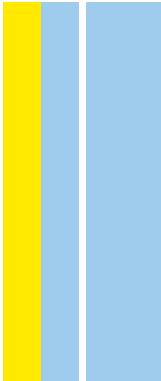


MESTRADO
TOXICOLOGIA E CONTAMINAÇÃO AMBIENTAIS

Emission of global warming gases from Northern Portugal estuaries: the contribution of CH₄ and N₂O

Alexandra Dinis Santos

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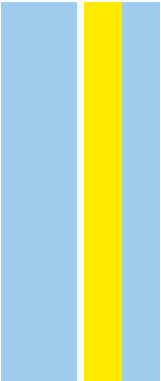


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Dissertação de Candidatura ao grau de Mestre em
Toxicologia e Contaminação Ambientais submetida
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Abstract

Global warming is one of the most important problems of the 21st century. Human activities cause an increase in greenhouse gases emissions to the atmosphere. The increase of greenhouse gases in the environment, such as carbon dioxide, methane, and nitrous oxide, leads to an increase in their concentration in the atmosphere with commensurable impact on the climate due to their ability to absorb infrared radiation. Our research focused on two major gases responsible for global warming, methane (CH₄), and nitrous oxide (N₂O). Estuaries are one of the natural sources of emissions of these gases to the atmosphere. As such, the objective of this work was to estimate the potential emissions of CH₄ and N₂O to the atmosphere from the Douro, Ave, and Cávado estuaries. Water and sediment samples were collected seasonally in intertidal areas of the three estuaries. Dissolved concentrations of CH₄ and N₂O in water were quantified by GC-FID and GC-ECD, respectively, and potential fluxes of CH₄ and N₂O from sediments were estimated using slurry incubations. Douro estuary presented the higher value of both dissolved methane (1.149 nmol L⁻¹) and nitrous oxide (0.104 nmol L⁻¹). In addition, Douro estuary sediments presented the higher values of methane production (26.48 nmol h⁻¹ g⁻¹ wet sed). Ave estuary sediments presented the higher value of nitrous oxide production (0.10 nmol h⁻¹ g⁻¹ wet sed). In spite of being located within the same geographic area, the estuaries presented substantially different results in what methane and nitrous oxide emissions are concerned. Further studies are necessary to better understand the magnitude of the problem and to design measures to remediate the impacts on the estuaries.

Resumo

O aquecimento global é um dos maiores problemas do século XXI. As atividades humanas causam um aumento nas emissões de gases de efeito estufa para a atmosfera. O aumento da produção de gases, como o dióxido de carbono, o metano e o óxido nitroso, leva a um aumento da sua concentração na atmosfera, com impactos comensuráveis para o clima. Estes gases de efeito de estufa são importantes devido à sua capacidade de absorverem radiação infravermelha. Este estudo concentrou-se em dois importantes gases responsáveis pelo aquecimento global, o metano (CH_4) e o óxido nitroso (N_2O). Os estuários são conhecidos como ambientes onde ocorrem emissões naturais destes gases para a atmosfera. Assim, o objetivo deste trabalho foi estimar as emissões de CH_4 e N_2O dos estuários do Douro, Ave e Cávado. Amostras de água e sedimento foram colhidas sazonalmente, em zonas intertidais dos três estuários. As concentrações de CH_4 e N_2O dissolvidos foram quantificadas por GC-FID e GC-ECD, respetivamente. Os fluxos de CH_4 e N_2O a partir dos sedimentos foram estimados usando *slurries*. O estuário do Douro apresentou os valores mais elevados quer de CH_4 ($1.149 \text{ nmol L}^{-1}$) quer de N_2O ($0.104 \text{ nmol L}^{-1}$) dissolvidos. Adicionalmente, o estuário do Douro também apresentou valores elevados para a produção de CH_4 em sedimentos ($26.48 \text{ nmol h}^{-1} \text{ g}^{-1} \text{ sed hum}$). Por sua vez, o estuário de Ave apresentou o maior valor de produção de N_2O ($0.10 \text{ nmol h}^{-1} \text{ g}^{-1} \text{ sed hum}$). Embora os estuários em estudo estejam localizados na mesma área geográfica, apresentam resultados substancialmente diferentes nas emissões de CH_4 e N_2O . Mais estudos são necessários para entender melhor a magnitude do problema e criar medidas para remediar potenciais impactos negativos.

