residual organic matter breakdown (Arsuffi et al., 1985). On the other hand, litter fragmentation produces smaller and smaller debris with C:N ratios significantly lower as compared to those of the original detritus (Sinsabaugh et al., 1990). Increasing nutrient content in smaller debris has been attributed to microbial bioaccumulation due to higher surface-area to volume ratios and thus a greater capacity for microbial nutrient immobilization and adsorption (Rinkes et al., 2014). Microorganisms condition the litter through nutritional enrichment, thereby increasing litter quality and, consequently, the rate of decomposition by invertebrate feeding (Villanueva et al., 2012). Recently, the number of ecological studies on intermittent streams has grown exponentially (Datry et al., 2011), in part, because of climate-change predictions of stream drying. Several authors have studied leaf breakdown in streams that seasonally dry compared to permanent streams (Pinna et al., 2004), along a natural gradient in immersion and emersion (Corti et al., 2011), during and after drying (Schlief et al., 2011), in different habitat resulting from flow fragmentation in temporary stream (Abril et al., 2016) and in stream mesocosms with flow reduction compared to natural flow regime (Schlief et al., 2009). However, these studies mainly used litter bags method or were focused on consequences to biotic community structure rather than to associated ecosystem processes. Studies based on microcosms generally tested the effects of drying on microbial processes, excluding interactions with other environmental or biological factors (Arroita et al., 2018; Amalfitano et al., 2008). Furthermore, while most studies about decomposition in intermittent conditions have been performed in arid or Mediterranean regions (Abril et al., 2016; Bonada et al., 2006; Mora-Gómez et al., 2014), studies on streams in temperate regions, where intermittency is a relatively new phenomenon, are scarce (Mariluan et al., 2015; Fritz et al., 2006). I performed a laboratory experiment using microcosms to test the combined effect of water intermittency, macroinvertebrates shredders and leaf size on organic matter mineralization, nutrient recycling and their ecological stoichiometry. Although these physical, chemical and biological factors are likely to be interacting, they are often not studied in concert. Moreover, I nested experimental approaches in the

laboratory to produce a deeper understanding of how processes are influenced by interacting factors. In particular, I nested short-term dark incubations to measure respiration and nutrient regeneration rates of leaf litter within longer-term incubations that measured biomass loss and changes in elemental stoichiometry. In the context of future climate change and given the importance of both biota and hydrology to biogeochemical cycling in ecosystems, a greater understanding of the effects of hydrological intermittency on functioning of benthic systems is needed. In this direction the aim of this work is to better understand how organic matter processing would be affected by the presence of water and to evaluate the role of benthic communities on leaves decomposition and nutrient recycling in relation to hydrological intermittency. I hypothesized that drying interrupts biological processes, including microbial and macrofaunal and results in lower leaf breakdown rates. I also hypothesized that shredders hasten organic matter mineralization, microbial activity, and nutrient recycling through direct (e.g., excretion) and indirect ways (litter conditioning). As leaf size may affect breakdown rates, I added this factor in the experimental design. I expected faster breakdown for smaller leaves due to higher colonization by microbes and fungi and higher consumption by shredders. The three interplaying factors drying, macrofauna and leaf size likely produce element-specific effects on organic carbon (C), total nitrogen (N) and total phosphorous (P), resulting in different stoichiometry along the decomposition process.

## METHODS

### *Experimental set-up*

All the material used in this experiment, including water, mineral substrate, macrofauna and leaves, was collected from the Parma stream, an intermittent third-order mountainous stream (44°27'53.3"N 10°02'53.9"E), located in the Tuscan-Emilian Apennines (Italy). Leaf disks were incubated in presence and absence of larval shredders and under different hydrological regimes (permanent vs intermittent). For each condition leaf disks of different diameter (1 and 5 cm) were used. All microcosms contained 7 ± 0.5 g of sterilized and ignited sand and sifted with a 1-mm mesh size sieve. The experimental set-up consisted in forty-five PVC microcosms provided with two different lids (Figure 1.1). I tested nine conditions, each with five replicates: C=Control; L1=Leaves 1 cm; L1_D=Leaves 1 cm + Drying simulation; L1_M=Leaves 1 cm + Macrofauna; L1_M_D=Leaves 1 cm + Macrofauna + Drying simulation; L5=Leaves 5 cm; L5_D=Leaves 5 cm + Drying

simulation; L5_M=Leaves 5 cm + Macrofauna; L5_M_D=Leaves 5 cm + Macrofauna + Drying simulation.

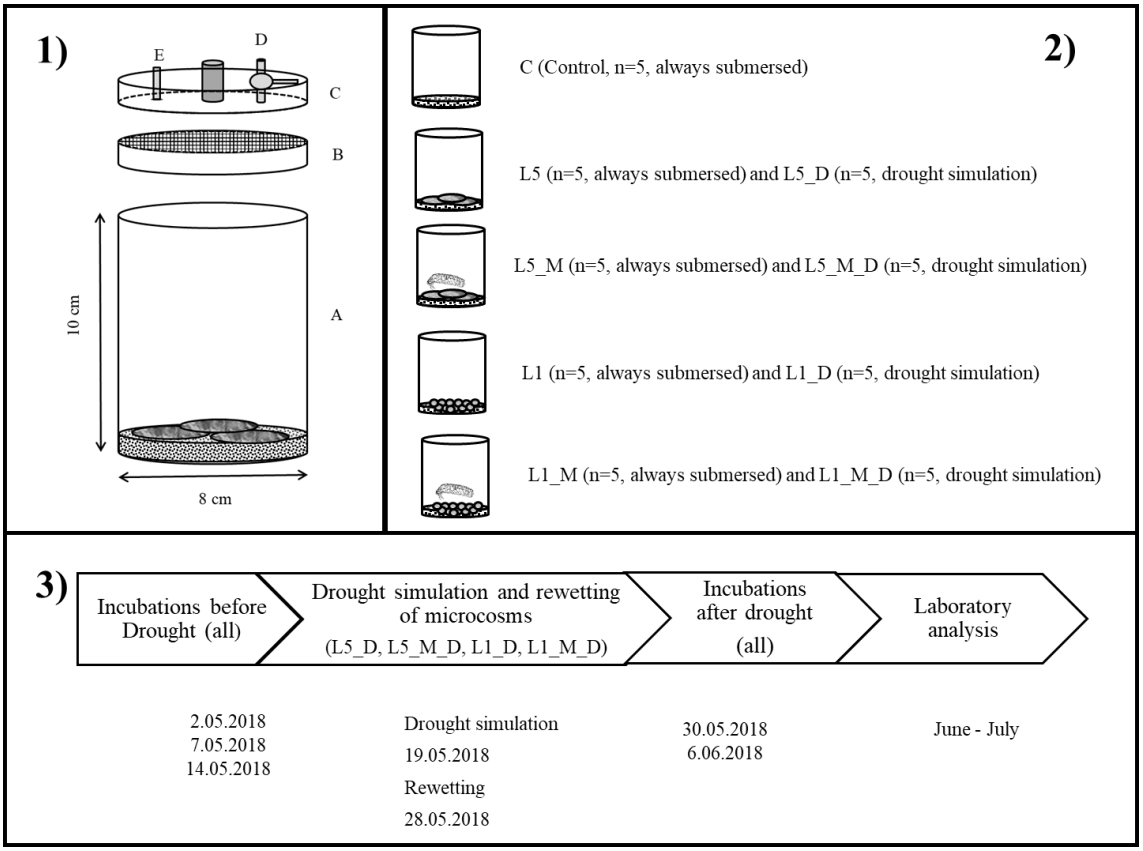


**Figure 1.1**. Microcosms set up (a), experimental design (b) and sequence of actions (c). The microcosms used in this experiment were made with opaque PVC (a1). The experimental design consisted of nine treatments, each with five replicates, with various combinations of leaves and macrofauna (b). During maintenance in the tank microcosms were closed with a lid provided with a net (a2) to allow water exchange between the inner microcosms and the tank. Such lid was replaced with a gas-tight lid (a3) during short-term dark measurements of oxygen and nutrients. The incubation lid was provided with a sampling port (a4) and a compensation valve (a5). Besides control microcosms, incubated to correct fluxes for processes in the water column, four combinations of small and large leaves with or without macrofauna were tested (b). These combinations became eight during the experiment (c) as half of the replicates for each condition underwent a drying simulation.

Microcosms were maintained for forty days in a 200 L incubation tank containing filtered (20 µm plankton net) stream water that was mixed and aerated continuously by aquarium pumps and aerators. Nearly 20% of the tank water was replaced every three days with fresh water from the stream to avoid significant changes in water chemistry. During most of this incubation period all microcosms were provided with a lid made of plastic net (mesh size 1-mm) allowing water exchange but not that of macrofauna. The tank was placed outdoor to reproduce light and temperature daily variations and water physical and chemical characteristics were monitored overtime to ensure stream water homogeneous conditions.

Macrofauna and leaves were added on April 28, 2018. On May 2 (day 5), May 7 (day 10), and May 14 (day 17) (before drying simulation), and on May 30 (day 33), and June 6 (day 40) (after drying simulation) all microcosms underwent a short (six hours) dark incubation (see detailed methods in next section). The drying simulation was started 22 days after the beginning of the experiment (May 19) (Figure 1.1). Drying was simulated removing with a syringe most of the water from each microcosm to simulate stream contraction phase, which characterizes a typical intermittent hydrological cycle, and which precedes a complete stream drying. Rapid water removal reflected what happens in permeable riverbeds, where water drains, does not accumulate and does not evaporate slowly. Where present, macrofauna was carefully removed using tweezers and counted whereas leaves, debris (including particulate matter and mineral substrate) were not removed. The removal of macrofauna was decided based on the specific traits of the chosen organism (see the discussion). Microcosms with all their content were maintained outdoor, outside the incubation tank, protected by a net, for ten days. The remaining water evaporated gradually, within the second day from the water removal as suggested by constant weight. After ten days of emersion, microcosms were rewetted by adding a small amount of filtered stream water but not macrofauna. Microcosms were finally re-submersed in the tank, with the net preventing any biomass loss. At the end of the experiment (day 40), microcosms were retrieved from the incubation tank and transferred to the laboratory. Macrofauna, leaves and debris were carefully separated, removed from microcosms and oven-dried at 30 °C to a constant weight. Thereafter leaves and debris were weighed and powdered for organic carbon, total nitrogen and total phosphorous analyses (see next section). Leaf biomass loss was calculated by difference between the initial and final dry weights.

*Leaves and Shredders*

I collected leaves of *Populus nigra*, a common species in the riparian zones of Tuscan-Emilian Apennines that almost exclusively contributes to allochthonous organic matter input at the study site. Leaves were collected in November 2017, just before abscission, were transported in laboratory and gently rinsed with deionized water. Petioles were removed, leaves were cut into disks of 5 and 1 cm of diameter using hollow cutter, dried at 25 °C to a constant dry mass and stored at room temperature until needed. Leaves material might have different size and size itself may affect organic matter breakdown rates. This is the reason underlying the choice of two different diameters. Before the start of the experiment, leaves were rehydrated in stream water and then immediately transferred into

the micrcosms. A total biomass of 0.5 ± 0.002 grams of leaves disks of both dimensions were added in each microcosm; to this purpose three leaves were added to each of the L5, L5_D, L5_M, L5_M_D and sixty-four leaves were added to each of the L1, L1_D, L1_M and L1_M_D microcosms. Last instar larvae of *Potamophylax cingulatus* (Trichoptera, Limnephilidae) were collected by hand picking. *Potamophylax cingulatus* is a case-building caddisfly that in the last larval instar construct its cases using entirely mineral particles (Otto et al., 1980). The organisms used were at the same developmental stage of the larval forms and similar-sized individuals (0.04 ± 0.003 gdw ind-1) were chosen for the experiment. This species is widely distributed in the study area, where it represents the most dominant shredder taxon participating in the recycling of organic matter and can have strong effects on ecosystem functioning and stream trophic structure. After sampling, shredders were transported in laboratory and placed in aquarium. The day after collection 20 individuals were added in each macrofauna treatment (L5_M, L5_M_D, L1_M, L1_M_D). The number of individuals in each microcosm was chosen on the basis of natural densities found in Parma stream, according to previous studies in the study area (Laini, unpublished).

*Measurement of Benthic Fluxes and Shredders Metabolism*

During the 40 days of maintenance in the tank all microcosms underwent five short-term dark incubations targeting dissolved $O_2$ and inorganic nutrients regenerated during mineralization process ($NH_4^+$, $PO_4^{3-}$) fluxes. We acknowledge that soluble organic compounds are released as well and might represent important pools of released C, N and P but in this study we focused only in the inorganic, more reactive fractions. This was also decided in order to discuss the relevance of macrofauna respiration and excretion to measured fluxes. Three incubations were carried out before drying simulation while two were carried out after drying a subsample of the microcosms (L5_D, L5_M_D, L1_D, L1_M_D). The incubations lasted six hours; at the starting water samples were collected in triplicate with 50-mL syringes from the incubation tank and the nets covering each microcosm was replaced with a gas-tight lid. At the end of the incubation the water samples were collected from each microcosm. An aliquot of collected water samples were transferred to 12-mL exetainers and poisoned with 100 µL 7 M $ZnCl_2$ to stop microbial activity (Dalsgaard et al., 2000). Another aliquot was filtered (GF/F Whatman filters) and transferred to 10-mL glass vials for soluble reactive phosphorous determination and to 20-mL scintillation vials for $NH_4^+$ determination. Dissolved $O_2$ was measured within two

hours by means of polarography with a microelectrode connected to a picoamperometer (Unisense, Denmark). The electrode was calibrated in 100% saturated water at the same incubation temperature and at 0% saturation ($N_2$ bubbling). Nutrient samples were immediately analyzed with standard spectrophotometric techniques (Golterman et al., 1980; Koroleff, 1970). At the end of the incubation the gas-tight lids were replaced with nets. Fluxes of $O_2$ and nutrients were calculated according to the equation below.

$$Flux\ x = \frac{([x]_f) - ([x]_i) \times V}{A \times t}$$

where $[x]_f$ and $[x]_i$, expressed in mM or µM, are the concentrations of the solute x at the end and at the start of the incubation, respectively, V (L) is the volume of the microcosm water phase, A ($m^2$) is the area of microcosm and t (h) is the incubation time.

Additional individuals of *Potamophylax cingulatus* sampled from the same study stream were also incubated singly and in groups in the dark to analyze $O_2$ consumption and inorganic nutrient excretion. Individuals were incubated in 50 mL glass vials containing filtered stream water, following a similar procedure as described above.

*Elemental Analysis*

C and N content in leaves at the beginning and in residual leaves and debris at the end of the experiment were measured with a EA/NA-1100 CHN elemental analyzer (Thermo Finnigan) coupled with a mass spectrometer. Total phosphorus on the same matrices was determined after ashing at 450 °C, P extraction from ashes with concentrated HCl and spectrophotometry (Aspila et al., 1976). The content of C, N and P in the leaves and in the produced debris was analyzed and data were presented as percentage and total content, for budgeting purposes. For the debris, as it was mixed with the mineral particles added to the microcosms, it was not possible to calculate percentages but only total content.

*Data Analyses*

A two-way factorial ANOVA was used to test the differences among treatments in nutrients fluxes before drying simulation, with leaves dimension and macrofauna as fixed factors. To test differences among treatments in nutrients fluxes after drying simulation, remaining leaf biomass and elemental composition a three-way ANOVA was used, with leaves dimension, presence of macrofauna and drying as fixed factors. The effects of single factors and interactions among treatments were examined. All tests were considered significant if the p-value was less than 0.05. Each analysis was performed after assumptions of normality and homoscedasticity were verified. All analyses were performed with statistical software R (R Core Team, 2018). Graphs were produced with Sigma Plot 11.0.

## RESULTS

*Measurements of Shredders Respiration and Excretion Rates*

Incubations of macrofauna alone allowed to calculate for organisms with dry weight (gdw) between 0.03 and 0.09 $ind^{-1}$ an average respiration rate of -13.06 ± 0.9 µmol $O_2$ $gdw^{-1}h^{-1}$ (average ± standard error, n=9) and an average $NH_4^+$ excretion of 0.75 ± 0.09 µmol $NH_4^+$ gdw $ind^{-1}$. $PO_4^{3-}$ excretion was undetectable, due to difference between initial and final concentrations below the analytical precision of the methods. The literature reports $PO_4^{3-}$ excretion rates for the Limnephilidae family averaging 0.2 ± 0.09 µmol $PO_4^{3-}$ $m^{-2}h^{-1}$ (Hall et al., 2007).

*Benthic Respiration and Nutrient Fluxes*

Data for two out of the five incubations were presented: the first, on day 5, and the fourth, on day 33, two days after rewetting. Results from second and third incubation provide rates that lay in between those reported, with consistent outputs of the statistical tests showing the same differences among treatments. Results from fifth incubation suggest very low fluxes likely due to exhaustion of more reactive organic matter pools. Even if they are not shown here, all measured fluxes are reported as additional material.

Oxygen respiration and nutrient fluxes in the microcosms varied temporally and among treatments (Figure 1.2).

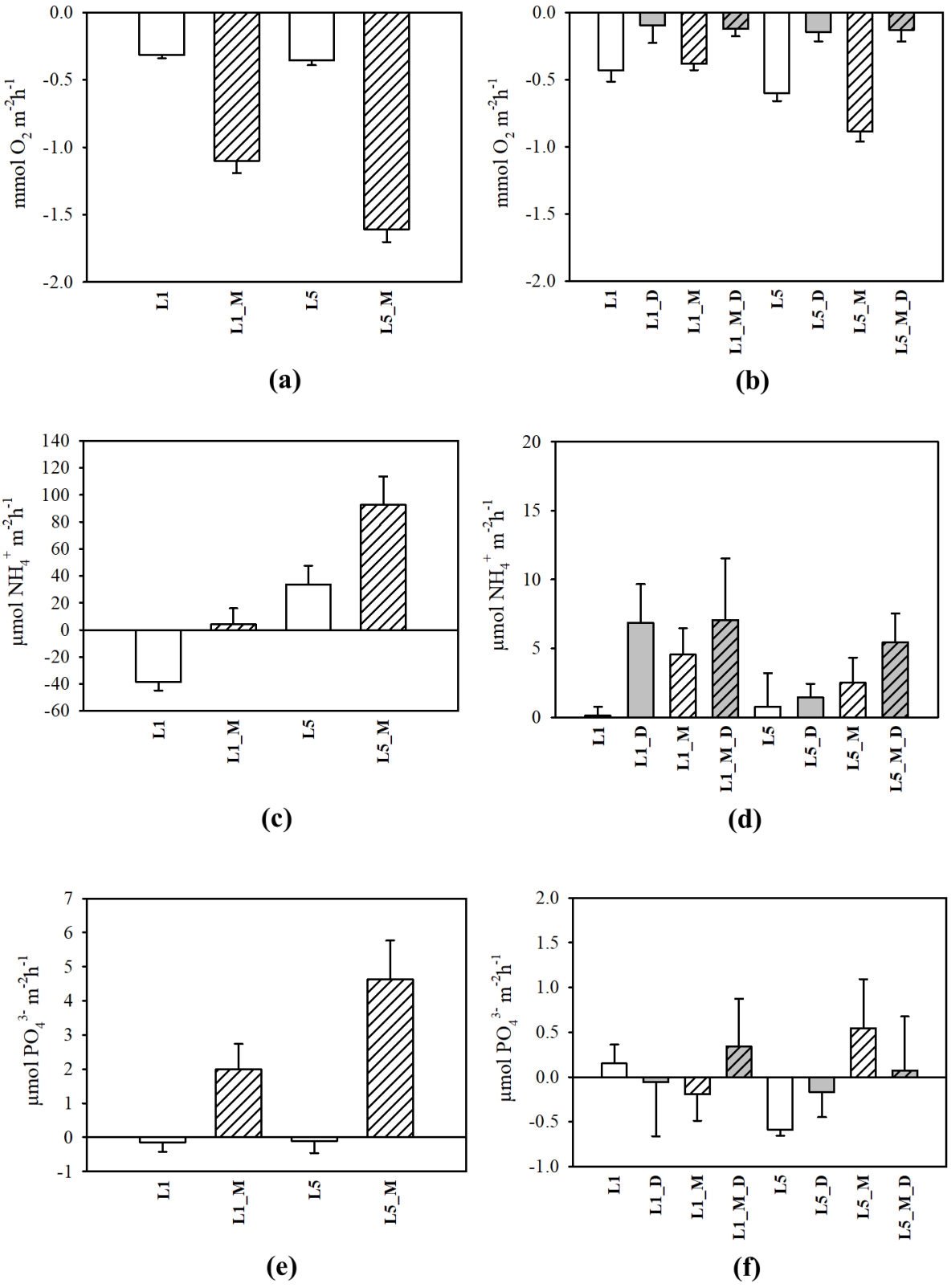


**Figure 1.2**. Dark net fluxes of $O_2$ (a, b), $NH_4^+$ (c, d), $PO_4^{3-}$ (e, f) at day 5 (a, c, e, g) and at day 33 (b, d, f, h). Mean fluxes and standard error (n = 10 at day 5 and n=5 at day 33) of each condition are reported. All fluxes are expressed in µmol $m^{-2}$ $h^{-1}$ or mmol $m^{-2}$ $h^{-1}$. White bars represent permanently submerged condition, grey bars represent dried conditions and hatched bars represent conditions with macrofauna.

At day 5 only four conditions are shown as all microcosms were submersed; at day 33 instead the four conditions are split in two as half the microcosms underwent 10 days of drying. Microcosms respiration and nutrient fluxes were generally higher at day 5, at the beginning of the experiment. Along the course of the experiment, there was a clear decrease of $O_2$ consumption rates and nutrient fluxes in all conditions, in particular during the last incubation, in which $O_2$, $NH_4^+$ and $PO_4^{3-}$ were very small. At the beginning of the experiment, the conditions with macrofauna displayed significantly higher $O_2$ consumption rates as compared to microcosms without macrofauna. At day 5, rates varied from a maximum of -1.61 ± 0.09 mmol $O_2$ $m^{-2}h^{-1}$, measured in L5_M, to a minimum of -0.31 ± 0.03 mmol $O_2$ $m^{-2}h^{-1}$, measured in L1 (Figure 1.2). At day 33 rates nearly halved and varied between -0.88 ± 0.08 (L5_M) and -0.09 ± 0.1 (L1_D). Statistical analyses (Table 1.1) revealed for day 5 significantly higher $O_2$ consumption in treatments with macrofauna as compared to treatments without macrofauna ($p<0.001$; $F=216.5$) and in treatments with leaves of 5 cm of diameter as compared to treatments with 1 cm ($p<0.001$; $F=15.7$), however, such differences depended upon the interaction of the two factors ($p<0.01$; $F=11.4$). Nutrient fluxes were highly variable ranging from positive to negative values. In the first incubation and in treatments without macrofauna, microbial conditioning of leaves determined negative $NH_4^+$ and $PO_4^{3-}$ fluxes, likely due to nutrient uptake from the water column. At day 5, $NH_4^+$ fluxes varied from -38.47 ± 6.3 µmol $NH_4^+$ $m^{-2}h^{-1}$ measured in L1 to 67.3 ±16 µmol $NH_4^+$ $m^{-2}h^{-1}$ measured in L5_M, while $PO_4^{3-}$ fluxes varied from 4.6 ± 1.2 µmol $PO_4^{3-}$ $m^{-2}h^{-1}$ measured in L5_M to -0.14 ± 0.28 µmol $PO_4^{3-}$ $m^{-2}h^{-1}$ measured in L1. At the beginning of the experiment the presence of macrofauna led to significantly higher $NH_4^+$ and $PO_4^{3-}$ fluxes ($p<0.001$; $F=13.2$ and $p<0.001$; $F=22.6$, respectively). Significantly higher $NH_4^+$ regeneration was measured in leaves of 5 cm of diameter as compared to leaves of 1 cm of diameter ($p<0.001$; $F=33.1$). At day 33, two days after rewetting, nutrient fluxes were very low (Figure 1.2). Treatments subjected to drying displayed significantly lower $O_2$ demand (Table 1.2) as compared to treatments permanently submersed ($p<0.001$; $F=73.9$). Leaf size also affected $O_2$ consumption, with significantly higher $O_2$ uptake in leaves of 5 cm of diameter at day 33, compared to treatments with leaves of 1 cm of diameter ($p<0.01$; $F=11.9$). However, such difference depended upon the interaction of the two factors ($p<0.01$; $F=8.6$). Drying produced also a significant effect on $NH_4^+$ regeneration ($p=0.02$; $F=5.8$), whereas the three tested factors did not produce significant effects for $PO_4^{3-}$.

