Regulation of CO₂ fluxes along gradients of water saturation in irrigation canal sediments

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Received: 22 April 2020 / Accepted: 30 December 2020 / Published online: 18 January 2021
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Abstract

Hydrological intermittency affects sediment biogeochemistry, organic carbon (OC) metabolism and carbon dioxide (CO₂) emission but the study of the effects of drying is generally confined to natural ecosystems. Agricultural canals are artificial, widespread elements in irrigated floodplains, and regularly subjected to water level fluctuations. The aim of this study was to quantify the CO₂ emissions along water saturation gradients in artificial canals to understand the environmental factors regulating these fluxes. CO₂ measurements were performed in five replicated canals within the Po River basin (Northern Italy). In each canal we analysed three sites: (i) a spot with exposed, dry sediments; (ii) a spot with inundated, saturated sediments and (iii) a spot with an intermediate level of saturation. Besides dark CO₂ flux measurements, net potential nitrification and denitrification rates were measured as proxies of sediment redox potential and due to their CO₂ sink and source role, respectively. We hypothesized a site-specific regulation of CO₂ emission, depending on the interplay among water saturation, sediment oxidation and organic matter content. Our results suggest that desiccation stimulates mineralization processes and CO₂ fluxes, that were mainly dependant on water and organic matter content and correlated with microbial N transformations. CO₂ emissions tended to increase along the considered water saturation gradients, almost tripling rates from inundated, saturated (158.2 ± 24.1 mmol CO₂ m⁻² days⁻¹) to dry, exposed sediments (416.5 ± 78.9 mmol CO₂ m⁻² days⁻¹). Results also suggest that net potential nitrification and denitrification allow tracing the effects of drying on N microbial communities involved in CO₂ fluxes. Net potential nitrification rates produce little effects on CO₂ fluxes, but is a good proxy of oxygen (O₂) availability, whereas potential denitrification may be responsible for variable fractions (up to 100%) of CO₂ production, in wetter sediments.

Keywords CO₂ emission · Net potential nitrification · Potential denitrification · Air-exposed sediments · Agricultural canals

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00027-020-00773-5>.

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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; Marcé et al. 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 CO₂ 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 CO₂ 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 fish-ponds, 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 CO₂ 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 CO₂ 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 and Marxen 2011). However, biogeochemical processes and in particular OC mineralization are demonstrated to continue in dried systems (Zoppini and Marxen 2011; Pohlson et al. 2013; Timoner et al. 2014), so that temporary streams can release significant amounts of CO₂ 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 CO₂ 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 CO₂ 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 O₂ penetration and the sediment volume where aerobic microbial activities

take place. O₂ microprofiles revealed in fact that in organic sediments O₂ penetration depth is confined to the upper few millimeters (de Klein et al. 2017). During water level draw-down, the enhanced O₂ 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 O₂ content can be measured with electrodes (Koschorreck 2005). Alternatively, specific microbial processes as nitrification and denitrification, that are sensitive to O₂ levels, can be measured as indirect proxies. Both processes respond to redox oscillations associated with varying levels of water saturation and O₂ availability in sediments (Martin et al. 2001; 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 O₂ conditions and is expected to increase along with water saturation and O₂ shortage (Canfield et al. 2005). Significant positive and negative correlations between nitrification and denitrification rates and O₂ 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 CO₂ 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 O₂ penetration on OC mineralization and on CO₂ emission. Taken together, these often-contrasting effects of water intermittency and drying on net CO₂ emissions support the idea of a complex and site-specific regulation.

The aim of this study is to quantify CO₂ 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 CO₂ 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 O₂ penetration, aerobic and anaerobic C processing and CO₂ diffusivities, regulating CO₂ emission. These issues make agricultural canals ideal sites to explore the effects of drought in artificial ecosystems, to study the mechanisms regulating CO₂ 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 CO₂ emissions in agricultural canals may help us to understand how CO₂ 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, we hypothesized a complex, local regulation of CO₂ fluxes. Previous studies have demonstrated increased CO₂ 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 CO₂ diffusivity. We 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 O₂ penetration and microbial processes as nitrification and denitrification, ultimately affecting net CO₂ emission. In particular, local conditions promoting chemoautotrophic nitrification should reduce CO₂ effluxes whereas conditions promoting heterotrophic denitrification should stimulate CO₂ 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 (Fig. 1) and CO₂ 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 (Fig. 2). Since the irrigation canals were

Fig. 1 Location of the Po river basin (Northern Italy) and of the five artificial canals studied in this work

Fig. 2 The sampling strategy consisted in a transect perpendicular to the shore including 3 sites with variable water saturation levels

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 four 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.