Introduction

Global warming and greenhouse effect

Global warming is one of the most important issues of the 21st century (Houghton, 2009). The term global warming is used to describe the increase in the average temperature of the Earth oceans and atmosphere (Bodri et al., 2007). Throughout history, Earth climate has changed. In the last 650 000 years, there have been seven cycles of glacial advance and retreat, fostered by natural causes, with the last ice age ending abruptly about 7 000 years ago, marking the beginning of the modern climate era (IPCC, 2014b). In these days, global warming refers to the rapid rising of the global temperature occurring during the last 100 to 150 years, believed to be related to anthropogenic activities (Bodri et al., 2007). Activities like the burn of fossil fuel, deforestation, and intense agriculture are increasing the concentration of greenhouse gases in the atmosphere, causing an increase in the greenhouse effect (Houghton, 2009).

The greenhouse effect is fundamental to the life on Earth regulating the temperature to around 18 °C, and without it the temperature would be -15 °C (Tremblay et al., 2005). In normal conditions, the solar radiation is absorbed by the Earth, warming it, and reflected by the atmosphere and the Earth. With the increase of the greenhouse gases in the atmosphere, the infrared radiation reflected and emitted by Earth is absorbed and re-emitted in all directions, causing the warm of Earth surface and lower atmosphere (Figure 1) (North, 2015).

Since the late 19th century, the average temperature has risen about 0.9 °C, mainly due to the large increase of emissions of greenhouse gases to the atmosphere. Records show that the warmest years occurred in the last 35 years, with the warmers being registered since 2010. The warmer year, on the record, was 2016, with eight of twelve months (January to September, with the exception of June) being the warmest on record for those months, respectively (IPCC, 2018).

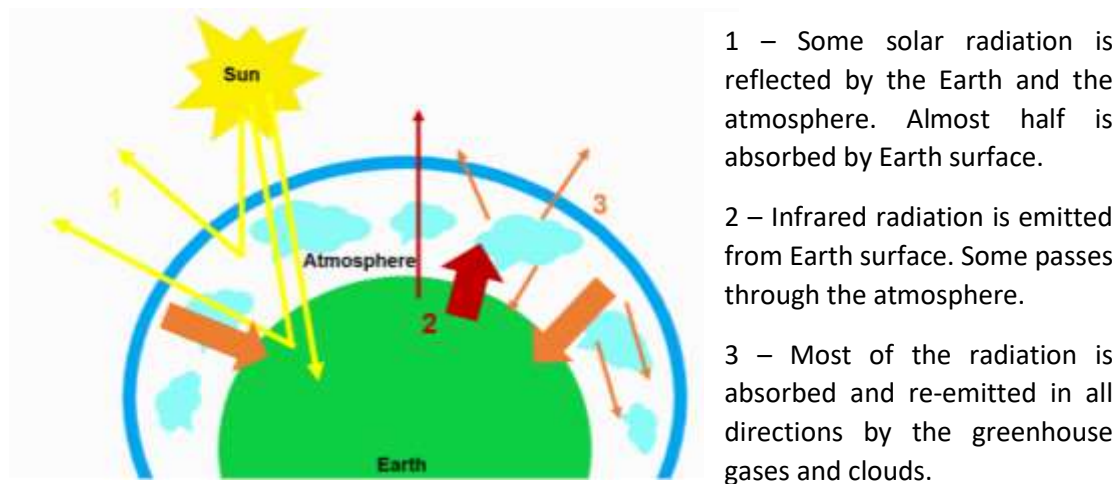


Figure 1 – Schematic of the natural greenhouse effect (original).

The warming of the Earth surface causes the melting of permafrost. Permafrost degradation can cause impacts locally and globally, one of the more important being the release of greenhouse gases to the atmosphere (Streletskiy et al., 2015). Additionally, the increase of the temperature can cause serious problems like melting of polar ice caps leading to sea levels rising and more coastal erosion, increase in the magnitude and extension of natural disasters, desertification, massive migration, and increase in epidemics (Houghton, 2009).