**Table 1.1**. Summary of results of two-way ANOVA testing the effects of factors macrofauna and leaf size on benthic respiration ($O_2$) and nutrient fluxes ($NH_4^+$, $PO_4^{3-}$) measured on day 5. Significant values are printed in bold.

| | **Df** | **$O_2$** | | **$NH_4^+$** | | **$PO_4^{3-}$** | |
|---|---|---|---|---|---|---|---|
| | | **p-value** | **F value** | **p-value** | **F value** | **p-value** | **F value** |
| Macrofauna | 1 | **< 0.001** | **216.5** | **< 0.001** | **13.2** | **<0.001** | **22.6** |
| Leaf size | 1 | **< 0.001** | **15.7** | **< 0.001** | **33.1** | 0.07 | 3.4 |
| Macrofauna:leaf size | 1 | **< 0.01** | **11.4** | 0.6 | 0.3 | 0.08 | 3.2 |
| Residuals | 36 | | | | | | |

**Table 1.2.** Summary of results of three-way ANOVA testing the effects of factors macrofauna, leaf size and drying on benthic respiration ($O_2$) and nutrient fluxes ($NH_4^+$, $PO_4^{3-}$) measured on day 33. Significant values are printed in bold.

| | **Df** | **$O_2$** | | **$NH_4^+$** | | **$PO_4^{3-}$** | |
|---|---|---|---|---|---|---|---|
| | | **p-value** | **F value** | **p-value** | **F value** | **p-value** | **F value** |
| Macrofauna | 1 | 0.2 | 1.4 | 0.3 | 1.1 | 0.3 | 1.3 |
| Leaf size | 1 | **<0.01** | **11.9** | 0.4 | 0.6 | 0.8 | 0.09 |
| Drying | 1 | **<0.001** | **73.9** | **0.02** | **5.8** | 0.8 | 0.05 |
| Macrofauna:leaf size | 1 | 0.2 | 1.9 | 0.8 | 0.06 | 0.3 | 1.1 |
| Macrofauna:drying | 1 | 0.3 | 1.1 | 0.6 | 0.3 | 0.9 | 0.02 |
| Drying:leaf size | 1 | **<0.01** | **8.6** | 0.2 | 1.8 | 0.8 | 0.09 |
| Macrofauna:drying:leaf size | 1 | 0.08 | 3.2 | 0.6 | 0.3 | 0.2 | 1.8 |
| Residuals | 32 | | | | | | |

*Residual Biomass and its Elemental Composition*

Assuming random differences in the initial dry weigh of the leaf disks and negligible differences among treatments in initial dry weigh of leaves, there were large, significant effects of treatments on remaining leaf biomass (Figure 1.3) and C, N and P content of leaves (Figure 1.4) at the end of the experiment. The percentage of biomass loss at the end of the experiment ranged from 12.8 to 51.8%, measured in L5_D and in L5_M, respectively. As compared to initial biomass of litter material (110 ± 0.4 gdw $m^{-2}$, corresponding to 0.5 ± 0.002 gdw per microcosm), the conditions that exhibited lower remaining leaf biomass were those permanently submersed and with macrofauna. For these treatments, the calculated leaf biomass loss was up to four-fold higher compared to conditions undergoing the drying simulation and without macrofauna. Macrofauna and drying significantly affected leaves biomass loss during the experiment (Table 1.3), with lower remaining leaf biomass in treatments with macrofauna and permanently submersed conditions ($p<0.001$; $F=32$ and $p<0.001$; $F=13.9$, respectively), while the effect of leaf size was very close to the significance level, with lower remaining leaf biomass in larger litter disks ($p=0.07$).

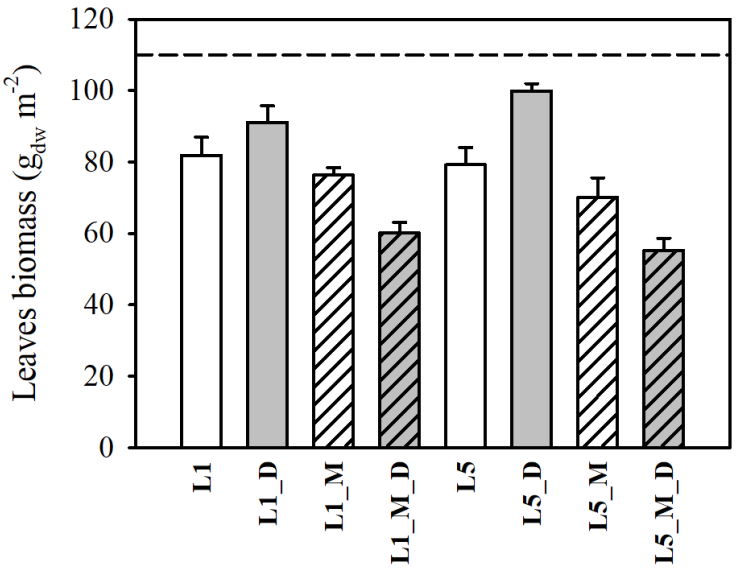


**Figure 1.3**. Remaining leaf biomass recovered at the end of the experimental period. Mean ± standard error (n=5) were reported. The horizontal dashed line is the reference value of the average leaf biomass at the beginning of the experiment.

**Table 1.3**. Results of three-way ANOVA testing the effects of the three factors macrofauna, leaf size and drying on residual leaf biomass and its C, N and P percentage and content at the end of the experimental period. Significant values are printed in bold.

| **Leaves** | | **Leaves biomass** | | **C (%)** | | **C (g $m^{-2}$)** | | **N (%)** | | **N (g $m^{-2}$)** | | **P (%)** | | **P (g $m^{-2}$)** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Df** | **p-value** | **F value** | **p-value** | **F value** | **p-value** | **F value** | **p-value** | **F value** | **p-value** | **F value** | **p-value** | **F value** | **p-value** | **F value** |
| Macrofauna | 1 | **<0.001** | **32** | 0.5 | 0.4 | **<0.001** | **48** | 0.4 | 0.6 | **<0.001** | **103** | 0.9 | 0.01 | **<0.001** | **24** |
| Leaf size | 1 | 0.07 | 3.4 | **<0.001** | **24** | **<0.001** | **18.1** | 0.06 | 3.6 | 0.2 | 2 | 0.2 | 2.2 | 0.06 | 3.6 |
| Drying | 1 | **<0.001** | **13.1** | 0.3 | 1.2 | **<0.001** | **13.6** | **<0.001** | **53.4** | 0.4 | 0.8 | **<0.001** | **30** | 0.2 | 1.8 |
| Macrofauna:leaf size | 1 | 0.3 | 1.2 | 0.4 | 0.6 | 0.1 | 2.2 | 0.9 | 0.007 | **0.02** | **6** | 0.3 | 1.2 | 0.6 | 0.3 |
| Macrofauna:drying | 1 | 0.9 | 0.005 | 0.7 | 0.2 | 0.6 | 0.3 | 1 | 0.001 | 0.2 | 2.1 | **0.004** | **9.4** | 0.07 | 3.5 |
| Drying:leaf size | 1 | 0.4 | 0.7 | 0.8 | 0.1 | 0.1 | 2.2 | 0.2 | 2 | 0.8 | 0.1 | 0.5 | 0.5 | 0.9 | 0.02 |
| Macrofauna:drying:leaf size | 1 | 0.4 | 0.7 | 0.8 | 0.1 | 0.4 | 0.7 | 0.5 | 0.6 | 0.7 | 0.1 | 0.8 | 0.09 | 0.9 | 0.02 |
| Residuals | 32 | | | | | | | | | | | | | | |

Assuming random differences in the initial elemental composition of the leaf disks and negligible differences among treatments, the leaves elemental composition and stoichiometry changed along the course of the experiment and among treatments. Both percentage and quantity of C in residual leaves decreased during the experiment compared to initial values, while N and P increased, resulting in lower C:N and C:P ratio (Figure 1.4 and Table 1.4).

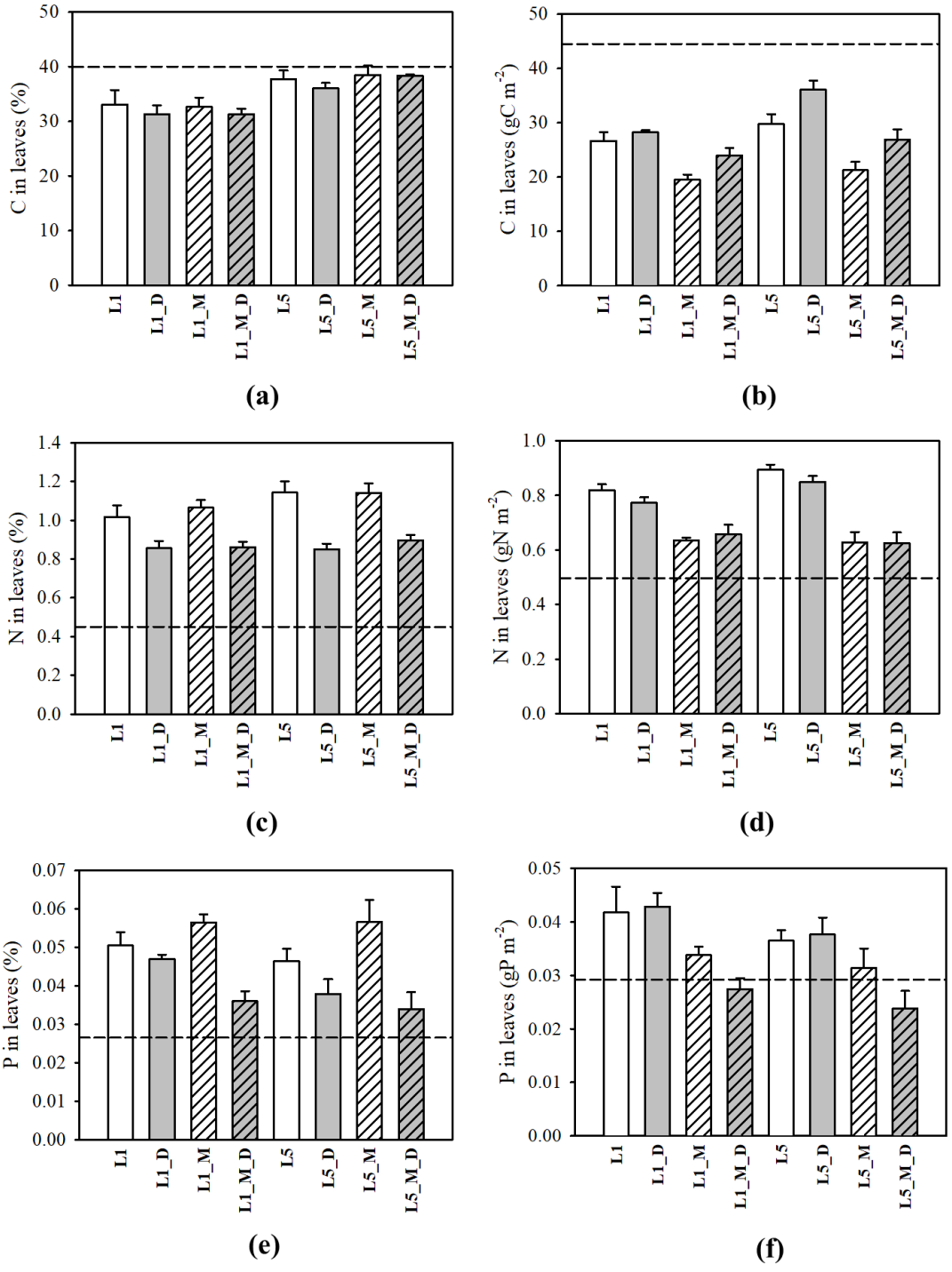

**Figure 1.4.** C, N and P percentage (a, c, e) and content (b, d, f) in residual litter at the end of the experimental period. Mean ± standard error (n=5) are reported. White bars represent permanently submerged condition, grey bars represent dried conditions and hatched bars represent conditions with macrofauna. The horizontal dashed line is the reference value of the average C, N, P percentage and content in leaves at the beginning of the experiment.

**Table 1.4.** Molar ratios of C, N and P of leaf biomass (mean ± standard error) at the beginning and at the end of the experimental period.

| | **C:N** | **C:P** | **N:P** |
|---|---|---|---|
| **Initial litter** | 104 ± 5 | 3933 ± 450 | 38 ± 4 |
| **L1** | 38 ± 2 | 1721 ± 183 | 46 ± 6 |
| **L1_D** | 43 ± 1 | 1729 ± 114 | 40 ± 2 |
| **L1_M** | 36 ± 2 | 1497 ± 71 | 42 ± 2 |
| **L1_M_D** | 43 ± 1 | 2291 ± 157 | 54 ± 5 |
| **L5** | 39 ± 2 | 2124 ± 144 | 55 ± 3 |
| **L5_D** | 50 ± 3 | 2566 ± 284 | 51 ± 3 |
| **L5_M** | 39 ± 2 | 1826 ± 190 | 46 ± 4 |
| **L5_M_D** | 50 ± 2 | 3185 ± 64 | 64 ± 11 |

Concerning organic carbon (C), the percentage in leaves at the end of experiment was lower in all conditions compared to the initial value (40.4% ± 2.56), ranging between 31% and 38%, with a clear tendency of leaves of 1 cm of diameter to have lower C percentage compared to leaves of 5 cm of diameter ($p<0.001$; $F=24$, Table 1.3). C loss, calculated from percentages and biomass at the end and the beginning of the experiment, was highest in L1_M and L1_M_D conditions (9%), while in the other conditions it was within 2%. Statistical analyses (Table 1.3) suggest significantly lower C percentage in treatments with leaves of 1 cm of diameter ($p<0.001$; $F=23.9$), while the effect of macrofauna and drying was not significant ($p=0.55$ and $p=0.3$, respectively). Compared to C content in leaves at the beginning of experiment (44.5 ± 2.8 g C $m^{-2}$), C quantity decreases in all conditions by values between 56% and 19%, with higher calculated C loss measured in L1_M, corresponding to a C quantity of 19.5 ± 0.9 g C $m^{-2}$. As for biomass, highest C loss was associated to the presence of macrofauna, with significant lower C content in leaves

conditioned by macrofauna ($p<0.001$; $F=47.85$). In addition, both factors drying and leaf size produced a significant effect on C content in residual leaves, with lower values in treatment with drying simulation and with leaves of 1 cm of diameter ($p<0.001$; $F=18.14$ and $p<0.001$; $F=13.6$, respectively). While C percentages decreased during the experimental period, N percentages in residual biomass almost doubled in all conditions compared to initial values (0.45% ± 0.01), with highest values measured in L5 and L5_M, in which N percentage increased by 2.5-fold (1.14% ± 0.05 in both conditions). Leaves in treatments subjected to drying displayed significantly lower increase in N percentage (0.85% ± 0.03 in L5_D condition), compared to treatment permanently submersed ($p<0.001$; $F=53.5$). Concerning N content in leaves, results show a general increase in calculated N amount in all conditions compared to initial content (0.5 ± 0.01 g N $m^{-2}$), varying between 0.6 ± 0.04 and 0.9 ± 0.02 g N $m^{-2}$, measured in L5_M_D and in L5, respectively. Leaves in treatments without macrofauna accumulated significantly more N compared to leaves subjected to their feeding activity, increasing on average by 40 and 22%, respectively ($p<0.001$, $F=102.9$). However, the effect of macrofauna depended upon the leaf size, and was greater for large than for small leaves ($p=0.02$, $F=6$). Similarly to N, P percentage and quantity in leaves increased during the course of the experiment in most conditions. At the beginning of the experiment, P percentage in leaves averaged 0.026% ± 0.003 (Figure 1.4e). After forty days, in conditions permanently submersed and with macrofauna (L1_M and L5_M) P percentages exhibited a two-fold increase, while conditions with macrofauna and subjected to drying showed a much smaller increase (L1_M_D and L5_M_D). The factor drying was significant ($p<0.001$; $F=30.05$) and resulted in decreased P percentages, but it depended upon the presence of macrofauna ($p<0.01$; $F=9.4$). Concerning calculated P content, the presence of macrofauna was the only significant factor, without interactions ($p<0.001$, $F=24$), (Table 1.3). The conditions with macrofauna and undergoing drying simulation (L1_M_D and L5_M_D) were the only with calculated P content lower than the initial value (Figure 1.4f). The initial nutrient amount in leaves was used as a reference value for nutrients analyzed in the debris recovered at the end of the experiment (Figure 1.5). I acknowledge that besides introduced leaves, additional C, N and P inputs along the course of the experiment may derive from the dissolved inorganic and organic nutrients in the water and from the pellets produced by macrofauna. Inorganic nutrient concentrations in the stream water were however very low (<5 μM for $NH_4^+$ and <1 μM for $PO_4^{3-}$) whereas the dissolved organic forms were not measured. Produced pellets depended upon introduced leaf material through macrofauna ingestion.

I calculated that the quantity of C accumulated in the fine material within the microcosms corresponded to values between 2.5% and 33% of the initial C content in leaves. Results of the effects of the three tested factors on debris elemental composition are reported in Table 1.5.

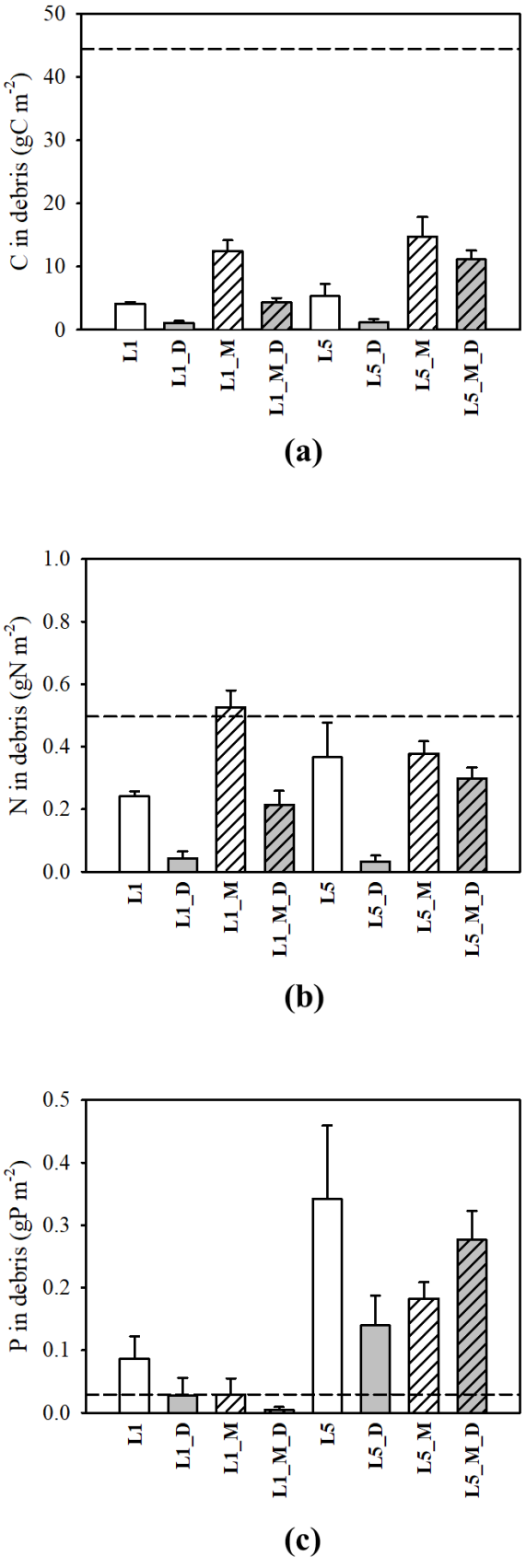


**Figure 1.5**. C, N and P content in debris (a, b, c) recovered from each microcosm at the end of the experimental period. Mean ± standard error (n=5) are reported. White bars represent permanently submerged condition, grey bars represent dried conditions and hatched bars represent conditions

with macrofauna. The horizontal dash line is the reference value of C, N, P content in leaves at the beginning of the experiment.

**Table 1.5**. Results of three-way ANOVA testing the effects of the three factors macrofauna, leaf size and drying on the C, N and P content in the debris recovered from each microcosm at the end of the experimental period. Significant values are printed in bold.

| **Debris** | | **C ($g\ m^{-2}$)** | | **N ($g\ m^{-2}$)** | | **P ($g\ m^{-2}$)** | |
|---|---|---|---|---|---|---|---|
| | **Df** | **p-value** | **F value** | **p-value** | **F value** | **p-value** | **F value** |
| Macrofauna | **1** | **<0.001** | **49.3** | **<0.001** | **26.6** | 0.5 | 0.4 |
| Leaf size | **1** | **0.02** | **5.6** | 0.5 | 0.4 | **<0.001** | **39** |
| Drying | **1** | **<0.001** | **17.2** | **<0.001** | **35.2** | 0.09 | 3 |
| Macrofauna:leaf size | 1 | 0.09 | 3 | 0.4 | 0.9 | 0.6 | 0.2 |
| Macrofauna:drying | 1 | 0.3 | 1.2 | 0.4 | 0.8 | **0.04** | **4.6** |
| Drying:leaf size | 1 | 0.09 | 3 | 0.8 | 0.1 | 0.6 | 0.3 |
| Macrofauna:drying:leaf size | 1 | 0.2 | 1.6 | **0.03** | **5.5** | 0.4 | 0.8 |
| Residuals | 31 | | | | | | |

A significantly higher C amount associated to the debris was found in treatments with macrofauna (10.7 ± 1.3 g C $m^{-2}$) compared to conditions without macrofauna (3 ± 0.7 g C $m^{-2}$) ($p<0.001$; $F=49.3$). In addition, the three-way ANOVA revealed a significant effect of drying and leaves on C amount in residual fine matter. These results show higher C accumulation in residual fine matter of treatments permanently submersed and with leaves with higher diameter ($p=0.001$; $F=17.2$ and $P=0.02$; $F=5.6$, respectively). Nitrogen enrichment in debris was significantly higher in treatments permanently submersed and with macrofauna ($p<0.001$; $F=35.2$ and $p<0.001$; $F=26.6$, respectively). Treatments undergoing drying simulation and without macrofauna showed the lowest values of N content, while highest values were found in treatments with macrofauna and permanently submersed, with values slightly higher in L1_M conditions compared to initial N content in leaves. Regarding P, results show significantly higher content in conditions with leaves with high diameter ($p<0.001$; $F=38.9$), with significant interaction between macrofauna

and drying ($p=0.02$; $F= 5.6$) and with P amounts exceeding the reference value and 10-fold higher compared to leaves of 1 cm of diameter.