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 min, 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 , $\text{mmol CO}_2 \text{ m}^{-2} \text{ days}^{-1}$) 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 days^{-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 ($\text{m}^3 \text{ atm K}^{-1} \text{ mmol}^{-1}$).

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 Plexi-glass 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 NH₄⁺ 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 NH₄⁺ 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 h incubation the slurry was subsampled, centrifuged at 3000 rpm for 10 min, filtered (Whatman GF/F filters), and analysed for combined nitrite and nitrate (NO_x⁻ = NO₂⁻ + NO₃⁻) via standard spectrophotometric techniques (APHA et al. 2017). Net potential nitrification rates (PN, µmol N cm⁻³ days⁻¹) were calculated from accumulation of NO₂⁻ + NO₃⁻ over time from the equation:

$$PN = \frac{d[\text{NO}_x^-]}{dt \times [S]}$$

where $d[\text{NO}_x^-]$ is the accumulation of NO₃⁻ and NO₂⁻ in the slurry (µM), dt is the incubation time (d), and $[S]$ is the concentration of the slurry (cm³ 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 then filled with water, previously bubbled with N₂ to remove O₂. 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 O₂ and nitrate traces; afterward, 100 µL of Na¹⁵NO₃ 20 mM were added through the exetainer lid septum and an accessory needle and the anoxic slurry was incubated for 8 h, as detailed in Moraes et al. (2019). At the end of the incubation 200 µL of 7 M ZnCl₂ was added to the exetainers to inhibit microbial activity. ¹⁵N abundance in N₂ was analysed by membrane inlet mass spectrometry (MIMS, Bay instruments, USA). The rates of potential denitrification (PD, µmol N cm⁻³ days⁻¹) were calculated as

$$PD = \frac{2[^{30}\text{N}_2]}{dt \times [S]}$$

where $[^{30}\text{N}_2]$ is the concentration of ¹⁵N-labelled N₂ gas at the end of the incubation (µM), dt is the incubation time (d), and $[S]$ is the concentration of the slurry (cm³ of fresh sediment L⁻¹). We assumed that at the beginning of the incubation concentrations of ¹⁵N-labelled N₂ were negligible.

Potential activities of nitrifiers and denitrifiers were converted into potential CO₂ 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 CO₂ production assuming a ratio between moles of C oxidized and moles of nitrate reduced of 1.25:1 (Atlas and Bartha 1998).

Statistical analyses

Within each canal, the effect of different sampling sites on CO₂ 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 CO₂ 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 Inflation 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 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 1). Within each canal, water content varied among sites by a factor of 3–4.

Table 1 Sedimentary features of sampling sites along the transects of each studied canal and of the pooled canals (mean $\pm$ SE are reported, $n=4$ and $n=20$, respectively)

Canal code	Site	T ($^{\circ}$ 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 $\pm$ SE									
Site 1		18.7 $\pm$ 0.11		14.5 $\pm$ 0.71		6.03 $\pm$ 0.4		59.1 $\pm$ 14.7	
Site 2		17.6 $\pm$ 0.08		36.2 $\pm$ 2.03		6.51 $\pm$ 0.42		535 $\pm$ 166.4	
Site 3		16.9 $\pm$ 0.07		47 $\pm$ 2.12		5.55 $\pm$ 0.33		1588 $\pm$ 196.3	

Sedimentary organic matter content varied from $3.7 \pm 0.4\%$ to $10.7 \pm 0.7\%$ 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 1).

CO₂ emissions, net potential nitrification and potential denitrification rates

All sites in all canals were net CO₂ emitters to the atmosphere (mean $\pm$ SE = 326.1 ± 36.8 mmol CO₂ m⁻² days⁻¹, $n=60$; range = 27.6–1200.8 mmol CO₂ m⁻² days⁻¹). Fluxes were extremely variable among canals and among sites (data from single canals are reported in the supplementary Fig. S1); however, as a general tendency, they tended to decrease with increasing levels of water content (Fig. 3). Such pattern was rather evident in canals 2, 4 and 5 whereas in canals 1 and 3 CO₂ emissions peaked at the intermediate water content (Supplementary materials, Fig. S1).

Rates of net potential nitrification (mean $\pm$ SE = 2.9 ± 0.2 $\mu\text{mol N cm}^{-3}$ days⁻¹, $n=60$; range = 0.6–8.3 $\mu\text{mol N cm}^{-3}$ days⁻¹) varied also among canals and sites and in general tended to decrease, as CO₂ fluxes, along with increasing levels of water content (Fig. 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, Fig. S1). Rates of CO₂ potentially fixed by nitrifiers were estimated to vary between 1.1 and 6.9 mmol C m⁻² days⁻¹. This means that net potential nitrification may attenuate CO₂ emission by exposed sediments by 0.2–3%.