Greenhouse gases

Greenhouse gases affect the Earth climate by trapping the radiation in the atmosphere, inhibiting the cooling of the Earth (North, 2015). Thus, the increase of greenhouse gases in the atmosphere, such as water vapour, CO_2 , methane (CH_4), and nitrous oxide (N_2O) is important due to their ability to retain the heat. The emissions of these gases can occur naturally in rivers, lakes, estuaries, soils, and forests, but anthropogenic activities are increasing emissions in rates never seen before (Lashof et al., 1990).

Acting as a feedback mechanism to the climate, the water vapour is one of the most important gases in the atmosphere. Its concentration in the atmosphere increases as the Earth is getting warmer but reduces because of clouds formation and precipitation (IPCC, 2014b). It is one of the most important greenhouse gases being capable of absorbing and emitting infrared radiation. As an important component of the hydrological cycle, it is naturally present in the atmosphere. Its evaporation to the atmosphere can occur from

water and soils, there it condenses and forms clouds, returning to Earth surface by precipitation (Brusseau et al., 2019).

As part of the Earth carbon cycle, CO₂ is also naturally present in the atmosphere, but human activities are adding more CO₂, affecting the ability of natural sinks to remove it (EPA, 2019). CO₂ is produced naturally by respiration and volcano eruptions, and anthropogenically by deforestations, land use alterations, and the burning of fossil fuels (IPCC, 2014b). With a lifetime of about 100 years, the CO₂ is the most abundant greenhouse gas present in the atmosphere, causing about 63% of global warming (Houghton, 2009; IPCC, 2007b).

Methane is a key component of natural gas. Natural sources of CH₄ to the atmosphere include wetlands, natural transfer of CH₄ from the soil by plants, methanogenic activity in soil and sediments, and animal digestive processes such as termites and ruminants (Figure 2). With a land occupation of only 6 %, wetlands represent 63 % of the natural sources of CH₄ to the atmosphere; this emission is affected by abiotic and biotic factors. Nonetheless, human activities continue to be the main contributor to CH₄ presence in the atmosphere, with activities such as the burning of fossil fuel, agriculture, burning or destruction of biomass, wastewater management, and indirectly the melting of permafrost (Houghton, 2009; Hu et al., 2019; IPCC, 2013; Rasmussen et al., 1983). Agriculture is one of the biggest sources of CH₄ mainly due to rice production, livestock production, enteric fermentation, and landfills (IPCC, 2007a). In 1997, it was estimated that agriculture was responsible for two-thirds of the CH₄ sources to the atmosphere. The previsions indicate that if no measures are taken, the CH₄ emissions will increase 60 % until to 2030 (Cole et al., 1997; IPCC, 2007a). CH₄ is a major by-product of organic matter degradation (Bastviken, 2009). In sediments, under aerobic conditions, CH₄ is formed by microorganisms that convert organic compounds to CH₄. However, under anaerobic conditions, CH₄ can be produced by a process called methanogenesis. This process occurs in sediment, marine environments and freshwater, and is carried out by anaerobic bacteria as free-living or as symbionts with protists or animals (Cui et al., 2015; Fenchel et al., 2012). CH₄ degradation can occur by two processes, photochemical elimination and microbial oxidation (Yoon et al., 2009). In the first, reactions in the stratosphere with hydroxyl radicals or electronically excited singlet oxygen, degrade CH₄. In the microbial process, the oxidation of CH₄ is carried out by methanotrophs, since this bacterial group uses CH₄ as energy and carbon source. Before 1800, the concentration of CH₄ in the atmosphere was twice lower than the levels present in our days (Houghton, 2009; Yoon et al., 2009). The average lifetime of CH₄ in the atmosphere is about 12

years shorter than CO₂, being responsible for 21% of the greenhouse effect (EPA, 2019; IPCC, 2007b).