## DISCUSSION

Studies reporting rates of litter decomposition have recently included drying as a factor due to the increasing frequency of climatic anomalies resulting in temporary periods of water absence. Such periods, despite being short, may produce relevant effects of organic matter conditioning and mineralization, that are consequences of the disappearance of macrofauna and the ecosystem services associated (e.g. nutrient recycling) and of the strong limitation induced by drying on fungal and microbial activity. During drying in a natural environment several factors interact and affect species and ecosystem processes and our knowledge of combined effects are limited (Leberfinger et al., 2010). The experiment described in the present work has two main elements of novelty: it considers simultaneously three interplaying factors: drying, presence of macrofauna and leaf size and it combines a traditional, static approach (remaining leaf biomass evaluation and the analysis of changes in elemental composition) with an approach based on process rates measurement (oxygen and inorganic nutrient fluxes). To the best of our knowledge, such approach was never used in previously published papers. The present experiment was conducted in microcosms that inevitably simplify communities and processes occurring in riverine ecosystems, but the controlled conditions in which I operated allowed to compare rates of oxygen and nutrient exchange along the course of the experiment and to measure after a 40-d period the remaining leaf biomass and the C, N, and P content in the remaining leaf and in debris.

### *Submersion, Macrofauna and Large Areas Promote Leaf Litter Decomposition*

The mineralization of litter material is a well-studied topic, in particular in perennial rivers as it mobilizes nutrients and represents the engine of downstream processes as pelagic and benthic primary production by phytoplankton, algae and macrophytes (Vannote et al., 1980). Under submersion conditions, mineralization primarily depends on the activity of macrofauna, and its rate is regulated by organic matter quality, water temperature and nutrient availability (Dehedin et al., 2013; Ferreira et al., 2011; Mas-Martí et al., 2015). Recent intermittent stream studies as that presented here have a different perspective as they focus the effects of river discontinuum on litter mineralization (Lake, 2003; Datry et al., 2014). Of special concern for riverine ecology is the understanding of how hydrologic interruptions locally affect river processes and communities and, as a consequence, affect downstream functioning. Drying is expected to be a major concern for low-order and

eventually for high order streams due to climate change. Stream intermittency represents a major discontinuum (Datry et al., 2014) producing poorly studied cascade of consequences for whole riverine ecosystems, in particular on allochtonous organic matter processing (Lake, 2003; Tzoraki et al., 2007). This study demonstrates interesting combined effects of a short-term drying event, presence of macrofauna and leaf size, on leaf litter mineralization and its elemental composition. Results of the statistical analyses suggest that these three factors are sometimes interplaying, as indicated by significant interaction terms (e.g. macrofauna x leaf size for oxygen demand and N content in residual biomass or macrofauna x drying on P percentage in residual biomass). Results confirm findings from different studies addressing single factors as regulators of decomposition rates (Corti et al., 2011; de Souza Rezende et al., 2018; Dehedin et al., 2013). In particular, highest rates of biomass loss during the nearly 40 days of incubation of the litter material were recorded in microcosms permanently submerged containing leaves with larger diameter and macrofauna. On the contrary, the lowest biomass loss was recorded in dried leaves incubated without macrofauna. Underlying reasons are from one side the larger surface available for macrofauna shredding activity, resulting in leaves fragmentation, higher release of nutrients and higher colonization and combined activity by fungi and microbes and, at the other extreme, the disappearance of macrofauna and the interruption of the activity of biofilms. Results from this experiments also demonstrate the different paths of leaf-associated C, N and P along the course of the incubation, with the dynamics of the residual matter largely overlapping C content and the dynamics of N and P showing different patterns, ruled by the high C:N and C:P ratios of the original material and the need to import (from the aquatic compartment) additional N and P to allow for microbial decomposition of otherwise recalcitrant material. Here is the key role of macrofauna as facilitator of microbial activity through nutrient recycling. Slower decomposition rates are often reported under dry conditions primarily because emersion causes the cessation of water-dependent processes (Corti et al., 2011; Kaushik et al., 1971), with negative impacts on macrofauna community and its stimulatory activity on microbes and changes of litter quality (Corti et al., 2011; Mora-Gómez, 2014). During dry periods abiotic processes such as physical abrasion, and photodegradation or biotic processes as degradation by terrestrial macrofauna (Corti et al., 2010) may become dominant in driving leaf-litter decomposition (Austin et al., 2006). In this study the most critical aspect of drying regards changes in microbial decomposers and invertebrate detritivores activity, as suggested by low rates of respiration measured after rewetting. In agreement with previous studies in which negative relationship between litter breakdown and emersion was primarily attributed to negative

effects of drying on decomposers communities and to elimination of drying-sensitive shredders (Corti et al., 2011; Foulquier et al., 2015), results show a two-fold higher biomass loss in the microcosms with macrofauna. Drying strongly alters growth, activity and functional aspects of decomposers community colonizing and decomposing leaf litter. Such alteration affects organic matter mineralization by temporarily limiting metabolic activity and reducing microbial biomass (Mora-Gómez et al., 2018; Bruder et al., 2011; Langhans et al., 2006). The net effects of drying events likely depend on their duration [50] and severity [49] as macrofauna can only temporary find refuge areas as temporary pools or migrate vertically in the hyporheic zone (Datry et al., 2012). Mora-Gómez et al. (Mora-Gómez et al., 2018) demonstrated that short term emersion of leaf litter in temperate streams may reduce decomposition rates by 34% 54% and 72% after 7, 14 and 21 days of drying. In the same study it was demonstrated that fungal and bacterial biomass dynamics changed significantly only after 21 days of emersion and recovered quickly after rewetting (Mora-Gómez et al., 2018; Langhans et al., 2006). However, only a minor part of the initial live cell biomass was available to immediately start the reactivation of the aquatic microbial food web (Amalfitano et al., 2008) and some bacterial enzyme activities were significantly reduced after seven days of emersion and may remain affected after rewetting (Mora-Gómez et al., 2018). Results from the present study confirm that even short/term drying events, lasting ten days, are enough to slow biological processes, reducing litter breakdown by nearly 50%. Flux measurements performed after the drying simulation suggest that emersion would not only decelerate decomposition and enlarging the time leaf litter takes to be decomposed in the stream. Emersion also affects microbial assemblages and activity on leaf substrate, reduces quality of leaf litter as a resource with consequences to detrital food webs (Mora-Gómez et al., 2018). The results obtained are realistic for areas where drying sets to zero the macrofauna community. *Potamophylax cingulatus* has a univoltine reproductive cycle so, there is one generation per year. If the streambed becomes completely dry, organisms can find refuge in residual pools, die or become adults as a response of environmental stress, and the community temporarily disappears. Data from monitoring macrofauna in intermittent streams suggest that the recovery of community needs at least few weeks, whereas more time is needed for restoring the initial density of organisms. By removing macrofauna after drying I simulated a situation in which shredders community migrates or emerges as adults, which is realistic for different areas of the study site and shortly after rewetting. I acknowledge that under other environmental settings or with other organisms the effects of drying can be less marked as macrofauna community can restore rapidly. Moreover, at some sites macrofauna can contribute nutrient cycling

with its dead biomass, something that was not considered here. The relevance of macrofauna for leaf breakdown process is well known (Gessner et al., 1999; Graça et al., 2001) as well as their role in recycle and translocation of nutrients (Vanni et al., 2002). Nutrient flux measurements, in particular during the early stage of this experiment, suggest that shredders living on leaf litter caused local nutrient enrichment in the sand used as substrate in microcosms and within leaf pack in which they feed through excretion and nutrient regeneration, stimulating microbial growth. Nutrient excreted by shredders, especially in oligotrophic conditions, are rapidly immobilized by microbes growing on leaves and decomposing the same resource (Villanueva et al., 2012). Because shredders nutrition is highly dependent on leaf-litter microorganisms (Graça et al., 2001), conditioned leaves become more attractive as food source to aquatic consumers (Cummins, 1974). This positive effect on fungi and bacteria would imply a positive feedback on shredders and in turn on decomposition rates (Villanueva et al., 2012).

In addition, leaf size influenced decomposition processes due to litter surface available for microbial colonization, that increases leaf litter palatability to shredders (Cummins, 1974). In the present experiment I used two different leaf sizes and I found higher respiration, nitrogen regeneration and slightly higher values of biomass loss in larger leaves. Results may be realistic for the litter material employed and for the macrofauna species selected but not always. In fact, other studies report higher soluble compounds release and faster degradation in small litter fragments characterized by high surface-area-to-volume ratio, due to higher microbial colonization (de Souza Rezende et al., 2018; Bärlocher et al., 1983). Overall, results from this study are relevant as under climate change scenario drying events will be more frequent and more prolonged (Datry et al., 2014; Poff et al., 2010) and the understanding of their consequences for low order stream metabolism is central.

*What Fluxes Tell us About Decomposition*

Results from single organism incubations were used to assess the share of *P. cingulatus* metabolic activity in the measured benthic oxygen demand and nutrient release, and the percentage of measured flux due to the contribution of macrofauna activity. Oxygen consumption and ammonium excretion by *P. cingulatus* individuals were upscaled and converted to square meter, using the macrofauna densities adopted in this study (4000 ind $m^{-2}$). Oxygen consumption and ammonium excretion by macrofauna was estimated in -2.2 ± 0.3 mmol $O_2$ $m^{-2}$ $h^{-1}$ and 127 ± 25 µmol $NH_4^+$ $m^{-2}$ $h^{-1}$, respectively, whereas phosphorous excretion, calculated from literature data (Hall et al., 2007) averaged 34.6 µmol $PO_4^{3-}$ $m^{-2}h^{-1}$. Macrofauna-mediated fluxes derived from *P. cingulatus* alone incubations are much

larger than those calculated combining measured fluxes in this experiment (e.g. L1_M-L1 oxygen, ammonium or reactive phosphorous fluxes in Figure 1.2). In particular, they overestimate animal contribution by 216% ± 48, 368% ± 246 and 1018% ± 690.5, respectively. Such overestimation may be due to higher activity of macrofauna when incubated without a substrate: organisms likely do not feel comfortable in a glass bottle with filtered water and move much more than under natural conditions. This was found also in other analogous experiments (Ruginis et al., 2014). Concerning nutrients, I add another explanation. In fact, the degree of overestimation should be similar to that of oxygen, but it's much higher. My interpretation is that in microcosms with litter and macrofauna, at the beginning of the experiment, a major fraction of the excreted $NH_4^+$ and $PO_4^{3-}$ is immediately recycled by growing biofilms in the litter biomass and does not accumulate in the water column. Towards the end of the experiment the large difference in the fluxes measured in the conditions with and without macrofauna recorded at day 5 are on the contrary much smaller, likely due to a general decrease of the activity of macrofauna and the microbial communities, following a marked decrease in the quality of residual litter or due to the aging of the experiment. Overall, large differences between calculated and measured fluxes may be due to macrofauna excretion-mediated higher retention of nutrient in the early stage of decomposition process, to microbial uptake from water column and biofilms growth, that rapidly immobilized nutrient excreted by macrofauna. If this is true for the early stage of decomposition process, towards the end of the experiment litter quality is poor and macrofauna and biofilm activity decreases. Such explanation fits with the results of the leaf litter analysis at the end of the experiment, that is highly enriched in N and P and displays a different nutrient stoichiometry at the end of the experiment as compared to the original material (Melillo et al., 1984; Chauvet, 1987). Microbial processing of leaf litter may liberate or sequester nutrients depending on characteristics of the microbes and their resources (Kaushik et al., 1971). Leaves with high C:N and C:P ratios (nearly 105 and 4013, respectively) were used and microbes may satisfy part of their nutrient demand by removing nutrients from the water column if either N or P is insufficient in the substrate. Results revealed a general decrease in C:N and C:P molar ratio in leaf litter after forty days of decomposition as a result of N and P increase, coupled with C decrease. Other studies have found this pattern for N and P, while C remained constant (Abril et al., 2016; Webster et al., 2009; Martínez et al., 2015). Permanently submersed conditions and the presence of macrofauna allowed the continuous colonization of leaf litter resulting in higher quality of resources (lower C:N and C:P), compared to drying. Decreased benthic respiration after drying simulation suggests the interruption of

biological processes, slowing down leaves biomass loss and affecting leaves elemental composition and nutrient dynamics. On the other hand, measured fluxes after rewetting phase show that rewetting episodes can mobilize labile substances from residual leaves, lysing dead microbial cells and leaching dissolved nutrients and soluble compounds (Wilson et al., 2008). This could explain higher C:N and C:P in residual biomass subjected to drying, suggesting a possible downstream transport of low quality organic matter with potential effects on detrital food webs and associated ecosystem processes (Datry et al., 2018).

## CONCLUSIONS

Drying and the presence of macrofauna were the most important factors determining changes in leaves and debris elemental composition. Leaves permanently submerged and conditioned by macrofauna had lower C and N content, while sand was enriched of both elements. This underlines the importance of water and the role of macrofauna in facilitating nutrient recycling, with impacts on stream food web and on ecosystem processes across multiple trophic levels. Through feeding activity macrofauna lead to decreased C, N and P content in leaves, transforming CPOM in FPOM, increasing the organic and nutrient content of the substrate on which they feed. In the natural environment such nutrient enriched residual litter would be mobilized and transported downstream, becoming available to the stream food web and sustaining secondary consumers. Permanently submersed conditions promote C and N release, while drying followed by rewetting leads to higher retention and slow release of particulate downstream. This may cause a significant alteration of nutrients stoichiometry, with cascading implications for primary producers and water quality. Under predicted global change scenarios, which are expected to increase the frequency and intensity of drying events, these findings suggest that hydrological intermittence could change streams ecosystem functioning by altering the capacity of benthic fauna to process detritus. In turn, this will impair ecosystem processes and related services.

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# Chapter 2: Nutrient dynamics in hyporheic zone

In all streams and rivers, hydrological connectivity, defined as the "water-mediated transfer of matter, energy, or organisms within and/or between elements of the hydrologic cycle" (Pringle 2003), has three spatial dimensions—longitudinal, lateral, and vertical—that interact along a fourth dimension, time (Ward et al. 1989). In intermittent rivers, cessation of surface flow disrupts hydrological connectivity in one or more spatial dimensions, with repercussions for most physical, chemical, and biological processes. Seasonal shifts in vertical interactions profoundly affect stream water biological communities and physico-chemical features.

Vertically, most of the exchange of water between the surface channel and the shallow saturated sediments below (i.e. hyporheic zone, White 1993) ceases when surface flow stops, impairing hyporheic processes (Datry and Larned, 2008; Boulton et al. 2010). Changes in vertical water exchange alter dissolved oxygen levels in the hyporheic zone that, in turn, influence the dominance of subsurface aerobic and anaerobic biogeochemical processes (Stanley and Boulton, 1995). Furthermore, desiccation may affect the microbial community that constitutes the majority of the biomass and activity in hyporheic ecosystems (Pusch et al. 1998; Fischer and Pusch 2001). In hyporheic sediments, biofilms are responsible for most of the metabolic activity, organic matter processing and biogeochemical processes (Findlay et al. 1993; Storey et al. 1999; Mermillod-Blondin et al. 2005).

Hyporheic biofilms are considered crucial components of the global biogeochemical fluxes of carbon, nitrogen, and phosphorous (Battin et al. 2008). Biofilms degrade large quantities of organic matter, releasing carbon dioxide to the atmosphere, and also denitrify nitrate, emitting nitrous oxide and nitrogen gas to the atmosphere (Mulholland et al. 2008).

Sediment desiccation has been shown to directly affect biofilms, leading changes in microbial biomass, community composition, metabolic rates and functioning (Fierer et al. 2003; Rees et al. 2006; Amalfitano et al. 2008; Zoppini et al. 2010).

In this chapter I will focus on the effects of hyporheic sediment desiccation on oxygen demand, denitrification rates and nutrient regeneration. To this purpose, permanently inundated and hydrologically intermittent river sediments were collected, packed in columns and incubated for several weeks.

# Effects of drying and rewetting on stream hyporheic metabolism and nutrient cycling

## INTRODUCTION

Water scarcity and drought are among the most pressing environmental challenges of the 21st century. Intermittency affects not only Mediterranean and arid regions but also streams and rivers in temperate regions, where extended periods of desiccation have been observed (Wilby et al. 2006; Sutherland et al. 2008). In the last few decades, the intensity and frequency of droughts have increased dramatically also across Europe, due to global climate change and enhanced anthropic pressures causing perennial streams to shift to intermittent flow regime, with cascading effects on subsurface flow, water and oxygen saturation level, biological communities and associated processes (Lange and Haensler 2012).

Flow is a key driver of the structure and function of aquatic ecosystems (e.g. Vannote *et al.*, 1980; Poff & Zimmerman, 2010) and intermittency, as an extreme form of flow variation, has pervasive ecological effects on freshwater ecosystems (Larned et al. 2010). During droughts, hydrological connectivity along the stream network is broken as some stream reaches undergo total desiccation. With increasing of dry periods groundwater inputs might be reduced causing the hyporheic sediments desiccation.

The hyporheic zone is a principal component of stream ecosystems (Grimm and Fisher 1984; Jones and Holmes 1996; Krause 2011) and because of the high exchange of water, the large surface of biofilm-water interfaces and the high activity of microbes, it plays a key role as a reactive zone, promoting high turnover rates of organic matter and the exchange of oxygen and nutrients, as well as providing habitat for benthic communities of macroinvertebrates (Boulton et al. 1998; McClain et al. 2003).

Sediment desiccation has been shown to lead to changes in microbial biomass, community composition, metabolic rates and functioning, which could have an impact on biogeochemical processes (Fierer et al. 2003; Rees et al. 2006; Amalfitano et al. 2008; Zoppini et al. 2010). Desiccation deeply alter the chemical environment of the hyporheic zone as it removes water and introduces air, changing dramatically the levels of oxygen.

During the dry phase, increasing oxic conditions may favour aerobic pathways, such as nitrification, that leads to $NO_3^-$ production. Anaerobic activities such as denitrification are inhibited and hyporheic sediments change from a $NO_3^-$ sink to a potential $NO_3^-$ source to surface waters (Stanley and Boulton 1995; Gómez et al. 2012).

Furthermore, studies targeting the effects of desiccation on stream epilithic biofilms report high bacterial mortality at extreme sediment desiccation and the increase of N and P released by intracellular solutes (Baldwin and Mitchell 2000), resulting in enhanced N and P availability.

With flow resumption, rewetting of desiccated sediments can dissolve these mineral nutrients and can rapidly stimulate sediment biogeochemical processes that may result in a release of inorganic carbon ($CO_2$), inorganic nitrogen (N) and phosphorous ($PO_4^{3-}$) to streamwater column, that may move downstream (Steinman et al. 2014) leading to eutrophication into receiving downstream water bodies. Furthermore, nitrate release and availability have also been shown to rapidly stimulate denitrification (Austin et al. 2004; Schwinning and Sala 2004; Arce et al. 2013, 2014, 2015), with variable time lag, depending on the microbial communities capacity to recovery after desiccation and on oxygen levels. Therefore, the shift between dry and wet phases might results in pronounced differences in the capacity of the hyporheic zone to process the organic matter, in alternating periods characterized by nutrient retention and nutrient release and in different capacity to remove nitrogen via denitrification. Given the importance of the hyporheic zone in streams and rivers processes and its potential in controlling $NO_3^-$ export through high denitrification rates, the study of its dynamics is a fundamental step toward a better understanding of lotic ecosystem functioning.

The effects of drying on microbial metabolism and the implications on nutrients dynamics have been widely investigated in rivers and streams (e.g. Amalfitano *et al.*, 2008; Marxsen, Zoppini & Wilczek, 2010; Timoner *et al.*, 2012, 2014; Sabater *et al.*, 2016) but this knowledge is limited to the surface processes and communities while few studies have evaluated the effects of intermittency on subsurface processes.

Here I wanted to study short-term temporal dynamics of biogeochemical processes during rewetting of hyporheic dried sediments, focusing on how processes and nutrient release from hyporheic zone to surface water change after rewetting, in order to quantifying the contribution of sub-surface sediments to flush peaks of nutrients that may affect stream water quality (Skoulikidis and Amaxidis 2009; Merbt et al. 2016). Furthermore, I wanted to compare perennial and intermittent stream processes in their respective resistance (the degree to which processes remains unchanged after a disturbance) and resilience (the rate at which processes returns to its initial state) to non-flow period to understand whether repeated exposition to drying changes the response of the stream community. The resistance and recovery of the microbial processes after rewetting are relevant for the maintenance of the river function. Communities inhabiting intermittent rivers may be adapted to the

environmental stress of drying-rewetting periods and may be more resilient if compared to stream ecosystems that rarely undergo water level fluctuations (Schimel et al. 2007; Amalfitano et al. 2008; Marxsen et al. 2010; Arce et al. 2015).

This study was designed to explore the response of microbial community processes to rewetting of hyporheic sediments using the sediment core perfusion technique (Fiebig and Marxsen 1992; Marxsen and Fiebig 1993; Marxsen et al. 2010). This approach, that simulates the natural process of groundwater diffusion through the sediments, allows to measure microbial activities in hyporheic sediments under controlled conditions.

Temporal dynamics of nutrient regeneration after rewetting process were observed and analysed to answer four main questions. (1) Are microbial activity and nutrient recycling in hyporheic zone influenced by desiccation and rewetting? (2) Are microbial activities different in perennial and intermittent streams at the beginning of rewetting? (3) Does microbial activity recover differently from desiccation in perennial and intermittent streams? And (4) How fast does microbial metabolism recover?

I hypothesize that a) hyporheic biofilms and microbial communities and their associated activities are influenced by desiccation and re-wetting, with effect on nutrient dynamics and biogeochemical processes due to a change in sediment redox conditions; b) intermittent and perennial microbial communities and their associated activities differ in their response to desiccation and in their capacity to recover after drying due to communities adaptions to drying periods.

METHODS

*Study site and sampling strategy*

Sediment samples were collected from 10 perennial and 10 intermittent streams located in Austria (Figure 2.1; Table 2.1) in the region of Burgenland (2 perennial, 2 intermittent), Styria (3 perennial, 3 intermittent), Carinthia (2 perennial, 2 intermittent) and Lower Austria (3 perennial, 3 intermittent), during the flowing phase (May 2019). The selected streams were located across a land use gradient: Streams in Burgenland and in Styria were influenced by agriculture, small, shaded and dominated by fine sediments, while streams located in Carinthia and in Lower Austria were large, open, oligotrophic and dominated by gravel. Sediments were collected to a depth of 10 to 20 cm, removing the upper sediment layer, sieved (2 – 4 mm mesh size sieve) and packed into the reactors in the field.