Rates of potential denitrification (mean $\pm$ SE = 2.9 ± 0.3 $\mu\text{mol N cm}^{-3}$ days⁻¹, $n=60$; range = 0.0001–9.2 $\mu\text{mol N cm}^{-3}$ days⁻¹) exhibited a different pattern as they always tended to increase along with the level of water saturation (Fig. 3).

The production of CO₂ calculated from potential denitrification varied between 0.3 and 288.8 mmol C m⁻² days⁻¹. CO₂ 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.

Drivers of CO₂ emissions

The analysis of CO₂ fluxes and net potential nitrification and denitrification rates as a linear function of sediment

Fig. 3 Efflux of CO₂ (a) and rates of net potential nitrification (b) and denitrification (c) measured in exposed, dry sediments (site 1), in sediments with intermediate level of water content (site 2) and in inundated, saturated sediments (site 3) measured in 4 spots of 5 replicated

canals (n = 20). 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

water content revealed large variability in the five canals. Results from these linear regressions are reported for each canal in Table 2. Slopes of CO₂ emissions VS water content were significantly different from zero in canals 1, 2, 4 and 5 (Table 2), those of net potential nitrification rates were significant in canals 3, 4 and 5 (Table 2), whereas slopes of denitrification were significant and positive in all canals (Table 2).

Furthermore, we constructed a simple model including all measured variables in order to analyse quantitatively the fraction of CO₂ flux variance explained by the measured sedimentary features and by N transformation rates. We developed a series of multiple regression models initially 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).

The results emphasize the predominant influence of water content, organic matter content and their interaction on the CO₂ efflux rates in the air-exposed sediments. Increasing water content had a negative effect on the CO₂ fluxes while increasing organic matter content resulted in increasing CO₂ fluxes from sediments to the atmosphere (Table 3). However, these effects depended upon the interaction of the two factors (Table 3; Fig. 4). Our results show a decrease in CO₂ fluxes with increasing water content at lower values of organic matter, while under elevated organic matter content the effect of water content on CO₂ emission is mitigated and less clear (Fig. 4).

The effect of nitrification and denitrification processes on CO₂ emissions was not so marked. Despite the effect was not statistically significant, our results showed a weakly negative and positive effect of net potential nitrification

Table 2 Linear regression between CO₂ fluxes and net potential nitrification and net potential denitrification rates and water content (mean ± SE, n = 12)

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

Significant values (*p* < 0.05) are printed in bold

Table 3 Results from multiple linear regressions testing the effects of sediment water and organic matter contents, and net potential nitrification and denitrification rates on CO₂ fluxes

	Slope	SE	<i>t</i> value	<i>p</i> 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

and denitrification rate on the CO₂ emissions, respectively (Table 3, Fig. 5a, b).

Discussion

Desiccation increases CO₂ emission in irrigation canals

Experimental results from this study suggest that in irrigation canal sediments desiccation stimulates OC mineralization processes and CO₂ 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 CO₂ fluxes, but allows to trace oxygen availability, whereas potential denitrification may support large CO₂ production in wetter sediments. Results from the model testing the effect of all measured variables

on CO₂ fluxes show that sediment moisture, organic matter content and the interaction between the two factors were the strongest predictors of CO₂ fluxes, as recently found by Keller et al. (2020). At lower sediment organic matter content there is a predominant effect of water in driving CO₂ emissions, with decreasing CO₂ emissions along with increasing water content. With lower organic matter content, the negative effect of water content on CO₂ emissions can be explained though the effect of lower O₂ 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. We speculate that variable O₂ penetration may determine different effects on CO₂ 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 O₂ availability, resulting in higher CO₂ efflux (Fujikawa and Miyazaki 2005; Luo and Zhou 2010; Kosten et al. 2018). Under this circumstance, the regulation of CO₂ 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 CO₂, 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 CO₂ emissions is

Fig. 4 Efflux of CO₂ as a function of sediment water content at different sediment organic matter content. Water content and organic matter values reported are mean-centered

masked due to the higher availability of organic matter that fuels CO₂ production, resulting in high CO₂ emissions even under water saturation conditions (Keller et al. 2020).