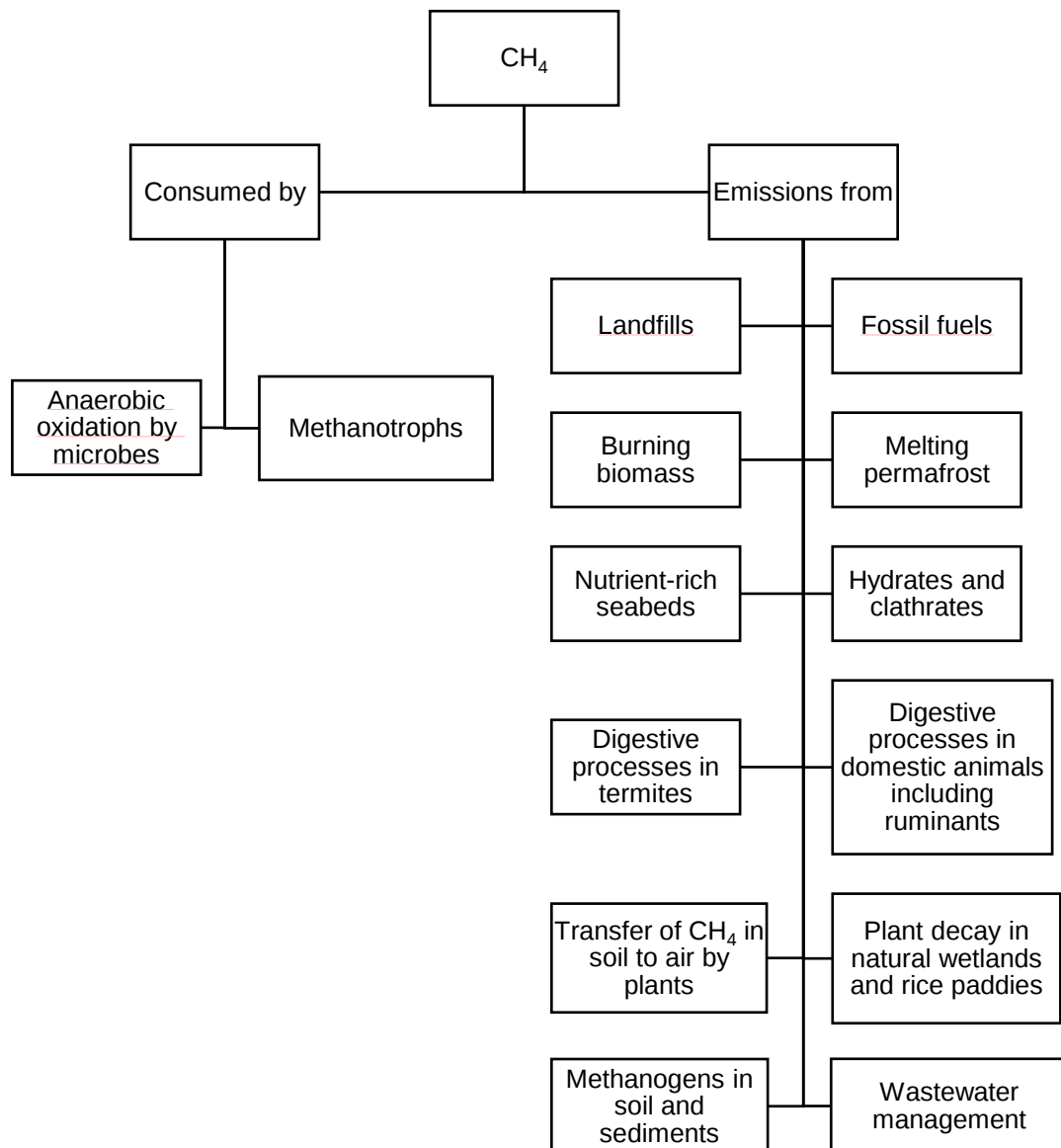


Figure 2 – Schematic of CH₄ emissions and consumption processes (original).

Nitrous oxide is naturally present in the atmosphere as part of the nitrogen cycle, but human activities have been increasing its amount (EPA, 2019). In the early 2000s, the main source of N₂O to the atmosphere where soils and oceans, with emissions dependent on the activity of microorganisms (Bange, 2000). Presently, the main sources are from human activities like agriculture, wastewater management, and fuel combustion (Houghton, 2009). The two most important biogenic processes of N₂O production are nitrification and denitrification (Bange, 2000). In nitrification, the nitrate is obtained by the