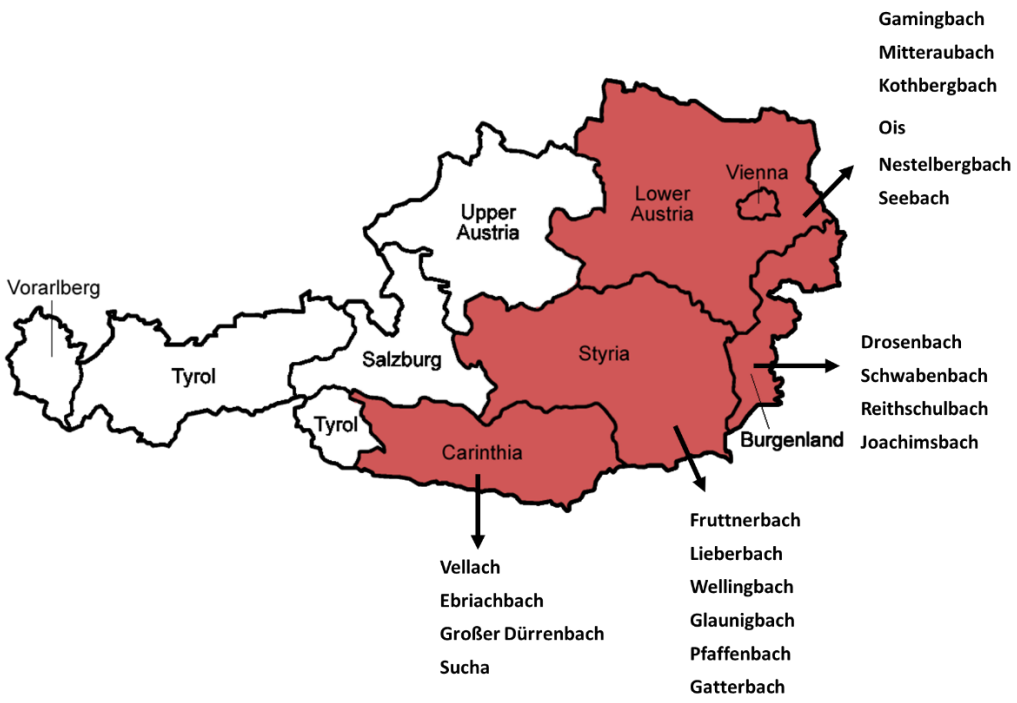


**Figure 2.1.** Sampling area (red) and location of the selected streams.

**Table 2.1** Sampling sites, hydrology (perennial/intermittent) and stream code.

| Region | Name | ID | Type | Coordinates |
|---|---|---|---|---|
| **Burgenland** | Reithschulbach | RB | Intermittent | 46°55'35.2"N 16°10'27.6"E |
| | Joachimsbach | JB | Intermittent | 46°55'23.7"N 16°09'11.7"E |
| | Drosenbach | DB | Perennial | 46°54'17.4"N 16°08'03.7"E |
| | Schwabenbach | SB | Perennial | 46°55'08.2"N 16°04'35.7"E |
| **Lower Austria** | Ois | OIS | Intermittent | 47°49'23.5"N 15°07'45.3"E |
| | Nestelbergbach | NB | Intermittent | 47°53'31.6"N 15°11'55.5"E |
| | Gamingbach | GB | Perennial | 47°54'39.2"N 15°05'31.8"E |
| | Mitteraubach | MB | Perennial | 47°55'00.8"N 15°04'34.6"E |
| | Seebach | MSB | Intermittent | 47°50'39.6"N 15°04'17.2"E |
| | Kothbergbach | KBB | Perennial | 47°52'58.3"N 15°00'21.2"E |
| **Styria** | Fruttnerbach | FB | Perennial | 46°47'37.0"N 15°56'51.9"E |
| | Glaunigbach | GLB | Intermittent | 46°44'41.6"N 15°48'06.4"E |
| | Lieberbach | LB | Perennial | 46°46'30.6"N 15°39'34.8"E |
| | Pfaffenbach | PB | Intermittent | 46°45'34.30"N,15°37'35.36"E |
| | Gatterbach | GAB | Intermittent | 46°48'03.3"N 15°36'36.3"E |
| | Wellingbach | WB | Perennial | 46°46'10.0"N 15°29'24.3"E |
| **Carinthia** | Sucha | SU | Intermittent | 46°33'36.0"N 14°39'00.3"E |
| | Vellach | VE | Perennial | 46°27'02.0"N 14°34'09.9"E |
| | Ebriachbach | EB | Perennial | 46°28'35.1"N 14°31'51.2"E |
| | Großer Dürrenbach | GDB | Intermittent | 46°31'28.4"N 14°06'15.3"E |

*Experimental setup and procedures*

The experimental set-up consisted in 20 transparent Plexiglass cores (50 cm long, 4 cm inner diameter), covered immediately after sediment sampling with black plastic coating, closed airtight and installed in a vertical position (Figure 2.2). Well-mixed and aerated water was pumped from one storage tank through the cores from bottom to top via a peristaltic multi-channel pump operated in flow-through mode (pump rate 1 ml/min). As it was practically impossible to collect water from the 20 sampling sites in amounts sufficient for the entire experimental period (11 weeks), the reservoir that feed the reactors contained water collected from a surficial well in the proximity of the laboratory. Such water had a comparable chemistry to the water at the sampling sites. Water temperature was monitored continuously by HOBO temperature data logger (Onset Computer Corp., Pocasset, MA).

All mesocosms were exposed to three consecutive hydrological conditions (Figure 2.3): a) flowing phase, in which water was pumped for two weeks to guarantee the biofilm adaption to the chemistry of the reservoir water and in general to the laboratory conditions and to test the reference conditions for each stream; b) drying phase, in which mesocosms were left open without water for 7 weeks (dry phase). During this phase, sediment water content in each reactor was monitored continuously with humidity soil sensors (Capacitive Soil Moisture Sensor v1.2) placed in the sediment at the top of each reactor; c) rewetting phase, in which water was recirculated in each mesocosm for 2 weeks. During the rewetting phase four samplings were carried out: at day 1, 3, 7 and 14 after rewetting (Figure 2.3). Before each sampling, 4 mg/L of DOC in the form of leaf leachate and $Na^{15}NO_3$ 20 mM stock solution to a final concentration of 50% of initial nitrate pool in groundwater were added to the tank (Figure 2.2c), in order to increase the dissolved organic pool and measure denitrification rates, respectively. Based on preliminary tests addressing the water residence time in the reactors, water samples at the reactors inlet and outlet were collected after a time interval of 12 hours. Such lag was the time needed for water at the inlet to cross the whole reactor at the flow rate set by the peristaltic pump.

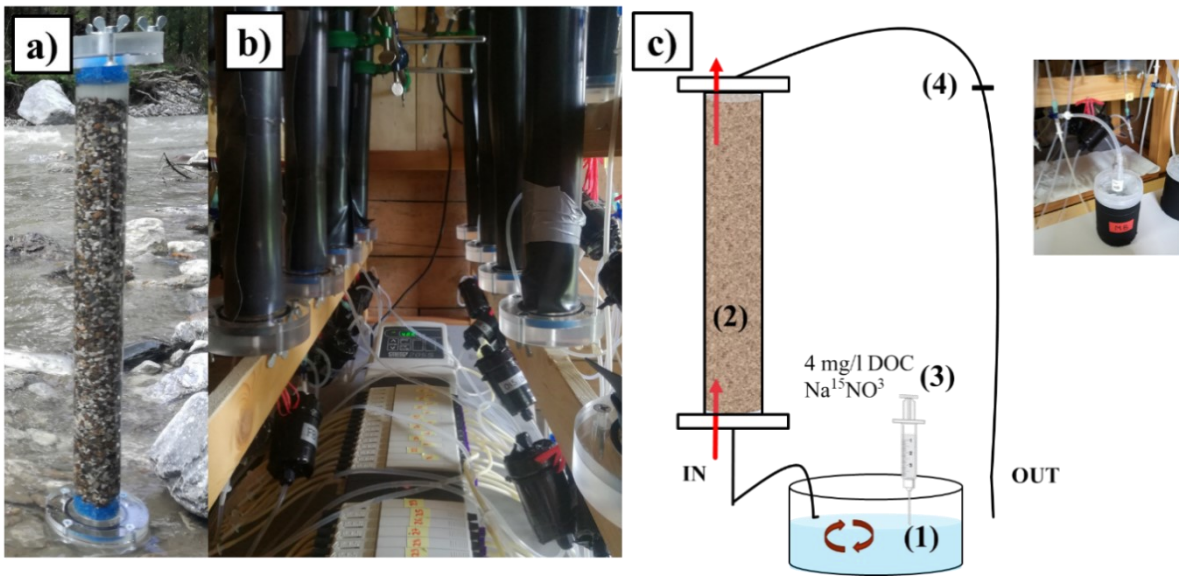


**Figure 2.2.** Experimental set-up: hyporheic reactor (a), experimental set-up (b) and sampling procedures (c). The hyporheic reactors used in this experiment were made with transparent Plexiglass and filled with stream hyporheic sediment (a). After sediment sampling, all 20 mesocosms were covered with black plastic coating in order to keep sediments in dark conditions and connected to a peristaltic multi-channel pump operated in flow-through mode (b). During the flowing phase groudwater was pumped from a 20 L tank (c1) through the cores from bottom to top (c2). Before each water sampling 4 mg/L of leaf leachate and $Na^{15}NO^3$ 20 mM were added to the tank (c3) and water samples were collected from the tank (inlet, c1) and the outlet (c4) of each reactor nearly 12 hours later.

The 20 reactors underwent the same experimental procedure and timing reported in figure 2.3.

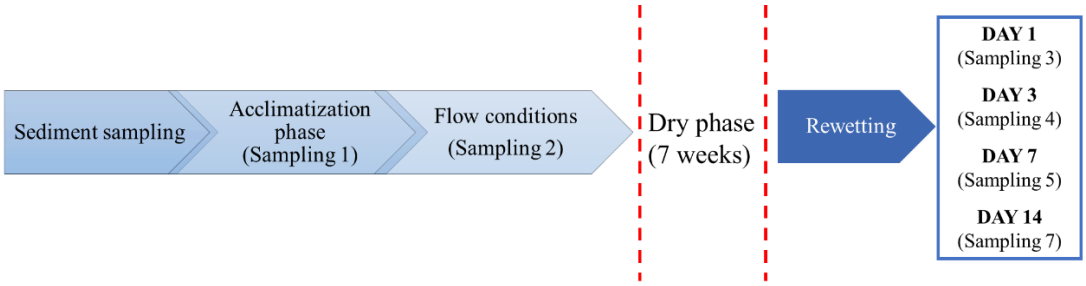


**Figure 2.3.** Experimental steps and timing

At the end of the experiment, sediments from each stream were analyzed for organic matter content and grain size.

*Analytical methods*

Each water sample from each reactor underwent the same processing at each sampling date: an aliquot of 60 ml was transferred to three 12-ml exetainers (Labco Scientific, UK), the first for $CO_2$, which was immediately titrated via six end points 0.1 N HCl microtitration (Anderson et al. 1986), the second for dissolved $O_2$, $^{29}N$, $^{30}N$ and $CH_4$ and the third for dissolved metals ($Fe^{2+}$ and $Mn^{2+}$) analysis. The sample for dissolved gas was added with 100-µl of 7 M $ZnCl_2$ to stop microbial activity whereas the sample for metals was added with 100 µl of concentrated $HNO_3^-$. Another aliquot of 20-ml was collected from the inlet and from the outlet, filtered (GF/F glass-fibre filters, 0.7 µm) and transferred into 20-ml plastic vials in order to analyse dissolved inorganic nutrients. Dissolved gas were analysed by membrane inlet mass spectrometer (MIMS, Bay instruments, USA) (Schlüter and Gentz 2008). Dissolved $N_2$ concentrations were calculated from obtained $N_2$:Ar ratio and theoretical Ar concentration (Weiss 1970). Dissolved metals were analysed by atomic absorption (Varian AA240). Dissolved inorganic nutrients ($NH_4^+$, $NO_3^-$, $NO_2^-$, $PO_4^{3-}$, $SiO_2$) were analysed spectrophotometrically using standard colorimetric methods (Koroleff and F 1970; Golterman et al. 1978).

Gas, metals and nutrient fluxes (F) were calculated by the general flux equation at steady state:

$$F = \frac{([x]_f) - ([x]_i)}{V} \times R$$

where $[x]_f$ and $[x]_i$ are the concentrations in the effluent and influent water (µM), R the flow rate (L/h), and V is the sediment volume in each reactor ($m^3$).

Denitrification was measured by the isotope pairing technique in flow-through systems and rates of denitrification were calculated from $^{29}N_2$ and $^{30}N_2$ fluxes (29F and 30F, respectively) (Risgaard-Petersen et al. 1998).
Denitrification of $^{15}NO_3$ (D15) was calculated directly:

$$D15 = 29F + 2 * 30F$$

Denitrification of $^{14}NO_3^-$ (D14) was calculated using the expression of Nielsen (1992) (Nielsen 1992), assuming ideal binomial distribution of 28F, 29F and 30F:

$$D14 = \frac{29F}{2 \times 30F} \times D15$$

Total denitrification ($D_{tot}$) was then calculated as the sum of D14 and D15.

*Data analysis*

The simultaneous effects of stream type, day and organic matter content on the measured parameters ($O_2$ and $CO_2$ fluxes, nutrient fluxes and denitrification rates) were analysed by a linear mixed effect model to take into account temporal dependency among replicates, using the function lmer of the R statistical environment.

Stream hydrology (perennial/intermittent), the sampling day and sediment organic matter content were selected as fixed effects, while the sampling site was included as a random effect. Statistical tests were considered significant at $P < 0.05$.

All analyses and graphs were performed with the statistical software R (R Core Team 2018), with base version and with ggplot2 (Wickham 2009) and lmerTest packages (Kuznetsova et al. 2017).

RESULTS

*Stream sediments features*

In terms of grain size, sediments extruded from the reactors were dominated (60-95%) by coarse fractions (2-4 mm), while sediment organic matter content ranged from 0.37 to 2.27%. Sedimentary features of each sampling site are presented in Table 2.2.

During the 7 weeks desiccation period the level of humidity in the reactors ranged between 3.8% and 58%. Such level of humidity was measured in the upper portion of the reactors using humidity sensors. From visual inspections the lower portion of the reactors contained variable levels of water.

**Table 2.2** Sediment organic matter content (OM) and grain size of the selected streams

| | **OM (%)** | **Grain size classes (%)** | | |
|---|---|---|---|---|
| | | **4 – 2 mm** | **1 – 0.125 mm** | **<0.125 mm** |
| **MB** | 0.37 | 93.52 | 6.36 | 0.12 |
| **GB** | 1.49 | 86.33 | 13.51 | 0.16 |
| **DB** | 0.41 | 83.59 | 16.04 | 0.37 |
| **SB** | 0.23 | 80.44 | 19.36 | 0.19 |
| **KBB** | 0.35 | 82.9 | 16.89 | 0.2 |
| **LB** | 0.21 | 69.48 | 30.33 | 0.19 |
| **FB** | 2.27 | 92.38 | 6.59 | 1.03 |
| **EB** | 0.38 | 80.76 | 19.19 | 0.04 |
| **VE** | 0.67 | 93.19 | 6.67 | 0.14 |
| **WB** | 1.08 | 62.07 | 37.6 | 0.33 |
| **OIS** | 0.27 | 71.5 | 28.42 | 0.07 |
| **RB** | 0.59 | 61.31 | 37.16 | 1.53 |
| **NB** | 0.43 | 93.33 | 6.55 | 0.12 |
| **PB** | 1.02 | 91.35 | 5.9 | 2.74 |
| **GDB** | 0.25 | 87.64 | 12.33 | 0.03 |
| **GLB** | 0.75 | 94.32 | 5.22 | 0.46 |
| **GAB** | 0.53 | 73.81 | 25.2 | 0.98 |
| **JB** | 1.26 | 95.3 | 4.18 | 0.52 |
| **MSB** | 0.34 | 92.54 | 7.43 | 0.03 |
| **SU** | 0.31 | 90.25 | 9.72 | 0.03 |

*Microbial respiration and nutrient dynamics*

Results from the mixed effect models (Table 2.4) showed a temporal variation of the measured parameters after drought, without significant differences between perennial and intermittent streams (Table 2.4). Therefore, only graphs from pooled data (intermittent and perennial streams together) are reported, in order to highlight nutrient and processes temporal dynamics after the drought simulation. The analyses revealed insignificant production of $CH_4$, $Fe^{2+}$ and $Mn^{2+}$ during the whole experiment; in most of the samples concentrations were below the instrumental detection limits (2 nM, 1 µM and 0.5 µM, respectively). Fluxes of these chemical forms are therefore not reported.

Oxygen consumption, $CO_2$ and nutrient fluxes varied temporally (Figure 2.4). At day 1 and 3 oxygen consumption was lower than pre-drought conditions, showing a decrease from -490.9 ± 47.4 µmol $O_2$ $m^{-3}$ $h^{-1}$ (mean ± standard error) during pre-drought condition to -223.2 ± 17.9 µmol $O_2$ $m^{-3}$ $h^{-1}$ at day 1 ($p<0.001$; Table 2.4) and -271.6 ± 35.8 µmol $O_2$ $m^{-3}$ $h^{-1}$ at day 3 ($p<0.001$; Table 2.4). At day 7 microbial respiration gradually increased until reaching values near pre-drought conditions at day 14. Statistical analyses revealed significant effects of sediment organic matter content on oxygen consumption, with increasing oxygen consumption along with increasing organic matter content ($p=0.03$; Table 2.4).

In general, measured $CO_2$ fluxes were very variable (Figure 2.4), and ranged from 374.2 ± 303.4 µmol $CO_2$ $m^{-3}$ $h^{-1}$ during pre-drought condition to 1188.3 ± 169.8 µmol $CO_2$ $m^{-3}$ $h^{-1}$ measured at day 14. Compared to the initial condition, $CO_2$ fluxes decreased at day 1 while they peaked at day 3, 7 and 14, with statistical differences between the pre-drought condition and the three sampling days ($p<0.001$; Table 2.4).

Also nutrient fluxes were very variable and, as a general tendency, a rapid nutrient mobilization was observed at day 1, followed by a quick return to the initial conditions.

The day after rewetting, $PO_4^{3-}$, $NH_4^+$ and $NO_3^-$ release in water increased, while $SiO_2$ release decreased (Figure 2.4). Despite the large variability between streams, $PO_4^{3-}$ fluxes slightly increased at day 1 compared to pre-drought condition (from 1.7 ± 0.5 to 2.4 ± 1.1 µmol P-$PO_4^{3-}$ $m^{-3}$ $h^{-1}$, respectively), but after the initial nutrient release, sediments became a P sink (-3.3 ± 0.7 µmol P- $PO_4^{3-}$ $m^{-3}$ $h^{-1}$ at day 14), with measured fluxes at day 3, 7 and 14 significantly lower than the initial condition ($p= 0.005$; $p<0.001$; $p<0.001$, respectively).

Results showed a rapid increase of $NH_4^+$ fluxes after rewetting, with values peaking from 4.3 ± 1.8 µmol N-$NH_4^+$ $m^{-3}$ $h^{-1}$ during pre-drought conditions to 47.8 ± 7.4 µmol N-$NH_4^+$ $m^{-3}$ $h^{-1}$ at day 1 ($p<0.001$; Table 2.4). From day 3 fluxes tended to decrease with lower values measured at days 3 and 14 compared to initial conditions ($p=0.0002$; $p<0.001$, respectively).

Furthermore, $NH_4^+$ fluxes tended to increase with increasing sediment organic matter content (p=0.01; Table 2.4). A similar trend was observed for $NO_3^-$ fluxes. As for $NH_4^+$, sediments changed from $NO_3^-$ sink to source, with higher nitrate release in water at day 1 (111. 2 ± 33.1 µmol N-$NO_3^-$ $m^{-3}$ $h^{-1}$; p<0.001, Figure 2.4, Table 2.4) and at day 3 they returned to pre-drought levels. A slight increase was observed also in $NO_2^-$ fluxes. Compared to pre-drought condition, results showed a significant increase of $NO_2^-$ fluxes at day 1, 3, 7 and 14 (p=0.002; p=0.001; p=0.0001; p=0.0004, respectively). In contrast with the general trend, reactive silica regeneration progressively decreased after rewetting, from 353.5 ± 40.6 µmol Si-$SiO_2$ $m^{-3}$ $h^{-1}$ measured before desiccation to -16.2 ± 37 µmol Si-$SiO_2$ $m^{-3}$ $h^{-1}$ measured at day 14 (Figure 2.4) and compared to the initial condition, $SiO_2$ fluxes were lower at day 1, 3, 7 and 14 (Table 2.4).

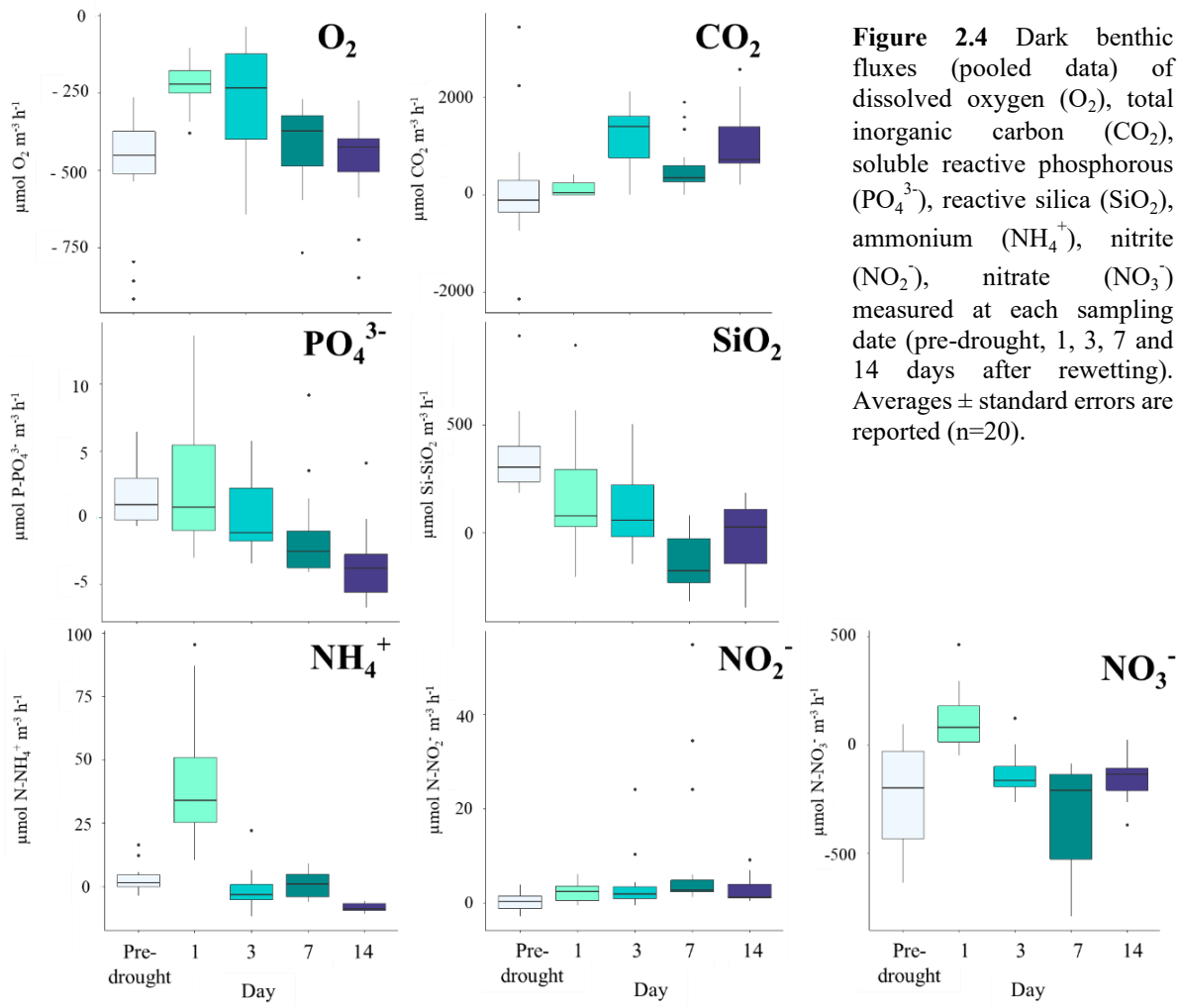


**Figure 2.4** Dark benthic fluxes (pooled data) of dissolved oxygen ($O_2$), total inorganic carbon ($CO_2$), soluble reactive phosphorous ($PO_4^{3-}$), reactive silica ($SiO_2$), ammonium ($NH_4^+$), nitrite ($NO_2^-$), nitrate ($NO_3^-$) measured at each sampling date (pre-drought, 1, 3, 7 and 14 days after rewetting). Averages ± standard errors are reported (n=20).