CO₂ fluxes regulation in irrigation canals

The amount of organic matter has been identified as an important factor affecting CO₂ 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). We 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 CO₂ 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 CO₂ 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 CO₂ emission from sediments. This is also likely

Fig. 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

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 we 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 $\mu\text{mol N m}^{-2} \text{h}^{-1}$) and they are regulated by pH and temperature and by the availability of NH_4^+ and O_2 (Prosser 2005). We hypothesized an increase of the potential rates along with increasing sediment desiccation, but our 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 we 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). Our 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 CO₂ fluxes, likely due to different reasons. A possible explanation is the presence of water that limits CO₂ evasion despite potentially high production. Alternatively, measurements of denitrification (and nitrification) are potential, which means that for both we have added large amounts of nitrate (and NH₄⁺) 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 days⁻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 CO₂ fluxes. Elsewhere similar calculations in riparian areas suggest that denitrification support 25–30% of CO₂, 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 NH₄⁺ oxidation in dry soils (Vybernaite-Lubiene et al. 2018).

Irrigation canals as model systems to study the effects of desiccation on C turnover and CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 NH₄⁺, 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 NH₄⁺ availability along the saturation gradients suggests in fact uncoupled ammonification and nitrification when O₂ penetration is low (e.g. under water saturated conditions). On the other hand, the low NH₄⁺ concentrations in drier sediment suggests air penetration and increased conversion of NH₄⁺ to NO₃⁻.

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 O₂ availability in sediments. Also exchangeable NH₄⁺, 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 CO₂ 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. We 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. We 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, we considered in our calculations only the upper 0–3 cm sediment layer, which underestimates the true C sedimentary pool, and we 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. Our measurements of CO_2 efflux from dry streambeds are in the same range of those measured in dry streambed sediment in the Fluvía River in Spain (Gómez-Gener et al. 2016) and higher than those observed in dry sediment in different locations (Table 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; Holgersson 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.

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. Our 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, our 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. We 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

Table 4 CO₂ 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 days ⁻¹ , mean ± SE)	CO ₂ fluxes range (mmol m ⁻² days ⁻¹)	Water content (%)	Organic matter content (%)
von Shiller et al. (2014)	Intermittent stream, Spain, summer	Dry water-courses	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 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 Range 3.4 ± 1.2 20.3 ± 5.6	Mean 2.28 ± 1.4 Range 0.52 ± 0.2 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 Range 11.6 ± 1.6 27.0 ± 6.2	Mean 8.89 ± 3.0 Range 5.9 ± 0.7 15.8 ± 8.5
Obrador et al. (2018)	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. (2017)	Po River floodplain, backwater system, Italy, October	Aquatic zone	Range: 23.8 ± 5.7	0.3	n.d	n.d	n.d
		Periodically exposed sediments (< 1 month)	21.1 ± 3.1	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

Table 4 (continued)

References	Location, condition	System	Temperature (°C, mean $\pm$ SE)	CO ₂ fluxes (mmol m ⁻² days ⁻¹ , mean $\pm$ SE)	CO ₂ fluxes range (mmol m ⁻² days ⁻¹)	Water content (%)	Organic matter content (%)
This study	Po river basin, agricultural canals, autumn	Dry sediments	18.7 $\pm$ 0.1	416.5 $\pm$ 78.8	88.9–1200.8	Mean 14.5 $\pm$ 0.7 Range 8.2–18.6	Mean 6.0 $\pm$ 0.4 Range 3.5–10.0
		Sediments at the interface	17.6 $\pm$ 0.08	403.7 $\pm$ 60.2	88.4–1060.1	Mean 36.2 $\pm$ 2.0 Range 22.9–49.2	Mean 6.5 $\pm$ 0.4 Range 4.1–10.5
		Saturated sediments	16.9 $\pm$ 0.07	158.1 $\pm$ 24.0	27.6–385.2	Mean 49.9 $\pm$ 2.1 Range 34.8–66.4	Mean 5.5 $\pm$ 0.3 Range 2.6–8.2

uptake. Denitrification activity was favored under submerged conditions, and was potentially responsible for a major fraction of CO₂ production in inundated sediments. However, CO₂ 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 CO₂ 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; our preliminary data suggest that the maintenance of an even small water flow during non-irrigation periods might be important to reduce both CO₂ emissions and inorganic N loads. However, the large scale implications of saturation and the emission of greenhouse gas as N₂O and CH₄ 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.

Acknowledgements The authors wish to thank the two anonymous reviewers for their suggestions and positive critics that highly contributed to the improvement of this work. We thank C. Lozano Fondón for helping with GIS elaboration.

Author contributions BP and MB carried out the study conception and design. Material preparation, data collection, field sampling and laboratory analysis were carried out by BP. The first draft of the manuscript was written by BP. SL performed statistical analysis and revised the paper draft. PV provided funding and revised the paper draft. MB contributed to data interpretation and to paper writing. All authors read and approved the final manuscript.

Funding Open Access funding provided by Università degli Studi di Ferrara. This study was funded by NOACQUA project—responses of communities and processes in intermittent rivers—funded by the Italian Ministry of Education and University (PRIN 2015, Prot. 201572HW8F).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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