transformation of ammonium. Soil organic matter mineralization and chemical fertilizers are the two main sources of ammonium in the soil. The N_2O is formed as a secondary product of nitrification, in the transformation of ammonium into nitrite (Figure 3) (Prosser, 2005). The microbial process of transforming nitrate and nitrite into dinitrogen (N_2) or N_2O is called denitrification. The amount of oxygen (O_2) available in the surrounding environment determines the occurrence of denitrification. Only in the presence of a low amount of O_2 will the denitrify bacteria switch from aerobic to anaerobic respiration, using nitrate as an electron acceptor (Martens, 2005). N_2O emissions can occur by incomplete denitrification due to inhibition of the last step of the process (Skiba, 2008). The removal of N_2O from the atmosphere occurs by natural sinks, but mostly by chemical reactions (EPA, 2019). Chemical removal can only occur in the stratosphere, where N_2O is photochemically destroyed, but can also form NO radicals that attack the ozone layer, which protects the earth against ultraviolet radiation. N_2O in the atmosphere has a lifetime of 120 years and cause a greenhouse effect of 7% (Houghton, 2009).

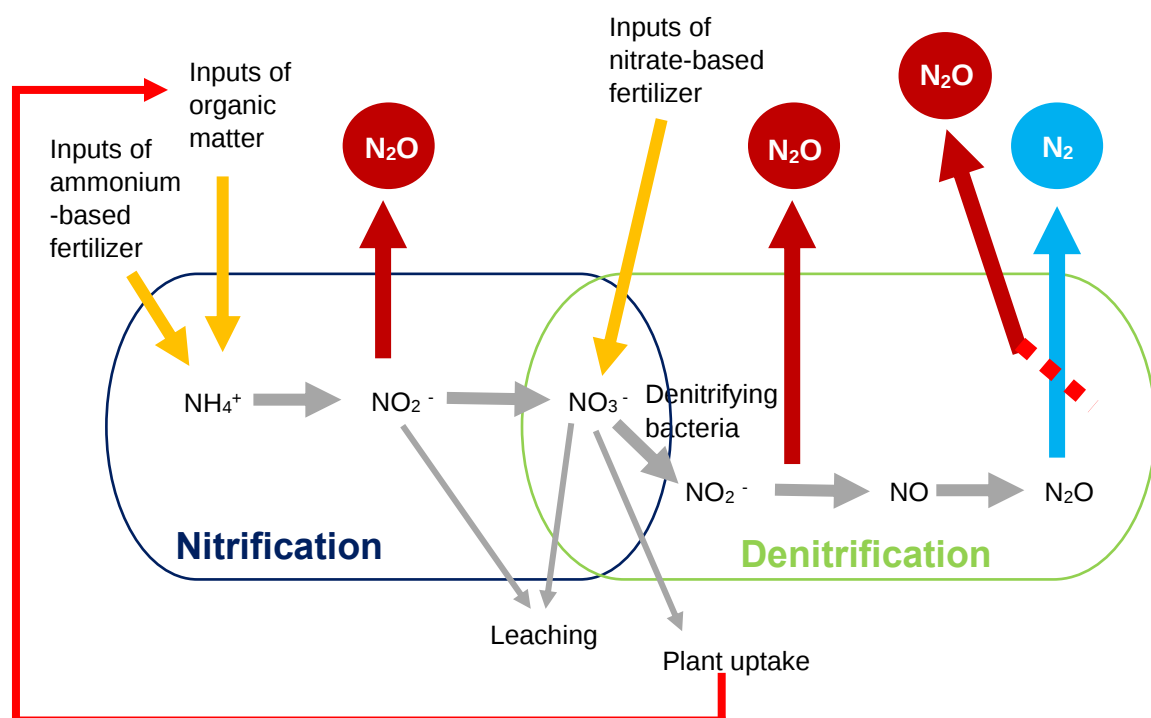


Figure 3 – Schematic of N_2O emissions and consume processes (original).