**Table 2.4** Results of the mixed effects models by testing the effect of day, stream hydrology and sediment organic matter content on the measured parameters ($O_2$, $CO_2$, $NH_4^+$, $PO_4^{3-}$, $SiO_2$, $NO_2^-$, $NO_3^-$); significant values are marked in bold.

| | $O_2$ | | | $CO_2$ | | | $PO_4^{3-}$ | | | $SiO_2$ | | | $NH_4^+$ | | | $NO^{2-}$ | | | $NO^{3-}$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Estimate** | **Std. Err.** | **p-value** | **Estimate** | **Std. Err.** | **p-value** | **Estimate** | **Std. Err.** | **p-value** | **Estimate** | **Std. Err.** | **p-value** | **Estimate** | **Std. Err.** | **p-value** | **Estimate** | **Std. Err.** | **p-value** | **Estimate** | **Std. Err.** | **p-value** |
| **Intercept** | -427.20 | 50.46 | **<0.001** | -203.33 | 175.28 | 0.25 | 1.59 | 1.1 | 0.16 | 248.4 | 49.8 | **<0.001** | 0.64 | 1.35 | 0.63 | -0.23 | 0.66 | 0.73 | -226.48 | 51.75 | **<0.001** |
| **Hydrology perennial** | 56.57 | 50.14 | 0.27 | 95 | 127.49 | 0.46 | 0.1 | 1.1 | 0.93 | 34.5 | 36.6 | 0.35 | 0.67 | 1.1 | 0.55 | -0.17 | 0.55 | 0.14 | -20.04 | 38.41 | 0.6 |
| **Organic content** | -11581.6 | 4876.5 | **0.03** | -8278.34 | 12194.56 | **0.5** | 50.24 | 107.6 | 0.64 | 8343.9 | 3590.5 | **0.02** | 305.92 | 108.86 | **0.01** | 60.32 | 54.65 | 0.28 | 1184.65 | 3706.11 | 0.75 |
| **Day 1** | 222.67 | 38.03 | **<0.001** | 330.93 | 206.96 | 0.11 | 0.46 | 0.75 | 0.54 | -181.36 | 59.02 | **0.002** | 32.15 | 1.78 | **<0.001** | 2.22 | 0.7 | **0.002** | 338.75 | 60.93 | **<0.001** |
| **Day 3** | 196.16 | 36.40 | **<0.001** | 1334.34 | 201.56 | **<0.001** | -2.2 | 0.75 | **0.005** | -275.8 | 56.6 | **<0.001** | -5.63 | 1.41 | **0.0002** | 2.29 | 0.7 | **0.001** | 90.54 | 59.22 | 0.13 |
| **Day 7** | 48.33 | 36.05 | 0.18 | 742.54 | 199.03 | **<0.001** | -3.3 | 0.75 | **<0.001** | -499.5 | 56.6 | **<0.001** | -2.18 | 1.37 | 0.12 | 2.84 | 0.7 | **0.0001** | -104.64 | 58.47 | 0.07 |
| **Day 14** | -19.61 | 38.55 | 0.61 | 1247.06 | 209.95 | **<0.001** | -5.26 | 0.75 | **<0.001** | -362.5 | 57.3 | **<0.001** | -10.72 | 1.46 | **<0.001** | 2.66 | 0.71 | **0.0004** | 77.25 | 63.03 | 0.22 |

*Denitrification rates*

Denitrification rates were in general very low and variable among streams (Figure 2.5). Rates varied from the limit of detection to 8.44 µmol N $m^{-3}$ $h^{-1}$. In general denitrification was close to zero during pre-drought condition (0.08 ± 0.03 µmol N $m^{-3}$ $h^{-1}$) and a slight increase in denitrification was observed immediately after rewetting at day 1 (0.7 ± 0.2 µmol N $m^{-3}$ $h^{-1}$). Denitrification was a minor N path in the reactors as rates resulted 1-3 orders of magnitude lower than nitrate fluxes reported in Figure 2.4.

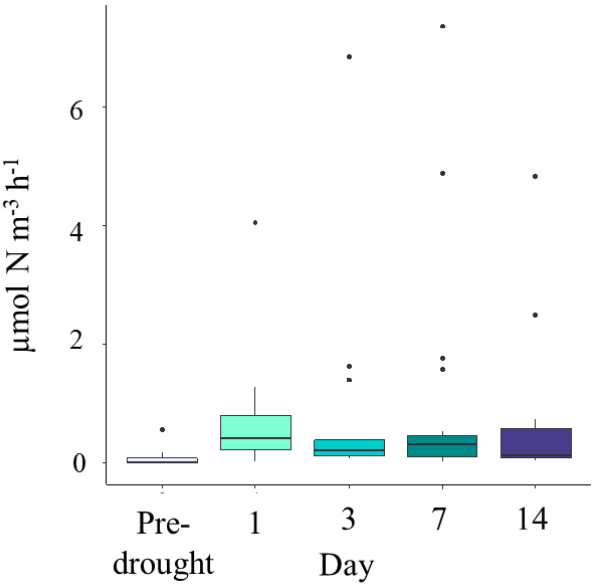


**Figure 2.5** Denitrification rates measured at each sampling date (pre-drought, 1, 3, 7 and 14 days after rewetting). Averages ± standard errors are reported (n=20).

Statistical analyses revealed higher denitrification rates 14 days after rewetting, compared to initial conditions ($p=0.006$) and a statistically significant effect of sediment organic matter content ($p<0.001$) in increasing these rates.

**Table 2.5** Results of the mixed effects models by testing the effect of day, stream hydrology and sediment organic matter content on denitrification rates; significant values are marked in bold.

| | Denitrification rates | | |
|---|---|---|---|
| | **Estimate** | **Std. Err.** | **p-value** |
| **Intercept** | 0.5 | 0.3 | 0.07 |
| **Hydrology perennial** | 0.04 | 0.23 | 0.87 |
| **Organic content** | 89.5 | 22.23 | **<0.001** |
| **Day 1** | 0.59 | 0.33 | 0.08 |
| **Day 3** | 0.63 | 0.34 | 0.06 |
| **Day 7** | 0.55 | 0.34 | 0.1 |
| **Day 14** | 0.95 | 0.34 | **0.006** |

## DISCUSSION

### *Suitability of the perfused cores technique*

This study was designed to explore the response of microbial community processes to rewetting of hyporheic sediments using the sediment core perfusion technique (Fiebig and Marxsen 1992; Marxsen et al. 2010). A perfused core system was applied in this study to explore the process of recovery and re-establishment of microbial processes in streambed sediments after desiccation. This approach, that simulates the natural process of groundwater percolation through the sediments, allows the development of microbial community and the measurement of microbial activities in hyporheic sediments under controlled conditions (Fiebig 1992, 1997; Marxsen and Fiebig 1993; Marxsen and Schmidt 1993; Marxsen 1996, 2001; Marxsen et al. 2010).

This experiment was conducted in mesocosms that inevitably simplify the processes occurring in the hyporheic volume, that is characterized by upwelling and downwelling zones and by carbon and nutrients inputs from surface and groundwater. However, the controlled conditions in which we operated allowed us to test experimentally the effects of desiccation in riverine systems that are permanently inundated and in systems that undergo hydrological intermittency.

As compared to in situ conditions, the major limits of the method employed deals with the loss of reactive organic matter and the difficulty of drying a closed mesocosm. Sediment

sieving is a necessary step before packing the columns to avoid their clogging and in order to have comparable conditions in terms of grain size across mesocosms. Sieving inevitably results in the loss of the more 'active', labile pool of particulate OM, which may affect (decrease) the rates of microbial activities. To contrast such effect, during each incubation, 4 mg/L of leaf leachate were added to the reservoir to ensure an adequate input of dissolved organic carbon, as it happens under in situ conditions. However, the low microbial metabolic rates that were measured during the experiment suggest either organic carbon or nutrient limitation or low nutritional quality of the added organic matter. The leaf leachate is likely enriched with C (2.851 g/l DOC) but it is N and P poor, which may explain the very low nutrient release during the experiment as compared to oxygen uptake, suggesting their assimilation and retention in microbial biomass. During a leaf decomposition experiments, an enrichment of N and P in the residual biomass was measured, contrasting the progressive C losses. Such N and P were retained from the water column or from the sediments due to low nutritional quality of the leaf material (Palmia et al., 2019). Another possible limitation of the method employed deals with the difficulty of reproducing in situ desiccation. In the natural environment, in the absence of surficial flow, the groundwater table progressively migrates downwards and the dry hyporheic volume increases. Under the experimental conditions that were reproduced, the 7 weeks during which the hydrological intermittency was simulated were not enough to remove all the interstitial water and dry all the sediment column, resulting in partial desiccation of biofilms.

*Microbial metabolism and nutrient fluxes*

Experimental results from this study confirms that 7-weeks desiccation affects microbial activity but highlight no difference between the two groups of rivers from which sediments were collected, contrarily to what was hypothesized. Such result may depend upon two factors. The first was discussed in the last part of the previous paragraph and deals with what happens in the natural environments VS what can be realistically simulated into the lab. The second deals with the plasticity of microbial communities. The latter are metabolically versatile and can switch to alternative biochemical pathways (e.g. from anaerobic to aerobic and vice versa) in real time and respond promptly to even large environmental changes. Moreover, due to their high grow rates and very short life cycles, microbial communities display rapid turnover and become structured much more fast than communities of larger organisms and recover consequently faster after extreme events like for example desiccation. For these reasons results from the present experiment did not reveal significant differences

between the microbial response of permanently inundated versus intermittently inundated sediments. On the contrary, what seems statistically robust and general for the two groups of systems considered in this study is the effect of the desiccation per se, with the implication on single cells metabolic activity.

Decreased microbial oxygen consumption after rewetting suggests the interruption of biological processes during desiccation. However, after three days, oxygen consumption rates returned to pre-drought conditions, suggesting that communities can quickly recover after desiccation. Assuming a $O_2$:$CO_2$ respiration ratio of 1:1, a similar but opposite trend for $CO_2$ fluxes was expeted. Other studies reported that rewetting of dry sediments through rain, groundwater upwelling, or surface flow resumption may represent a respiration "hot moment" (McClain et al. 2003) and may result in large instantaneous pulse of $CO_2$ compared to both the preceding dry phase and the subsequent extended flowing phase (Gallo et al. 2014; Marcé et al. 2019). Measurements of $CO_2$ fluxes in dried lowland canals evidenced increasing effluxes along with drier conditions, suggesting the importance of interstitial oxygen level on organic carbon mineralization (Palmia et al. 2021). These studies suggest that the regulation of $CO_2$ fluxes is complex and site-specific and likely depends on combinations of oxygen and water availability, organic matter quality and other biogeochemical processes that may consume $CO_2$ as nitrification or methanogenesis (Palmia et al. 2021).

I did not measure the instantaneous $CO_2$ release, as in the experimental design the first sampling was carried out 1 day after rewetting, but contrarily with expectations, results showed a $CO_2$ peak three days after rewetting, suggesting a lag phase. However, $CO_2$ fluxes measured at day 7 and 14 were very variable and exhibited a non-consistent temporal trend. Despite variability between the studied streams, a $NH_4^+$, $NO_3^-$ and $PO_4^{3-}$ pulse release one day after rewetting was observed, that is attributed to the mineralization of previously unavailable, easily decomposable organic substrates (Birch 1958).

The most abundant N species during the initial flow pulse was either nitrate or ammonium (Obermann et al. 2009; Skoulikidis and Amaxidis 2009; von Schiller et al. 2011) and the predominance of one or the other N species may strongly depend on the relative importance of mineralization, nitrification, and denitrification rates in sediments. In agreement with these findings, other studies carried out in surface stream sediment reported that rewetting may result in a nutrient pulse to stream water column (e.g. Baldwin and Mitchell 2000).

Hyporheic nutrient and OM may move downstream when flow resumes and their concentrations can increase several times above baseflow concentrations (Fisher and Minckley 1978; Tzoraki et al. 2007; Wilson and Baldwin 2008; Obermann et al. 2009; von

Schiller et al. 2011). Nevertheless, after this nutrient mobilization, results showed that sediments become nutrient sink.

A large pulse in nutrient release has a different meaning for river functioning than a lower but constant release. In the first case, which is likely to be the rule under desiccation-rewetting cycles, uptake or transformation processes can be saturated and the pulse can be transported downstream with limited benefit for the river community. In the second situation, moderate release can feed multiple processes along the longitudinal water path and contribute to element spiralling and river functioning.

The large uptake of nitrate after rewetting was uncoupled to $N_2$ production via denitrification. The absence of significant accumulation of molecular nitrogen, methane and reduced metals at the reactors outflow and the limited decrease of oxygen levels suggest very limited anaerobic metabolism in the pore water. This means that in the perspective of oxygen, water saturation or water absence affected only the availability of this electron acceptor but not its presence/absence.

Large nitrate uptake was therefore not due to respiratory needs of the microbial communities but to uptake and use of oxidized inorganic N as N source to bacteria in a N-limited environments as evidenced in other studies (Palmia et al. 2019). As I stressed before, it is likely that sediment sieving might have either decreased a major fraction of the microbial biomass or of the more labile pools of organic matter. The leaves leachate might also be nutrient poor, impairing C, N and P cycles in the hyporheic zone. A microbial strategy to overcome very high C:N or C:P ratios is to use inorganic nutrients from the water column, augmenting the nutritional quality of the organic substrate.

The peak in P concentration at day 1 tends to be lower compared to that of N, probably as a result of the organic matter elemental composition, the higher relative release of N over P from sediments and the presence of geochemical mechanisms (e.g. precipitation, complexation) that retain reactive P into the sediments (Tzoraki et al. 2007; Skoulikidis and Amaxidis 2009; von Schiller et al. 2011).

The results from reactive Si suggest as a general trend a decrease of its release along with the course of the experiment. At the beginning of measurements, large Si release tended to constantly attenuate. The biogeochemical regulation of Si is poorly understood in sediments and at present there are no univocal opinions on the net effects of the redox environment on Si retention/release (Siipola et al. 2016). Silica is generally described as an element with similar behaviour to that of phosphorus, but differently from the latter it doesn't undergo the same oxygen dependent retention/release. Results suggest limited effects of the desiccation/rewetting on its dynamics that seem more related to the initial sedimentary pool

of leachable Si. This is supported by the continuous decrease of production, which is also due to the experimental conditions (closed reactors without lithological inputs).

*Denitrification rates*

As in soils, the nutrient content and biogeochemical processing in the streambed sediments are strongly affected by desiccation. When sediments dry, the contact with the air creates an oxygenated environment that favours aerobic transformation processes (Mitchell and Baldwin 1999; Baldwin and Mitchell 2000). Along with the changes in nutrient concentrations and forms, many in-stream N transformation processes such as denitrification and nitrification are rapidly activated upon rewetting (McIntyre et al. 2009; Austin and Strauss 2011; Arce et al. 2015).

Despite the relatively low microbial activity, several studies have shown that sediment drying increased oxic conditions, that may favour aerobic pathways, such as nitrification, and restricts denitrification to anaerobic microsites, leading to the accumulation of inorganic N mainly in the form of nitrate (Gómez et al. 2012; Arce et al. 2015; Merbt et al. 2016). Thus, continuous nitrification during a period of general low biological N demand can provide $NO_3^-$ accumulation in stream sediments that may stimulate denitrification when flow returns (Gómez et al. 2012; Steward et al. 2012; Arce et al. 2014, 2015). Based on these findings, I expected a substantial $NO_3^-$ release from hyporheic sediments to the water column after rewetting, followed by a rapid increase of denitrification. I also predicted that sediment denitrification would be rapidly activated after rewetting, with the consequent modulation of $NO_3^-$ availability in water column. According to expectations, denitrification slightly increased after rewetting, but rates were very low and $NO_3^-$ accumulated in desiccated sediments was not processed immediately after rewetting via denitrification but was released to the water column.

However, this mobilization is transient and $NO_3^-$ fluxes returned to pre-drought level after three days.

In surface stream sediments, rewetting has been shown to rapidly stimulate denitrification in Mediterranean temporary streams (Austin et al. 2004; Schwinning and Sala 2004; Arce et al. 2013, 2014, 2015). Marxsen et al. (2010) observed the persistence of extracellular enzymes in sediments during drought and their reactivation after 4 days of rewetting in a Mediterranean stream; however, the abundance and activity of microbial communities had not completely restored. Other studies reported a rapid denitrification recovery after 4 h of

sediment rewetting, that was detected in a temporary headwater stream affected by a natural dry period of 4 months (Arce et al. 2013).

On the other hand, other experiments examining the response of denitrification after rewetting in temperate streams, reported more than 30 days lag between rewetting and rates returning to pre-dry levels (Zaman and Chang 2004), depending on the degree of sediment moisture which allows microorganisms to maintain their activity during desiccation periods, and to rapidly recover after rewetting. Austin & Strauss (2011) performed a desiccation-inundation experiment in a small headwater stream and they found that after a 28-day sediment desiccation period, denitrification rates had still not recovered. Similarly, studies examining the effect of desiccation on denitrification in stream sediments obtained decreased denitrification due to increased aerobic conditions (Cavanaugh et al. 2006; Gómez et al. 2012).

Results from this study showed very low denitrification rates also in pre-drought conditions, suggesting low denitrification capacity of the studied streams. Scarce N loss via denitrification in low order streams is not surprising and represents a strategy of the system as a macroorganism to retain and transfer downstream N instead of loosing this precious nutrient to the atmosphere. Low order streams receive huge amounts of allochthonous, low quality organic matter that is slowly processed due to combined macrofauna-bacterial activity. Such activity is supported by large amounts of organic C but suffers from limited availability of other macro and micronutrients, among which N. It would be a bad strategy to loose N to the atmosphere via denitrification and this might explain the very low rates measured at time zero in this experiments. In high order rivers and in eutrophic canals that are located much downstream the study areas of this work, high rates of denitrification (either in situ or potential rates) are reported (Pinardi et al. 2009; Palmia et al. 2021). High order rivers and canals receive from upstream as well as from cultivated lands much more labile organic matter in the form of phytoplankton and macrophytes and high inputs of nitrate from unused fertilizers (Soana et al. 2011). They are therefore characterized by an opposite situation as compared to low order streams as they have large amounts of labile organic matter (low C:N ratios) and they have large excess of dissolved inorganic N. This might explain the high denitrification rates and the non-necessity to retain N.

# CONCLUSIONS

This study provided valuable results on the hyporheic sediments metabolism dynamics and the re-establishment of microbial processes after rewetting following 7 weeks drought in both intermittent and perennial streams.

Dry periods may affect the functioning of hyporheic ecosystems and the exchange of nutrients in water column due to nutrient mobilization that follows rewetting phase. The nutrient pulses dynamics are similar to those measured in surface sediments or in isolated pools, peaking within the first day after rewetting. However, in the hyporheic zone, sediments can maintain a certain degree of humidity, allowing microbial communities to quickly recover after desiccation. This may explain why streams hydrology (perennial or intermittent flow regime) did not influence the resistance and recovery of microbial processes after rewetting and why communities inhabiting intermittent rivers did not show higher adaptation to environmental stress as compared to perennial rivers.

Understanding the effects of drying-rewetting events on hyporheic microbial processes is important to our understanding of stream ecosystem and nutrient dynamics, of the potential effects on stream communities and downstream ecosystems and will help to formulate predictions on the biogeochemical and ecological implications of the increasing flow intermittency.

Future experiments should be addressed to investigate nutrient dynamics and processes during rewetting phase at shorter temporal scales, to understand how hyporheic ecosystems respond immediately after rewetting. In situ samplings along whole river courses should be intensified during climate extremes and in particular in rainy periods following prolonged drought events. Such samplings might allow to catch peak loads of nutrients, that are likely transported downstream shortly after rewetting and restart of river flow. As samplings are generally performed during stable climatic conditions the risk is to underestimate true loads and miss the functioning of coupled river-watersheds systems. Frequent and spatially close samplings of river water after rewetting might also allow to explore the effect of nutrient pulses to their spiralling within the watercourses and understand whether the efficiency in their use (the length of uptake spirals) changes along with their concentrations.

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# Chapter 3: Artificial aquatic ecosystems

Hydrological intermittency as well as extreme events are expected to become more common as the climate changes. Climate-induced changes in lotic hydrology are likely to increase the occurrence, frequency and extent of sediment drying not only in natural environments but also in the artificial water bodies like ditches, fish ponds, weirs, reservoirs and irrigation channels, due mainly to water abstraction. This kind of "artificial intermittency" may alter biogeochemical cycles and functioning of these environments and the ecosystem services they provide.

Farm ditches are human-made linear elements that constitute a great part of the hydrographic networks in agricultural landscapes. Ditches are characterized by an intensive exchange of organic matter from the surrounding terrestrial matrix and play a key role in processing and recycling nutrients (Herzon and Helenius 2008). Furthermore, they were recently identified as hotspots of GHG emissions to the atmosphere (Vermaat et al. 2011; Hyvönen et al. 2013; Luan and Wu 2015; Tang et al. 2021).

Most of the ditches are characterized by marked fluctuations in water levels, that make them intermittent ecosystems. In fact, to ensure an adequate water supply during the summer crops growing season, water is diverted from natural freshwater ecosystems to feed irrigation canals. At the end of crops growing the whole network is artificially dry up.

These water levels fluctuations exposed large areas of sediments to the atmosphere, that should result in changing microbial metabolism, biogeochemical processes, like nitrification and denitrification, and in large $CO_2$ emissions.

Although artificial canals play an important role in organic matter transformation, nitrate removal and nutrient recycling, knowledge about their functioning and consequences of periodical drought is poor, especially compared to natural ecosystems.

Here I wanted to focus on the effects of sediment air-exposure on $CO_2$ emissions regulation, in order to understand the importance of ditches network with respect to $CO_2$ emissions, to identify the main drivers of the variability in emissions and the mechanisms by which moisture controls C fluxes.

# Regulation of $CO_2$ fluxes along gradients of water saturation in irrigation canal sediments

## INTRODUCTION

Over the past thirty years, the frequency and duration of droughts have increased worldwide due to climate change, water abstraction, and land use alteration, causing occasional, recurrent or even permanent drying of inland waters (Pekel et al. 2016; Marcè 2019). Drought and hydrological intermittency deeply affect all inland water ecosystems, including natural and artificial ones, and all aquatic biological components, their activities and, consequently, the biogeochemical processes and ecosystem services they provide. As inland waters receive and process large amounts of organic carbon (OC) from watersheds, there is a growing body of research analysing the effects of drying on OC processing and $CO_2$ emissions (Marcé et al. 2019). Increasing evidence has demonstrated the active role of inland waters in the global carbon (C) cycle and their capacity to emit significant amounts of $CO_2$ even during the dry phase (Gómez-Gener et al. 2015; Marcé et al. 2019). Most experimental activities were carried out in natural environments, whereas anthropogenic aquatic ecosystems, including fishponds, weirs, reservoirs, ditches and agricultural channels are comparatively understudied (Koschorreck et al. 2020).

Irrigation canals represent a major and expanding fraction of artificial inland waters, with a potentially important contribution to $CO_2$ emission and C budgets. Irrigation accounts for roughly 70% of total freshwater withdrawals globally (FAO 2011) and the irrigated area has expanded to over 270 Mha worldwide, about 18% of the total cultivated land surface (Fischer et al. 2007). Irrigation infrastructures are capillary distributed and are expected to increase together with water demand for food production. In heavily exploited agricultural areas such the Po River watershed, Northern Italy, irrigation canals undergo artificial wet and dry annual cycles. They are active for 4-5 months, during the summer growing season, whereas due to hydraulic safety the whole network dries up during autumn, winter and early spring non irrigation periods. Such management offers a unique opportunity to investigate the effects of intermittency on $CO_2$ emission in widespread artificial aquatic ecosystems.

Sediment desiccation causes bacterial activities and biomass decline, and changes in their community structure and composition (Amalfitano et al. 2008; Zoppini et al. 2011). However, biogeochemical processes and in particular OC mineralization are demonstrated to continue in dried systems (Zoppini, A., Marxen 2011; Pohlon et al. 2013; Timoner et al. 2014b), so that temporary streams can release significant amounts of $CO_2$ even when they

are dry (Gallo et al. 2014; von Schiller et al. 2014; Gómez-Gener et al. 2015). Carbon dioxide emissions from dry systems may be regulated by different processes that occur in sediments, both biotic and abiotic and may depend on environmental features as sediment temperature (Gómez-Gener et al. 2016), texture (Gallo et al. 2014; Gómez-Gener et al. 2016), organic matter and water contents (Gallo et al. 2014; Gómez-Gener et al. 2016; Bolpagni et al. 2017; Keller et al. 2020), and presence of vegetation or microphytobenthos (Bolpagni et al. 2017; Obrador et al. 2018). Water saturation might play a dual role as regulator of $CO_2$ fluxes (Gómez-Gener et al. 2015): high water content enhances C respiration by facilitating the contact between microorganisms and available substrates (Koschorreck and Darwich 2003); on the other hand, as diffusion in air is >10,000 times faster than in water (Haynes and Lide 2012), water saturation slows $CO_2$ efflux by decreased gas diffusivity through sediments (Howard and Howard 1993; Fujikawa and Miyazaki 2005; Gómez-Gener et al. 2015). In impermeable sediments like those in lowland, artificial irrigation canals, saturation may also affect OC mineralization by limiting $O_2$ penetration and the sediment volume where aerobic microbial activities take place. $O_2$ microprofiles revealed in fact that in organic sediments $O_2$ penetration depth is confined to the upper few millimeters (de Klein et al. 2017). During water level drawdown, the enhanced $O_2$ penetration is expected to expand the volume of sediments with aerobic microbial activity, and enhance the efficiency of mineralization as a consequence of higher energy yield (Baldwin and Mitchell 2000; Foulquier et al. 2013).

The effect of drying on sediment redox potential or $O_2$ content can be measured with electrodes (Koschorreck 2005). Alternatively, specific microbial processes as nitrification and denitrification, that are sensitive to $O_2$ levels, can be measured as indirect proxies. Both processes respond to redox oscillations associated with varying levels of water saturation and $O_2$ availability in sediments (Martin et al. 2001; Strauss et al. 2004).(Kemp and Dodds 2002; Strauss et al. 2004)(Kemp and Dodds 2002; Strauss et al. 2004)(Kemp and Dodds 2002; Strauss et al. 2004)(Kemp and Dodds 2002; Strauss et al. 2004) Nitrification, a strictly aerobic process, is expected to increase along with decreasing water saturation levels whereas denitrification is indicative of low $O_2$ conditions and is expected to increase along with water saturation and $O_2$ shortage (Canfield et al. 2005). Significant positive and negative correlations between nitrification and denitrification rates and $O_2$ availability have been demonstrated, respectively (Kemp and Dodds 2002; Strauss et al. 2004). Besides tracing the effects of water saturation, nitrification and denitrification produce opposite effects on $CO_2$ fluxes, either increasing the C sink role of sediments (nitrification) or enhancing its emission (denitrification). It can be expected that hydrological intermittency and sediment desiccation in artificial irrigation canals may stimulate nitrification and

suppress denitrification. Increased nitrification rates would attenuate the effects of $O_2$ penetration on OC mineralization and on $CO_2$ emission. Taken together, these often-contrasting effects of water intermittency and drying on net $CO_2$ emissions support the idea of a complex and site-specific regulation.

The aim of this study is to quantify $CO_2$ emission along water saturation gradients in sediments of agricultural canals within the secondary irrigation and drainage network of the Po River watershed and to understand which factors are involved in $CO_2$ emissions regulation. The Po is one of the major rivers in the Mediterranean region and the largest river in Italy and its watershed is one of the most densely populated and agriculturally productive areas in Europe (Viaroli et al. 2018). Nearly ~43% of the total surface is exploited for intensive agriculture and a network of >50,000 km of artificial canals with irrigation, drainage, and flood control purposes is operating (Soana et al. 2019). During the crops growing season (May–September), water is diverted from the Po River tributaries (e.g. sublacual rivers, rivers and creeks in the Alpine and Apennine sectors, respectively) and capillary distributed in the large network of irrigation canals, extending in the plain area for >18,500 km (Soana et al. 2019). At the end of the summer period irrigation is interrupted and the gradual sediment desiccation from the shores to the central part of the canals results in the coexistence of sediment spots exposed to the atmosphere, sediments spots with a shallow overlying water column and sediment spots with an intermediate level of saturation. Such spots differ for $O_2$ penetration, aerobic and anaerobic C processing and $CO_2$ diffusivities, regulating $CO_2$ emission. These issues make agricultural canals ideal sites to explore the effects of drought in artificial ecosystems, to study the mechanisms regulating $CO_2$ emissions and to explore the underlying processes that occur in natural ecosystems when sediments gradually dry out. Moreover, artificial canals are generally eutrophic and organic-rich and high metabolic rates are expected in sediments. Research on the consequences of drying on microbial activities and $CO_2$ emissions in agricultural canals may help us to understand how $CO_2$ emissions vary in response to changes in water saturation, which factors are involved, which are the implication at large spatial scales (e.g. entire watersheds, where these artificial elements represent a high proportion of lotic systems) and the contribution of these environments to C budgets.

In the specific sedimentary environments of irrigation canals, that deeply differ from exposed gravel bottom of intermittent rivers and creeks, I hypothesized a complex, local regulation of $CO_2$ fluxes. Previous studies have demonstrated increased $CO_2$ emission along with decreasing water saturation, due to the dominant effect of aerobiosis on C mineralization efficiency and to the effects of air lacunae in sediments, hastening $CO_2$

diffusivity. I hypothesized that such general rule might be different in impermeable irrigation canal sediments due to different amounts of sedimentary organic matter promoting water retention, acting upon $O_2$ penetration and microbial processes as nitrification and denitrification, ultimately affecting net $CO_2$ emission. In particular, local conditions promoting chemoautotrophic nitrification should reduce $CO_2$ effluxes whereas conditions promoting heterotrophic denitrification should stimulate $CO_2$ production.

## METHODS

### *Study area and experimental design*

The sampling campaign was conducted in autumn (last 2 weeks of October 2019), during the non-irrigation period, when the water supply to the Po River ditches network is interrupted and the areal extent of exposed sediments increases. Five replicated canals were considered (Figure 3.1) and $CO_2$ flux measurements were performed along transects perpendicular to the shores. Along each transect, three sampling sites were selected from the exposed, dry to the inundated, saturated sediments, to include variable levels of water content (Figure 3.2). Since the irrigation canals were dried up at the beginning of October, sediments at sites 1 and 2 had been exposed for a minimum of 2 weeks. Within each site, dark $CO_2$ fluxes and sediments density, porosity, water and organic matter content, exchangeable ammonium (exchangeable $NH_4^+$), net potential nitrification and denitrification rates were measured in 4 different spots. For each site, representative of 3 different levels of water saturation, a total of 20 measurements (4 spots in 5 canals) were therefore available.

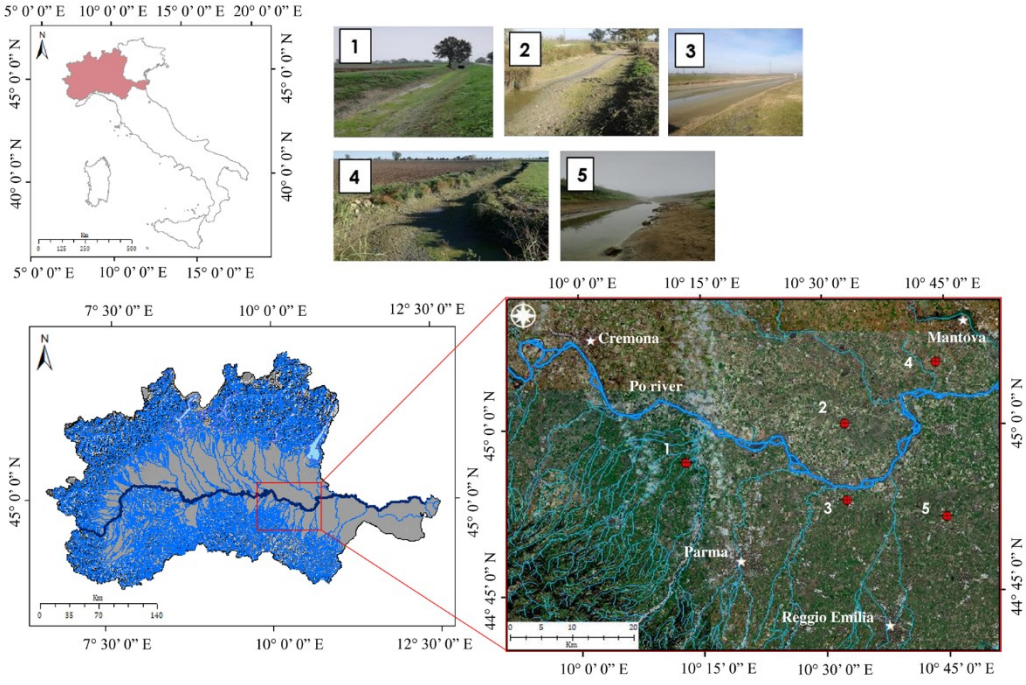

**Figure 3.1** Location of the Po river basin (Northern Italy) and of the five artificial canals studied in this work.

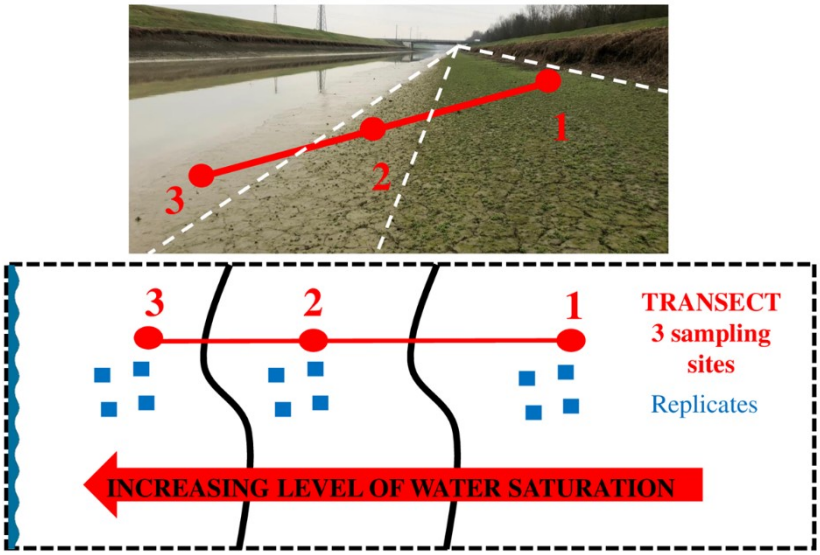


**Figure 3.2** The sampling strategy consisted in a transect perpendicular to the shore including 3 sites with variable water saturation levels.

*Determination of $CO_2$ fluxes*

Dark $CO_2$ flux measurements were generally performed in the central part of the day using an infrared gas analyser (EGM-4, PP Systems, 2002), powered by an internal 12 V battery, mounted on a rugged PVC Soil Respiration Chamber with stainless steel ring (SRC-1, PP Systems, Amesbury, USA). The chamber was equipped with a small fan, to maintain homogeneous the inner atmosphere, and a Soil Temperature Probe (STP-1, PP Systems, Amesbury, USA). At each site $CO_2$ fluxes were measured in 4 different spots and calculated as the slope of the regression equations of the $CO_2$ concentrations versus time. The gas concentration in the chamber was monitored every 4 s for a total of 2 minutes, flushing the chamber with ambient air between consecutive measurements. A total of 60 $CO_2$ flux measurements were made, 12 in each canal. Each linear regression was calculated on 27 points and linearity was always very good ($R^2$ >0.9, p<0.001). Carbon dioxide fluxes (F, mmol $CO_2$ $m^{-2}$ $d^{-1}$) were calculated from the rate of change of $CO_2$ inside the chamber:

$$F = \frac{dp}{dt} \cdot \frac{V}{RTS}$$

where *dp/dt* is the slope of the gas accumulation in the chamber (ppm $d^{-1}$), *V* is the volume of the chamber ($m^3$), *S* is the surface area of the chamber ($m^2$), *T* is the air temperature (K) and *R* is the ideal gas constant ($m^3$ atm $K^{-1}$ $mmol^{-1}$).

The turnover of organic C in exposed sediments (days) was calculated dividing the C pool in the upper sediment layer by the measured $CO_2$ fluxes. The organic C pool was calculated dividing the percentage of organic matter content obtained by loss on ignition by 2 (Sutherland 1998).

*Sedimentary features and potential microbial activities*

Four sediment cores were collected by transparent Plexiglass liners (internal diameter 4 cm, height 20 cm) in each sampling site for sediment characterization (n=12 for each transect). The upper 0-3 cm sediment layer was sub-sampled and analysed as most biological activity is expected to be concentrated in surface sediment rather than at greater depth (Gómez et al. 2012).

After homogenization, sediments subsamples were collected with cut-off syringes for different treatments: 5 ml were collected to determine water content, dry bulk density, porosity and organic matter content, 2 ml were collected for exchangeable $NH_4^+$ determination; 2.5 ml were collected for net potential nitrification rates and 1 mL was collected for potential denitrification rates.

Bulk density was determined as the ratio between wet sediment weight and volume. The water content (WC) was determined after desiccation of the fresh sediment volume at 60 °C until constant weight; porosity was calculated as the ratio between the volume of water and that of fresh sediment. Organic matter content (OM) was measured as percentage of weight loss by ignition (450 °C, 8 h) from dried, powdered sediment (Davies 1974); exchangeable $NH_4^+$ was extracted from fresh sediment after treatment with 2 M KCl and analysed by spectrophotometry (Maynard et al. 2008).

Net potential nitrification rates were obtained by oxic slurries containing 2.5 ml of fresh sediment suspended in 20 ml of water enriched with $NH_4^+$ to a final concentration of 200 µM and incubated under constant shaking in the dark at 20 °C. At the beginning and at the end of the 8 hours' incubation the slurry was subsampled, centrifuged at 3000 rpm for 10 minutes, filtered (Whatman GF/F filters), and analysed for combined nitrite and nitrate ($NO_x^-$ = $NO_2^-$ + $NO_3^-$ ) via standard spectrophotometric techniques (APHA et al. 2017). Net potential nitrification rates (PN, µmol N $cm^{-3}$ $d^{-1}$) were calculated from accumulation of $NO_2^-$ + $NO_3^-$ over time from the equation:

$$PN = \frac{d[NOx^-]}{dt \times [S]}$$

where $d[NO_x^-]$ is the accumulation of $NO_3^-$ and $NO_2^-$ in the slurry (µM), *dt* is the incubation time (d), and [*S*] is the concentration of the slurry ($cm^3$ of fresh sediment $L^{-1}$).

In order to measure potential denitrification rates 1 ml of sediment was transferred to 12 mL exetainers, containing a glass bead. All exetainers were than filled with water, previously bubbled with $N_2$ to remove $O_2$. Once filled, all vials were capped leaving no air bubbles, transferred into a rotating shaker and incubated for 20 h in the dark at 20 °C to remove any $O_2$ and nitrate traces; afterward, 100 µL of $Na^{15}NO_3$ 20 mM were added through the exetainer lid septum and an accessory needle and the anoxic slurry was incubated for 8 hours, as detailed in Moraes et al. (2019). At the end of the incubation 200 µL of 7 M $ZnCl_2$ was added to the exetainers to inhibit microbial activity.$^{15}N$ abundance in $N_2$ was analysed by membrane inlet mass spectrometry (MIMS, Bay instruments, USA). The rates of potential denitrification (PD, µmol N $cm^{-3}$ $d^{-1}$) were calculated as

$$PD = \frac{2[^{30}N_2]}{dt \times [S]}$$

where $[^{30}N_2]$ is the concentration of $^{15}N$-labelled $N_2$ gas at the end of the incubation (µM), *dt* is the incubation time (d), and [*S*] is the concentration of the slurry ($cm^3$ of fresh sediment $L^{-1}$). I assumed that at the beginning of the incubation concentrations of $^{15}N$-labelled $N_2$ were negligible.

Potential activities of nitrifiers and denitrifiers were converted into potential $CO_2$ fluxes (negative rates for nitrifiers and positive rates for denitrifiers). Net potential nitrification rates were converted into rates of C assimilation by bacteria assuming a $C_{fixation}$:$N_{oxidation}$ ratio of 1:35 (mol:mol), whereas potential denitrification rates were converted into $CO_2$ production assuming a ration between moles of C oxidized and moles of nitrate reduced of 1.25:1 (Atlas and Bartha, 1993).

*Statistical analyses*

Within each canal, the effect of different sampling sites on $CO_2$ fluxes, net potential nitrification and potential denitrification rates was tested using one-way analysis of variance (ANOVA) and subsequent post hoc comparisons (Tukey's Honest Significant Differences test). The simultaneous effects of water and organic matter content in sediments, net

potential nitrification and potential denitrification rates on $CO_2$ efflux were tested using multiple linear regressions. Independent variables were all centred as suggested by Schielzeth (2010). Initially all double interactions between explanatory variables were included in the model, but they were subsequently dropped one by one if not significant. Heteroscedasticity and normality of residuals were checked on the final model. Multicollinearity was checked by using the Variance Inflaction Factor and all terms produced values below 5 with the exception of intercept which was just slightly superior (5.06). A similar model including a random effect on combination of canal and plots was also run and produced very similar coefficients (r=0.99) (data not shown).

All statistical analyses were conducted by using the "lm" function of the R statistical environment. (R Core Team 2018) while graphic plots were produced with the "effects" package (John and Sanford 2019).

## RESULTS

### *Sediments features*

During the sampling campaign, incubation temperature varied between 13.5 and 24.1 °C. Within each canal, sediment temperatures along the transect varied generally by less than 1 °C, with less saturated sediments showing slightly higher values as compared to more saturated sediments. In this respect, canal 2 was an exception with nearly 6 °C difference among sites (Table 3.1). Sediment water content (from ~10 to ~64%) always followed the expected increase along the transects from the site close to the canals shore towards the canals central portion (Table 3.1). Within each canal, water content varied among sites by a factor of 3 to 4.

Sedimentary organic matter content varied from 3.7 ± 0.4 % to 10.7 ± 0.7 % and was more erratic, with no significant differences among canals and no consistent trends along the water content gradients. The analysis of sediment exchangeable $NH_4^+$ showed a consistent and pronounced gradient in $NH_4^+$ availability along transects, with increasing concentrations along with increasing water content in sediments. Concentrations (from 59.1 ± 14.7 to 1587.7 ± 166.5 nmol $cm^{-3}$) varied by nearly one order of magnitude and suggested large effects of water saturation on microbial N transformations. Pooled data from the 5 canals suggest, despite local differences, consistent gradients of water and exchangeable $NH_4^+$ content from site 1 to site 3, whereas averaged organic matter contents overlap (Table 3.1).

**Table 3.1** Sedimentary features of sampling sites along the transects of each studied canal and of the pooled canals (mean ± SE are reported, n=4 and n=20, respectively).

| Canal code | site | T (°C) | | Water content (%) | | Organic matter content (%) | | Exchangeable $NH_4^+$ (nmol $cm^{-3}$) | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| **1** | 1 | 19.4 | 0.01 | 16.7 | 0.9 | 5.7 | 0.5 | 26.5 | 23.1 |
| | 2 | 19.1 | 0.02 | 27.3 | 2.6 | 5.6 | 0.3 | 62.2 | 36.2 |
| | 3 | 18.2 | 0.01 | 45.3 | 1 | 3.7 | 0.4 | 790.3 | 135.1 |
| **2** | 1 | 24.1 | 0.01 | 16.5 | 0.6 | 6.3 | 1.0 | 57.9 | 23.3 |
| | 2 | 18.8 | 0.01 | 46.1 | 1.4 | 9.4 | 0.5 | 326.5 | 56.1 |
| | 3 | 17.9 | 0.004 | 64.2 | 1 | 7.9 | 0.2 | 1780.2 | 214.2 |
| **3** | 1 | 18.2 | 0.004 | 12.6 | 1 | 4.7 | 0.7 | 83.8 | 48.3 |
| | 2 | 18 | 0.004 | 25.6 | 1.3 | 4.7 | 0.3 | 291.9 | 112.5 |
| | 3 | 17.9 | 0.002 | 37.6 | 1.4 | 5.2 | 0.3 | 568.4 | 57.3 |
| **4** | 1 | 17.7 | 0.004 | 16.2 | 0.8 | 10.7 | 0.7 | 103.6 | 36.9 |
| | 2 | 17.4 | 0.007 | 41.3 | 3.2 | 7.0 | 1.0 | 72.9 | 34.4 |
| | 3 | 17 | 0.008 | 43.2 | 1.6 | 5.9 | 0.4 | 2391.4 | 294.3 |
| **5** | 1 | 14.5 | 0.04 | 10.2 | 1.4 | 5.2 | 0.1 | 15.6 | 15.6 |
| | 2 | 13.7 | 0.01 | 40.7 | 1.2 | 5.8 | 0.1 | 1921.3 | 196.7 |
| | 3 | 13.5 | 0.003 | 44.3 | 0.7 | 5.1 | 0.1 | 2408.5 | 236.0 |
| **mean ± SE** | | | | | | | | | |
| **Site 1** | | 18.7±0.11 | | 14.5±0.71 | | 6.03±0.4 | | 59.1±14.7 | |
| **Site 2** | | 17.6±0.08 | | 36.2±2.03 | | 6.51±0.42 | | 535±166.4 | |
| **Site 3** | | 16.9±0.07 | | 47±2.12 | | 5.55±0.33 | | 1588±196. 3 | |

*$CO_2$ emissions, net potential nitrification and potential denitrification rates*

All sites in all canals were net $CO_2$ emitters to the atmosphere (mean ± SE = 326.1 ± 36.8 mmol $CO_2$ $m^{-2}$ $d^{-1}$, n=60; range = 27.6 – 1200.8 mmol $CO_2$ $m^{-2}$ $d^{-1}$). Fluxes were extremely variable among canals and among sites (data from single canals are reported in the supplementary Figure S3.1); however, as a general tendency, they tended to decrease with increasing levels of water content (Figure 3.3). Such pattern was rather evident in canals 2, 4 and 5 whereas in canals 1 and 3 $CO_2$ emissions peaked at the intermediate water content (Supplementary materials, Figure S3.1).

Rates of net potential nitrification (mean ± SE = 2.9 ± 0.2 µmol N $cm^{-3}$ $d^{-1}$, n=60; range = 0.6 – 8.3 µmol N $cm^{-3}$ $d^{-1}$) varied also among canals and sites and in general tended to decrease, as $CO_2$ fluxes, along with increasing levels of water content (Figure 3.3). Canals 1 and 2 were exception, as net potential nitrification rates were slightly higher at site 2. In

canal 1 there were no differences in nitrification rates between the three studied sites, while in canal 2 rates from site 3 were lower than those from sites 1 and 2 (Supplementary materials, Figure S3.1). Rates of $CO_2$ potentially fixed by nitrifiers were estimated to vary between 1.1 and 6.9 mmol C $m^{-2}$ $d^{-1}$. This means that net potential nitrification may attenuate $CO_2$ emission by exposed sediments by 0.2-3%.

Rates of potential denitrification (mean ± SE = 2.9 ± 0.3 µmol N $cm^{-3}$ $d^{-1}$, n=60; range = 0.0001 – 9.2 µmol N $cm^{-3}$ $d^{-1}$) exhibited a different pattern as they always tended to increase along with the level of water saturation (Figure 3.3).

The production of $CO_2$ calculated from potential denitrification varied between 0.3 and 288.8 mmol C $m^{-2}$ $d^{-1}$. $CO_2$ potentially generated by nitrate reduction ranged from 0.03 to 8.3% in drier sediments, from 7 to 87.9% at intermediate water content and from 83.4 to >100% in saturated sediments.

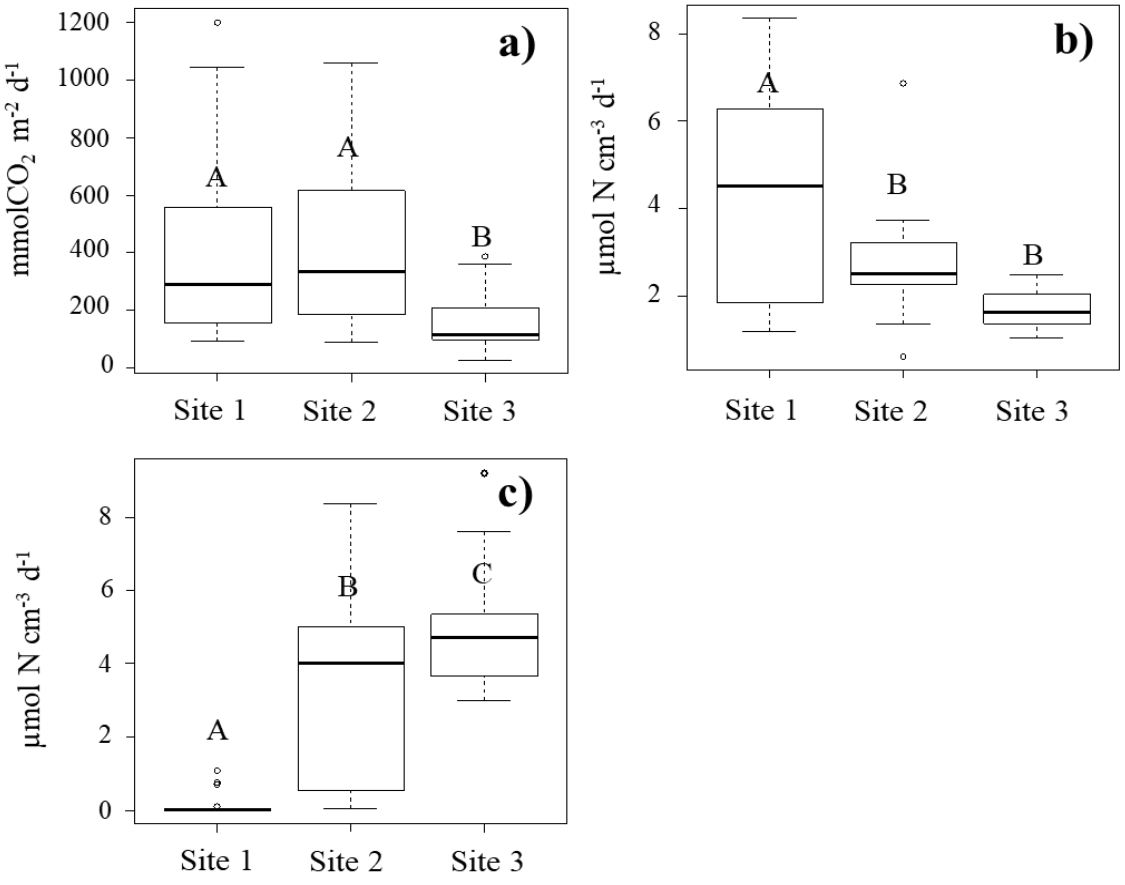


**Figure 3.3** Efflux of $CO_2$ (a) and rates of net potential nitrification (b) and denitrification (c) measured in exposed, dry sediments (site 1), in sediments with intermediate level of humidity (site 2) and in inundated, saturated sediments (site 3) of 5 replicated canals. Significant differences among sites for each parameter ($p<0.05$, Tukey's post hoc test) are marked with different capital letters above the box plots.

*Drivers of $CO_2$ emissions*

The analysis of $CO_2$ fluxes and net potential nitrification and denitrification rates as a linear function of sediment water content revealed large variability in the five canals. Results from these linear regressions are reported for each canal in Table 3.2. Slopes of $CO_2$ emissions VS water content were significantly different from zero in canals 1, 2, 4 and 5 (Table 3.2), those of net potential nitrification rates were significant in canals 3, 4 and 5 (Table 3.2), whereas slopes of denitrification were significant and positive in all canals (Table 3.2).

**Table 3.2** Linear regression between $CO_2$ fluxes, net potential nitrification and potential denitrification rates and water content (mean ± SE, n = 12). Significant values ($p<0.05$) are printed in bold.

| | $CO_2$ | | | Net potential nitrification | | | Potential denitrification | | |
|---|---|---|---|---|---|---|---|---|---|
| Canal 1 n=12 | Slope | -0.03 ± 0.01 | ***p*=0.04** | Slope | -0.01 ± 0.01 | *p*=0.54 | Slope | 0.17 ± 0.02 | ***p*<0.001** |
| | Intercept | 5.28 ± 0.37 | ***p*<0.001** | Intercept | 2.3 ± 0.5 | ***p*<0.001** | Intercept | -3.12 ± 0.66 | ***p*=0.001** |
| | $R^2$ | 0.35 | | $R^2$ | 0.04 | | $R^2$ | 0.9 | |
| Canal 2 n=12 | Slope | -0.01 ±0.005 | ***p*=0.04** | Slope | -0.005 ± 0.009 | *p*=0.6 | Slope | 0.16 ± 0.03 | ***p*=0.0002** |
| | Intercept | 6.74 ± 0.25 | ***p*<0.001** | Intercept | 2.09 ± 0.4 | ***p*<0.001** | Intercept | -2.32 ± 1.3 | *p*=0.11 |
| | $R^2$ | 0.38 | | $R^2$ | 0.03 | | $R^2$ | 0.76 | |
| Canal 3 n=12 | Slope | -0.008 ± 0.016 | *p*=0.6 | Slope | -0.18 ± 0.03 | ***p*=0.0003** | Slope | 0.13 ± 0.02 | ***p*<0.001** |
| | Intercept | 5.56 ± 0.45 | ***p*<0.001** | Intercept | 7.61 ± 0.87 | ***p*<0.001** | Intercept | -1.8 ± 0.46 | ***p*=0.004** |
| | $R^2$ | 0.03 | | $R^2$ | 0.77 | | $R^2$ | 0.87 | |
| Canal 4 n=12 | Slope | -0.2 ± 0.007 | ***p*=0.009** | Slope | -0.1 ± 0.02 | ***p*=0.0006** | Slope | 0.14 ± 0.01 | ***p*<0.001** |
| | Intercept | 5.92 ± 0.24 | ***p*<0.001** | Intercept | 6.9 ± 0.7 | ***p*<0.001** | Intercept | -1.7 ± 0.46 | ***p*=0.004** |
| | $R^2$ | 0.50 | | $R^2$ | 0.78 | | $R^2$ | 0.93 | |
| Canal 5 n=12 | Slope | -0.04 ± 0.13 | ***p*=0.02** | Slope | -0.17 ± 0.01 | ***p*<0.001** | Slope | 0.15 ± 0.01 | ***p*<0.001** |
| | Intercept | 7.05 ± 0.46 | ***p*<0.001** | Intercept | 9.87 ± 0.54 | ***p*<0.001** | Intercept | -1.61 ± 0.49 | ***p*=0.009** |
| | $R^2$ | 0.44 | | $R^2$ | 0.94 | | $R^2$ | 0.93 | |

Furthermore, a simple model including all measured variables was constructed, in order to analyse quantitatively the fraction of $CO_2$ flux variance explained by the measured sedimentary features and by N transformation rates. A series of multiple regression models were initially developed including all measured variables and their double interactions, that were thereafter simplified removing non-significant interaction terms in order to produce a parsimonious model interpretation. The final model contained sediment water content, organic matter content and their interaction, net potential nitrification and potential denitrification (Table 3.3).

**Table 3.3** Results from multiple linear regressions testing the effects of sediment water and organic matter contents, and net potential nitrification and denitrification rates on $CO_2$ fluxes.

| | **Slope** | **SE** | **t value** | **p value** |
|---|---|---|---|---|
| Intercept | **306.5** | **33.4** | **9.2** | **<0.001** |
| Water content | **-13** | **4.7** | **-2.8** | **0.008** |
| Organic matter content | **47.8** | **19.8** | **2.4** | **0.02** |
| Net potential nitrification | -17.8 | 20.2 | -0.9 | 0.4 |
| Net potential denitrification | 39.7 | 26.7 | 1.5 | 0.1 |
| Water content:organic matter content | **3.4** | **1.3** | **2.7** | **0.008** |

The results emphasize the predominant influence of water content, organic matter content and their interaction on the $CO_2$ efflux rates in the air-exposed sediments. Increasing water content had a negative effect on the $CO_2$ fluxes while increasing organic matter content resulted in increasing $CO_2$ fluxes from sediments to the atmosphere (Table 3.3). However, these effects depended upon the interaction of the two factors (Table 3.3; Figure 3.4). Results show a decrease in $CO_2$ fluxes with increasing water content at lower values of organic matter, while under elevated organic matter content the effect of water content on $CO_2$ emission is mitigated and less clear (Figure 3.4).

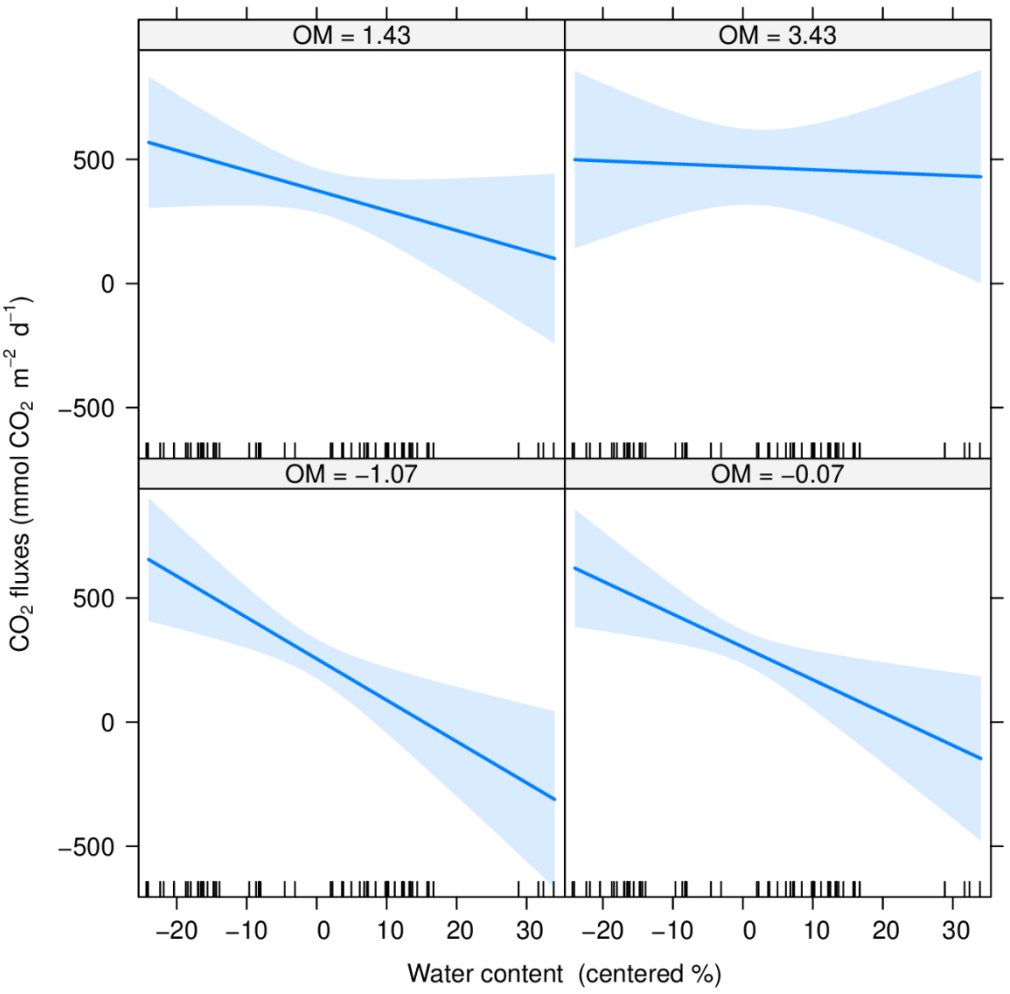


**Figure 3.4** Efflux of $CO_2$ as a function of sediment water content at different sediment organic matter content. Water content and organic matter values reported are mean-centered.

The effect of nitrification and denitrification processes on $CO_2$ emissions is not so marked. Despite the effect is not statistically significant, results showed a weakly negative and positive effect of net potential nitrification and denitrification rate on the $CO_2$ emissions, respectively (Table 3.3, Figure 3.5a, 3.5b).

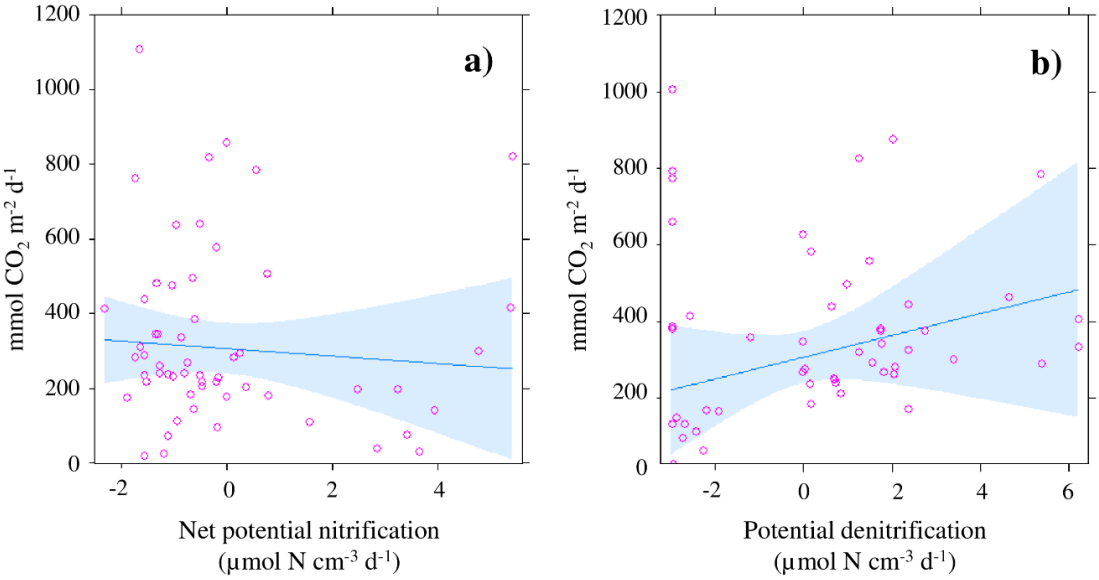


**Figure 3.5** Efflux of $CO_2$ as a function of net potential nitrification rate (a) and potential denitrification rate (b). Net potential nitrification and denitrification rate values reported are mean-centered.

## DISCUSSION

### *Desiccation increases $CO_2$ emission in irrigation canals*

Experimental results from this study suggest that in irrigation canal sediments desiccation stimulates OC mineralization processes and $CO_2$ fluxes. The latter increased by a factor of ~3 from inundated, saturated to dry, exposed sediments. Results also suggest net potential nitrification produces little effects on $CO_2$ fluxes, but allows to trace oxygen availability, whereas potential denitrification may support large $CO_2$ production in wetter sediments. Results from the model testing the effect of all measured variables on $CO_2$ fluxes show that sediment moisture, organic matter content and the interaction between the two factors were the strongest predictors of $CO_2$ fluxes, as recently found by Keller et al. (2020). At lower sediment organic matter content there is a predominant effect of water in driving $CO_2$ emissions, with decreasing $CO_2$ emissions along with increasing water content. With lower organic matter content, the negative effect of water content on $CO_2$ emissions can be explained though the effect of lower $O_2$ penetration and availability within the sediment that may decrease the efficiency of organic matter decomposition (Gómez et al. 2012; Kosten et al. 2018). This may be particularly true for the residual, refractory organic matter that undergoes different decomposition rates under unsaturated, oxic and saturated, anoxic conditions. I speculate that variable $O_2$ penetration may determine different effects on $CO_2$ emissions depending upon the mineralization efficiency, higher under oxic conditions but depending on the interaction with the organic matter quality (Longhi et al. 2016).

In addition, the sediment water content decrease might increase the gas diffusivity and $O_2$ availability, resulting in higher $CO_2$ efflux (Fujikawa and Miyazaki 2005; Luo and Zhou 2010; Kosten et al. 2018). Under this circumstance, the regulation of $CO_2$ release is likely driven by the bulk of the labile material released during drying periods from fresh materials through microbial cell lysis and physical processes (Fierer et al. 2003), enhancing microbial C respiration with subsequent release of $CO_2$, as seen in desiccated ponds (Fromin et al. 2010) and peatlands (Moore and Knowles 1989; Fenner and Freeman 2011). Under high loads of OM the effect of water content in regulating $CO_2$ emissions is masked due to the higher availability of organic matter that fuels $CO_2$ production, resulting in high $CO_2$ emissions even under water saturation conditions (Keller et al. 2020).

The amount of organic matter has been identified as an important factor affecting $CO_2$ emissions from soils and sediments (Gallo et al. 2014; Bolpagni et al. 2017), however these seem to be related to OM quality, intended as the degree to which the sediment organic C is resistant to microbial mineralization, rather than to OM quantity (Gómez-Gener et al. 2016). I did not analyse the organic matter quality; thus, it is possible that the variable quality of the organic matter masks the effect of water content on $CO_2$ emissions in the studied canals. The duration of air exposure can also represent an important, unaccounted for factor, acting upon sediment features, microbial communities and organic matter conditioning (Sasaki et al. 2009; Kosten et al. 2018). It is possible that without additional C inputs the $CO_2$ fluxes would tend to decrease along with the duration of the air exposure, as the supplies of readily decomposable C are exhausted, resulting in lower $CO_2$ emission from sediments. This is also likely due to progressively lower air temperatures from October to February.

The weakly negative and positive effect of net potential nitrification and denitrification rate on the $CO_2$ emissions I found is expected as nitrification is a chemoautotrophic process where $CO_2$ is incorporated during microbial growth (Kinsbursky and Salzman 1990; Denecke and Liebig 2003), acting as $CO_2$ sink, while denitrification is a microbial respiration carried out by heterotrophic bacteria that use organic matter as substrate, acting as $CO_2$ sources (Jalota et al. 2018). Nitrification rates in aquatic and terrestrial ecosystems are extremely variable (from a few up to some hundreds μmol N $m^{-2}h^{-1}$) and they are regulated by pH and temperature and by the availability of $NH_4^+$ and $O_2$ (Prosser 2005). I hypothesized an increase of the potential rates along with increasing sediment desiccation, but measured rates show that in some canals net potential nitrification activity peaked at intermediate conditions, likely due to a better combination between $O_2$ penetration and exchangeable $NH_4^+$ availability. This may be due to the different time of sediment exposure to air, that may cause asynchrony between C and N processes. Gómez et al. (2012) showed that the desiccation period plays a pivotal role in regulating N processes in dried sediments. Their results suggest a stimulation of net potential nitrification during the first days of sediment desiccation, with higher activity after 8 days, followed by a drop after 10 days of drying, while denitrification was immediately inhibited and significantly decreased after 2 days of drying. This lag between the onset of drying and the increase in nitrification rates may be explained by the slow growth of nitrifying bacteria and their generation times in the order of days (Canfield et al. 2005). Moreover, while nitrification rates reach a maximum after 8 days of drying (Gómez et al. 2012), $CO_2$ emissions peak immediately after the disappearance of the overlying water (Kosten et al. 2018). In the same way, sediment organic matter content

decrease in the first two days of drying, due to stimulation of mineralization (Merbt et al. 2016; von Schiller et al. 2017). The organic matter degradation and the low availability of $NH_4^+$ in the early stage of desiccation (Gómez et al. 2012), may decrease nitrification rates in drier sediments. The negative correlation between net potential nitrification rates and $CO_2$ emissions I found, can be explained by the reaction stoichiometry. The oxidation of $NH_4^+$ to nitrite and the oxidation of nitrite to nitrate yield a low amount of energy as 1 mol of $CO_2$ is fixed every 35 mol of $NH_4^+$ oxidized (Atlas and Bartha, 1993). Calculations of the amount of $CO_2$ potentially fixed by these bacteria reveal that this amount is negligible, representing at most 3% of the efflux, but generally much lower percentages. This means that potential net nitrification may be used as a proxy of air penetration but, at least in the considered canals, does not represent a significant $CO_2$ sink and is a weak predictor of fluxes, but could be an important $N_2O$ source. In conclusion aerobic (and anaerobic) processes responsible for carbon oxidation largely exceeds $CO_2$ assimilation by $NH_4^+$ oxidizers.

Under wetter conditions, denitrification has the potential to generate the whole amount of $CO_2$ emitted by sediments. However, as for nitrification, we have found a weak correlation between denitrification and measured $CO_2$ fluxes. Differently from nitrification the correlation was positive. Potential denitrification is a weak predictor of measured $CO_2$ fluxes, likely due to different reasons. A possible explanation is the presence of water that limits $CO_2$ evasion despite potentially high production. Alternatively, measurements of denitrification (and nitrification) are potential, which means that for both large amounts of nitrate (and $NH_4^+$) was added to put denitrifiers (and nitrifiers) in optimal conditions. As such, potential rates might largely overestimate in situ rates, whereas the information regarding their relative increase (or decrease) along the water content gradients remains a good proxy of the number of active cells. Microbial communities in sediments may switch among different biochemical pathways depending on the availability of electron acceptors; denitrifiers for example are typically facultative anaerobes and their community was more abundant in wetter sediments.

The presence of water in the secondary drainage system has an important role in this geographical area. Nitrate concentrations in the secondary irrigation and drainage system may in fact reach >50 mg $L^{-1}$ due to large excess of N fertilizers in the Po Basin (Bartoli et al. 2012; Viaroli et al. 2018). Such concentrations sustain elevated rates of denitrification, sometimes exceeding 26 mmol N $m^{-2}$ $d^{-1}$ (Soana et al. 2017). It was calculated by Racchetti et al., (2011), that under these circumstances denitrification rates may support large fractions of carbon mineralization, up to 80% and therefore sustain most of $CO_2$ fluxes. Elsewhere similar calculations in riparian areas suggest that denitrification support 25 to 30% of $CO_2$,

which are anyway relevant fractions (Seitzinger 1994; Laursen and Seitzinger 2002). The autumn drying of the canals results in a loss of permanent N removal via denitrification, as nitrification is favoured over N loss; this aligns with spring nitrate peaks generally found in rivers water after dry winters, due to high rates of $NH_4^+$ oxidation in dry soils (Vybernaite-Lubiene et al. 2018).

*Irrigation canals as model systems to study the effects of desiccation on C turnover and $CO_2$ emission*

Although it is well established that the recurrence of wet and dry cycles exert a strong effect on microbial activities and biogeochemical cycles within sediments (Baldwin and Mitchell 2000; Amalfitano et al. 2008; Austin and Strauss 2011; Gómez et al. 2012), with significant alteration of $CO_2$ fluxes to the atmosphere (Gallo et al. 2014; Gómez-Gener et al. 2015, 2016), there is a lack of knowledge about the processes involved in $CO_2$ emission regulation. The secondary drainage and irrigation network within heavily exploited basins as the Po River watershed offers a unique opportunity to study the fluxes of $CO_2$ under drying conditions in eutrophic and nitrate-rich settings and how such fluxes are regulated by sediment water and organic matter contents.

Recent studies targeting this topic generally focus on rivers and streams that are generally characterized by permeable sediments and rapid desiccation (Gómez-Gener et al. 2016; Looman et al. 2017; Boodoo et al. 2019). Irrigation canals as those considered in the present study are abundant in lowland plains and include a large variety of morphometries, bank slopes, water chemistry - when inundated -, and sedimentary features, only partially considered here. Most of them have water with high nitrate concentrations due to a generalized N excess over arable lands (Viaroli et al. 2018). They generally lay on fine sediments characterized by low permeability and suggesting much longer desiccation time as compared to streams.

However, the simple sampling design, including three sites along a transect orthogonal to the canals bank revealed steep water content gradients and the possibility to analyse within the same day sediments with significantly different water content in the same environmental settings (e.g. temperature, substrate). The steep gradient of water content, increasing from the shore to the central canal, was strongly correlated with the exchangeable $NH_4^+$, suggesting sharp differences in dominant microbial processes within benthic N cycling (e.g. ammonification, nitrification and denitrification), in turn depending on the sedimentary redox potential conditions. The increase of $NH_4^+$ availability along the saturation gradients suggests in fact uncoupled ammonification and nitrification when $O_2$ penetration is low (e.g.

under water saturated conditions). On the other hand, the low $NH_4^+$ concentrations in drier sediment suggests air penetration and increased conversion of $NH_4^+$ to $NO_3^-$.

Potential rates of nitrification and denitrification support this finding despite differences along the gradients were not always as sharp. Such results basically mean that the two microbial processes that were analysed can be considered as reliable proxies of $O_2$ availability in sediments. Also exchangeable $NH_4^+$, which is an even easier and fast parameter to analyse in sediments, reflected the steep gradients of N-related microbial metabolic activity along the canals transect.

Results from this study support the general hypotheses of a strong regulation operated by water saturation level on $CO_2$ emission in dried irrigation canals, with higher effluxes generally measured in drier sediments. The inter-canals comparison reveals some degree of variability, which is probably explained by other interacting factors, not considered in this study. Among them, are the organic matter pool and its macromolecular composition, that may vary among canals and among sites, the sediment permeability and the length of air exposure period. I speculate that increased exposure periods to the atmosphere may affect microbial communities and related activity, organic matter aging and sediment properties, with implication for fluxes (Amalfitano et al. 2008; Ylla et al. 2010; Palmia et al. 2019).

In general, metabolic rates should decrease along with the time of exposure due to progressive depletion of the more labile organic matter fraction or the increase of C:N ratios in the residual litter (Palmia et al. 2019). As during 2019 the 5 canals were dried out at the beginning of October and samplings occurred during the last 2 weeks of October, sediments were exposed for a period comprised between 2 and 4 weeks. Moreover, the site closer to the shore (site 1) was exposed for longer period to the atmosphere as compared to the other two sites.

This may have implication for leaching, mineralization of labile pools and nitrification, the latter peaking shortly after the exposure due to large $O_2$ and $NH_4^+$ availability and then limited by $NH_4^+$ scarcity, as exchangeable $NH_4^+$ data suggest. This may explain why nitrification in the drier stations of some canals has rates comparable to those of more wet sediments. I calculated theoretical turnover rates of the organic carbon sedimentary pools, from available data of organic matter content and measured $CO_2$ emission rates. These calculations are based on uncertain assumptions as the constancy over time of $CO_2$ production, which is unlikely due to varying quality of the organic C pool with time and to decreasing temperatures (Palmia et al., 2019). Moreover, I considered in these calculations only the upper 0-3 cm sediment layer, which underestimates the true C sedimentary pool, and I assumed that during the dry period additional inputs of organic matter to the canal

exposed sediments are negligible as they lay within ploughed lands. Results from such calculations reveal that the theoretical C turnover time varies between 1 and 9 months, which is to say that in some of the stations (e.g. the drier sediments of canals 2 and 5) the large $CO_2$ fluxes have the potential to consume all the sedimentary C pool in a few weeks, whereas in other stations (e.g. the drier and wetter sediments of canal 1) the sedimentary C pool can feed heterotrophs for much longer periods, comparable to the periods during which sediments are exposed (October to May). These calculations, despite the uncertainty, support the idea that exposure time is a key parameter to consider when attempting to compare (or to pool) results from different canals.

*Magnitude of $CO_2$ emissions*

Exposed sediments from agricultural canals and ditches are active sites in terms of $CO_2$ emissions to the atmosphere. Measurements of $CO_2$ efflux from dry streambeds are in the same range of those measured in dry streambed sediment in the Fluviá River in Spain (Gómez-Gener et al. 2016) and higher than those observed in dry sediment in different locations (Table 3.4). Fluxes from dried canals are also higher than those reported for ponds, lakes, reservoirs, wetlands and running waters (Fromin et al. 2010; Raymond et al. 2013; Gómez-Gener et al. 2015; Deemer et al. 2016; Holgerson and Raymond 2016; Marcé et al. 2019; Keller et al. 2020).

Results from the studies that have compared $CO_2$ emissions over dry and wet phases within the same ecosystems highlight the importance of dry phase fluxes (Gómez-Gener et al. 2016; Looman et al. 2017; Obrador et al. 2018) with $CO_2$ emission can be twice as much as the rates measured during flowing conditions (Gómez-Gener et al. 2016), in particular in the early stage of desiccation (Kosten et al. 2018) or immediately after rewetting (Gallo et al. 2014). This can result in higher $CO_2$ emissions at intermediate stages of drying (Sponseller 2007; Kosten et al. 2018) as found in two canals investigated in this study (canals 1 and 3).

The time of exposure as well as many other factors including solar irradiance, wind conditions, precipitation, temperature and slope of the exposed area and macromolecular quality and quantity of the organic pool contribute to influence microbial processes and $CO_2$ emissions from sediments. All these factors were likely variable among the studied canals and may explain the observed differences. Without significant organic inputs, $CO_2$ emission from dried canal sediments can progressively decrease with time and become similar to those saturated with water. Higher release under intermittency may therefore represent an important but transient phenomenon.

**Table 3.4** $CO_2$ fluxes from submerged and exposed sediments of different types of freshwater systems worldwide

| References | Location, condition | System | Temperature (°C, mean ± SE) | $CO_2$ fluxes (mmol $m^{-2}$ $d^{-1}$, mean ± SE) | $CO_2$ fluxes range (mmol $m^{-2}$ $d^{-1}$) | Water content (%) | Organic matter content (%) |
|---|---|---|---|---|---|---|---|
| von Shiller et al., 2014 | Intermittent stream, Spain, summer | Dry watercourses | n.d. | Median: 212 | 36 - 455 | n.d. | n.d. |
| | | Running waters | n.d. | Median: 79 | 41 - 96 | n.d. | n.d. |
| | | Stagnant waters | n.d. | Median: 24 | 22 - 41 | n.d. | n.d. |
| | | Mediterranean soils (Bond-Lamberty and Thompson 2012) | n.d. | Median: 188 | 44 - 371 | n.d. | n.d. |
| Gomez-Gener et al., 2015 | Intermittent stream, Spain, summer | Dry beds | n.d. | 209 ± 10 | n.d. | mean 22 ± 4<br>range 2.3 – 44.4 | range 0.06 – 0.1 |
| | | Running waters | 18.9 ± 0.9 | 120 ± 33 | n.d. | n.d. | n.d. |
| | | Isolated pools | 18.3 ± 0.6 | 17.2 ± 0.9 | n.d. | n.d. | n.d. |
| | | Impounded waters | 20.5 ± 1.5 | 36.6 ± 8.5 | n.d. | mean 0.58 ± 0.06 | range 1.25 – 2.4 |
| Gomez-Gener et al., 2016 | Intermittent stream, Spain, summer | Dry streambeds | 21.1 ± 1.9 | 781.4 ± 390.2 | 342 - 1533 | mean 11.0 ± 5.2<br>range 3.4 ± 1.2<br>20.3 ± 5.6 | mean 2.28 ± 1.4<br>range 0.52 ± 0.2<br>4.42 ± 2.9 |
| | | Flowing streambeds | n.d. | 305.6 ± 206.1 | n.d. | n.d. | n.d. |
| | | Upland soils | 20.6 ± 1.4 | 896.1 ± 263.2 | n.d. | mean 18.0 ± 4.9<br>range 11.6 ± 1.6<br>27.0 ± 6.2 | mean 8.89 ± 3.0<br>range 5.9 ± 0.7<br>15.8 ± 8.5 |
| Obrador et al., 2017 | Temporary ponds, Menorca, summer and autumn | Dry phase | n.d. | 131.3 ± 46.2 | 22.9 - 492.5 | n.d. | n.d. |
| | | Wet phase | n.d. | 101.7 ± 29.9 | 15.7 - 333.1 | n.d. | n.d. |
| | | Flooding phase | n.d. | 90.3 ± 18.1 | 20.1 - 172.4 | n.d. | n.d. |
| Bolpagni et al., 2018 | Po River floodplain, backwater system, Italy, September | Aquatic zone | Range: 23.8 ± 5.7<br>21.1 ± 3.1 | 0.3 | n.d. | n.d. | n.d. |
| | | Periodically exposed sediments (<1 month) | | 18.7 | n.d. | n.d. | 4.75 ± 0.10 |
| | | Periodically exposed sediments (<3 month) | | 24 | n.d. | n.d. | 3.02 ± 2.14 |
| Keller et al., 2020 | Global data | Dry inland water | n.d. | mean 186 ± 326 | -27 - 2968 | n.d. | 6 ± 7 |
| | | Previously inundated sediments and terrestrial soils | n.d. | mean 222 ± 277 | n.d. | n.d. | 8 ± 8 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| This study | Po river basin, agricultural canals, autumn | Dry sediments | 18.7 ± 0.1 | 416.5 ± 78.8 | 88.9 – 1200.8 | mean 14.5 ± 0.7<br>range 8.2 - 18.6 | mean 6.0 ± 0.4<br>range 3.5 - 10.0 |
| | | Sediments at the interface | 17.6 ± 0.08 | 403.7 ± 60.2 | 88.4 – 1060.1 | mean 36.2 ± 2.0<br>range 22.9 - 49.2 | mean 6.5 ± 0.4<br>range 4.1 - 10.5 |
| | | Saturated sediments | 16.9 ± 0.07 | 158.1 ± 24.0 | 27.6 – 385.2 | mean 49.9 ± 2.1<br>range 34.8 - 66.4 | mean 5.5 ± 0.3<br>range 2.6 - 8.2 |

## CONCLUSIONS

On a global scale, periodically dried sediments may play an important role in terms of C emissions, as the global land area subjected to seasonally drought is expected to increase under predicted global change scenarios. Despite the growing interest in studying the effects of drying on a wide range of aquatic ecosystems and, especially in the last years, in $CO_2$ emission from dry rivers, this is one of the few studies analyzing artificial lotic ecosystems. Results suggest that air-exposed sediments act as critical areas for C exchanges, with large C emissions measured in dry sediments, doubling those reported for Mediterranean soils (von Shiller et al., 2014). In line with other studies, results show that sediment water content and organic matter content are the most important drivers of $CO_2$ emissions, that influence $O_2$ availability, microbial activity and respiration. The mechanisms by which moisture controls C fluxes are complex, as they result from the interplay between changes in redox conditions, microbial activity and gas diffusivity. I demonstrated that during desiccation aerobic conditions stimulated nitrification rates, acting as a weak sink of $CO_2$, and producing a small effect on gas emissions through $CO_2$ uptake. Denitrification activity was favored under submerged conditions, and was potentially responsible for a major fraction of $CO_2$ production in inundated sediments. However, $CO_2$ fluxes under saturated conditions were much lower than those measured in exposed sediments. Organic matter mineralization rates and gas diffusivity through the sediment are probably the determinant factors regulating $CO_2$ emissions in these environments.

Due to large environmental variability, measurements should be intensified at both temporal and spatial scales, and include parameters as the macromolecular quality of the organic matter as well as the sedimentary nitrate content, not measured here. Due to their location within heavily exploited agricultural areas, artificial canals might be regulators of carbon and nitrogen cycling; preliminary data suggest that the maintenance of an even small water flow during non-irrigation periods might be important to reduce both $CO_2$ emissions and inorganic N loads. However, the large scale implications of saturation and the emission of greenhouse gas as $N_2O$ and $CH_4$ should be carefully evaluated.

What emerges is the importance to perform repeated gas flux measurements over longer periods (e.g. some months), in order to catch temporal patterns. Future assessments of C budgets should include the contribution of these small but widespread artificial lotic environments that, because of their spatial extension and their role in nutrient cycling, nitrogen in particular, cannot be ignored.

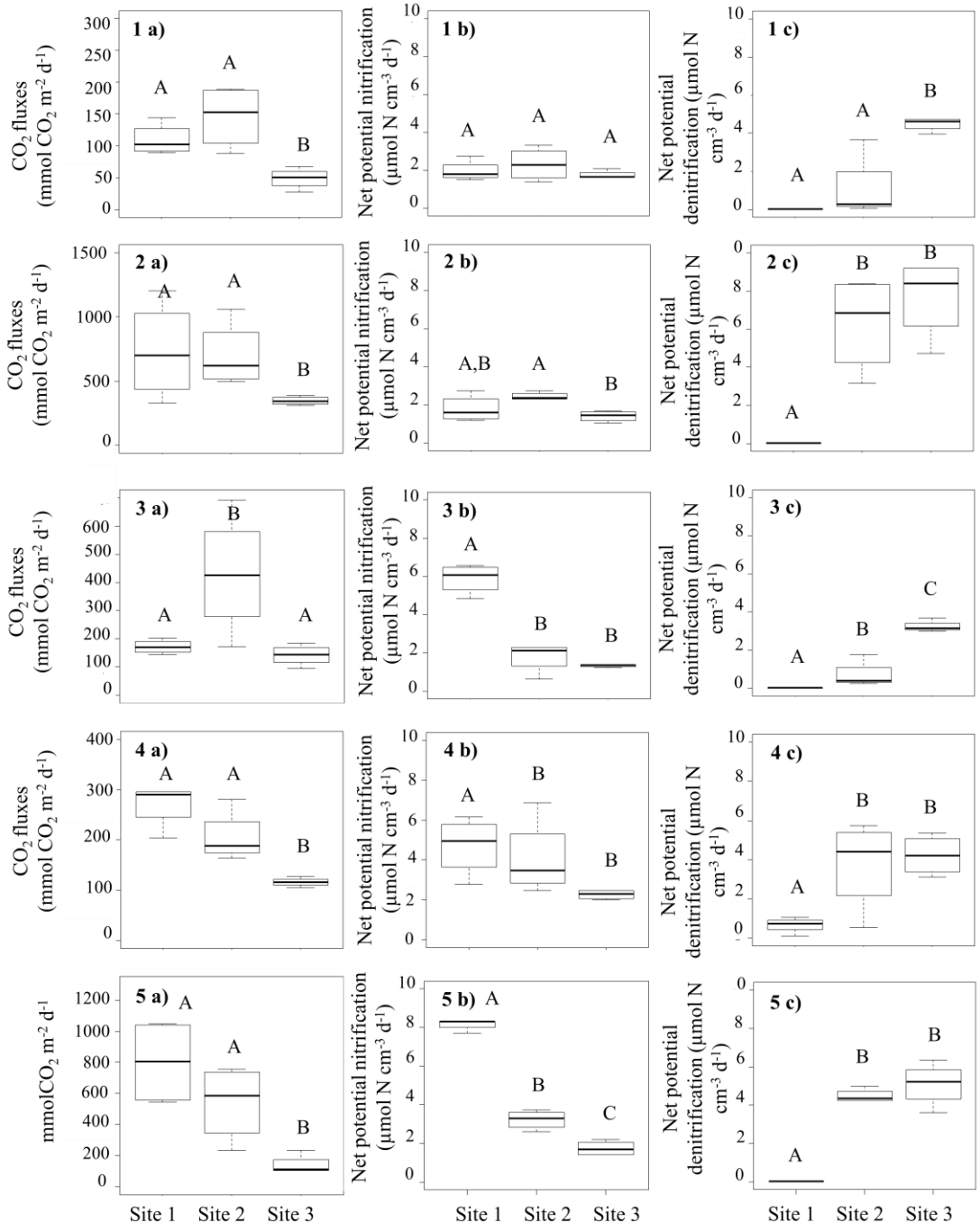


**Figure S3.1** Efflux of a $CO_2$ (a) and rates of net nitrification (b) and potential denitrification (c) measured in site 1, 2 and 3 of each studied canal (1,2,3,4,5). Significant differences between environment types for each parameter ($p<0.05$, Tukey's post hoc test) are marked with different capital letters above the box plots.

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# General Conclusions

Climate change and the effects that it is producing on ecosystems functioning are more and more evident and increasing in intensity, which suggests a series of positive feedbacks, as demonstrated for other large and global impacts as eutrophication or nitrogen release from agriculture. Climate change affects deeply the hydrological cycle and is expected to modify the patterns of precipitation in most Europe, with consequences on water availability for human use. In Italy, the projections of such changes forecast a decrease, by up to 50%, of river discharge during summer. In the Po River Plain water availability from the Alps and the Apennines is the engine of industrial, agricultural and animal farming strategic sectors. In the last years, water budget calculations suggest that water use by these sectors significantly exceeds water availability. This means that an increasing number of lotic ecosystems will undergo water shortage or hydrological intermittency and desiccation. For most Italian rivers, a minimum or an ecological vital flow, which is the minimum amount of discharge that allows organisms survival and biotic and abiotic processes to occur, was set based on experimental activities or as a percentage of the natural mean discharge. Water abstractions should always consider the thresholds defined for every single river, but climatic extremes as dry winter or spring periods allow for exception of this rule to guarantee human use. This means that under a scenario of much lower water availability abstractions will not decrease proportionally and river will run dry. Hydrological intermittency is therefore expected in years to come and little is known about the effects it will produce on the functioning of rivers. This thesis aimed at deepening our understanding of metabolic aspects related to this topic. To this purpose, I analysed the combined action of water and macrofauna on coarse organic matter decomposition, simulating the short-term disappearance of both these factors. The experiment I performed under controlled laboratory conditions applies to low order streams, which influence via macrofauna metabolic rates labile organic matter and nutrient transport downstream. The simulation of water intermittency in streams suggest a sharp interruption of decomposition processes, which means higher retention of coarse organic matter upstream and less transport of nutrients downstream. Future experiments should expand this initial effort and consider the timing required by macrofauna communities to recover after the desiccation stress, which may set to zero those species that are uncapable to contrast water shortage. Upon water return, a lag period, with variable length, is expected to occur before the community recover to the initial structure and perform the same ecosystem services. It appears also interesting, as a consequence of the temporary discontinuum, to monitor primary and secondary

production in high order river sectors, to analyze the downstream impact related to the interruption of dissimilative processes.

Closely related to this experiment is that performed on the effects of sediments desiccation in the stream hyporheic zone. The latter has a central role in river functioning due to multiple interfaces, high microbial density and elevated turnover of dissolved organic matter and inorganic nutrients. Again, I adopted a manipulative, experimental approach to analyse the effects of desiccation on the functioning of the hyporheic zone and in particular on microbial respiration (total, aerobic and anaerobic) and on inorganic nutrient (N, Si, P) cycling. Besides the factor desiccation, I added another potentially important element in the analysis, discriminating *the memory* of microbial consortia on water shortage. To this purpose, a large number of hyporheic reactors (10 per group) were realized using sediments from perennial streams and from naturally intermittent streams. The idea and the hypothesis underlying this choice was that different systems might have different memory and display different reactions to water shortage. Specifically, I expected a much larger impact on perennial streams, not used to desiccation. The experimental setup that was arranged to test these hypotheses was quite complex and required many weeks of work: the realistic simulation of interstitial water flow requires to simplify the natural hyporheic environment to avoid clogging and to ensure measurements. The main result of this experiment was that the activity of microbial consortia, differently from macrofauna, displayed no differences between the two groups of sampling sites. Microbes seems to react fast and to adapt to changes and have no memory for events as desiccation or perennial water flow. Another important result of the experiment is the impressive pulse of nutrient release that was measured upon water return. This pulse was observed also in the previous experiment and suggest that drought and rewetting alter the timing of nutrient transport. It is likely that an entire reach of river that undergoes desiccation is capable to release, upon rewetting, large amounts of nutrients exceeding the metabolic capacity (the uptake spirals) of downstream sectors. This means that a large fraction of nutrients can be transported without being metabolized. I discussed several potential limitations of the hyporheic reactors, that can be improved in the future but represent an opportunity to study subsurface processes altering the stoichiometry of nutrients or the nutritional quality of the organic matter or the level of oxygen to simulate suboxic or anoxic conditions. All these aspects and factors need to be urgently tackled to understand important metabolic aspects of river functioning.

Another part of my work focused on downstream, artificial lotic ecosystems that regularly undergo desiccation. Artificial irrigation canals are not as fascinating environments as low order mountain streams, but I introduced their relevance for global budgets and I stressed

the importance of measurements to be implemented in these systems. In the plain sectors of cultivated watersheds as the Po River Plain artificial canals represent the most abundant aquatic ecosystem and probably the quantitatively more important metabolic reactors. They are small, have multiple interfaces, accumulate large amounts of labile organic carbon and transport large amounts of nutrients. They also represent widespread examples of artificially intermittent systems, due to the regulation of their flow and the absence of water during non-irrigation period. Again, as in previous sections, I focussed on metabolic aspects as net $CO_2$ production and release to the atmosphere, which is the best proxy of total sediment respiration. The regulating factors of $CO_2$ fluxes that I considered were the level of water saturation, the organic matter content and two microbial processes of nitrogen cycle that are strongly linked to $CO_2$ production (denitrification) and fixation (nitrification). Differently from previous investigations I worked in situ, where I did $CO_2$ measurements along a gradient of water saturation in independent canals. Differently from previous environments, I focused on impermeable sediments where the dynamics triggered by water shortage and exposure to the atmosphere are completely different and diffusion mediated. The hypothesis here was that under decreasing water saturation muddy sediments become more and more oxidized, with implications for nitrification (stimulated) and denitrification (inhibited) but even more for the efficiency of mineralization processes (higher under increasing oxygen availability). Interestingly, I demonstrated that the main regulator of $CO_2$ efflux in lowland canals is water saturation, which is depressing $CO_2$ evasion to the atmosphere by a factor of 3. Desiccation in organic canal sediments produced significant effects on microbial N transformations, but not significant enough to impact $CO_2$ fluxes. In lowland canals, therefore, microbial processes which explain most of organic matter decomposition are enhanced during non-irrigation periods. Future studies in this field should expand the number of canals where metabolic measurements are performed, should upscale measured rates by the thousands of Kilometres canals in watersheds and should include other potentially important greenhouse gas as $CH_4$ and $N_2O$ in measurements. This might allow to analyse whether the maintenance of water in these canals during winter can produce a significant reduction of greenhouse gas emission and increase the removal of reactive nitrogen via denitrification. The desiccation of irrigation canals on the contrary results in large ammonium oxidation during drought and massive downstream transport of nitrate upon rewetting.