Methane and nitrous oxide are present in the atmosphere with concentrations that are two to six order of magnitude lower than CO_2 , but their high capacity of absorbing infrared

radiation produced or reflected by the Earth makes them important contributors to global warming. When comparing the three gases, CH₄ and N₂O have absorption capacities 34 and 298 times higher than CO₂, respectively (IPCC, 2014a). As such, the increase of their concentration in the atmosphere has been the focus of previous studies (Lashof et al., 1990; Tremblay et al., 2005). High levels and emissions of CH₄ were found in estuaries, streams surrounded by forests, agriculture lands, and peatlands (Sturm et al., 2016). While high levels and emissions of N₂O occur in estuaries, systems receiving freshwater from groundwater or streams, and especially systems where fertilizer had been applied (Sturm et al., 2016).

Study area

Estuaries have been defined as semi-enclosed coastal bodies of water, with a free connection with the sea, where seawater is commensurable diluted with freshwater (Pritchard, 1967). Estuaries are critical transition zones, linking sea, land, and freshwater habitats. They are one of the most important ecosystems on Earth, providing essential ecological functions like decomposition and nutrient cycling (Levin et al., 2001). These coastal areas were identified as important natural sources of emissions of CH₄ and N₂O (Bange et al., 1998). Estuaries present a higher physical-chemical variation than other aquatic systems. The variability of salinity and temperature over tidal cycles translates in a unique aquatic system (Elliott et al., 2011).

For the purpose of this study, three estuaries in Northern Portugal were selected: Douro, Cávado and Ave river estuaries. The Douro river is 927 km long with a watershed of 97, 667 km² shared between Portugal and Spain, making it one of the longest rivers in the Iberian Peninsula (Bordalo et al., 2005). Along the river watershed, there were built more than 50 dams for power generation and irrigation (Azevedo et al., 2010). Since his construction in 1985, the last dam located at 21.6 km from the mouth, is the limit of the Douro estuary (Azevedo et al., 2008). The estuary is located at 41.08° N and 8.42° W (mouth), presents a semi-diurnal mesotidal regime, and has been previously divided into 3 stretches due to seawater intrusion (Teixeira et al., 2012; Vieira & Bordalo., 2000). Living within the estuary area, in the last 8 km of the river, are more than 700,000 inhabitants (Carrola et al., 2014). Additionally, there are effluents from the eight existent wastewater treatment plants (WWTPs) without nutrient removal (Azevedo et al., 2006). The Cávado river has a watershed of 1,588 km², a length of 122 km, and its mouth is located at 41.5° N and 8.70° W (Gonçalves et al., 1994; Gredilla et al., 2015). Along its

course, nine dams have been built for power generation, agricultural and water supply. The river runs through heavy urbanized and industrialized areas with several WWTPs spread along the watershed (Moreira et al., 2006; Vieira et al., 1998). The Ave river is the smallest in this study, with a length of 90.9 km and a watershed of 1,388 km², flowing into the Atlantic Ocean at 41.26° N and 8.45° W (Araújo et al., 2010; Soares et al., 1999). Through the years, the river has been heavily modified with artificially constrained banks (Teixeira et al., 2013). The river runs through heavily populated areas with many industries (Araújo et al., 2010). Within his 1.5 km of extension, the estuary harbours industries and a shipyard that together with local agriculture and domestic pollution results in bad water quality (Barbosa-Vasconcelos et al., 2018).

Objective

The objective of this work was to estimate the potential emissions of CH₄ and N₂O to the atmosphere from Douro, Ave, and Cávado estuaries, through water and sediment analyses in the upper, middle, and lower stretches of each water body.

Materials and experimental procedures

Sampling

Sample collection was performed in estuarine intertidal areas of Douro, Ave and Cávado rivers during low tide. The sampling sites of the three estuaries were previously selected and represented the lower, middle and upper stretches (Figure 4, Table 1). In the Douro estuary, the three sites were located at the mouth of tributaries and close to the outlets of WWTPs. D1 was located at the mouth of Granja stream and near Sobreiras WWTP. D2 was located at the mouth of rivers Tinto and Torto, overlooking Freixo Marina, and under the influence of WWTPs of Freixo and Rio Tinto. D3 was located at the mouth of Gramido stream close to the effluent of the WWTP with the same name. In the Ave estuary, two sites were selected near the shipyard and downstream a weir where an effluent of unknown origin is located. In this estuary, only two points were selected due to the small length of the estuary. In the Cávado estuary three sites were selected, the first within a saltmarsh area, the second near a WWTP, and the most upstream, in a less impacted area but with the presence of recreational activities.

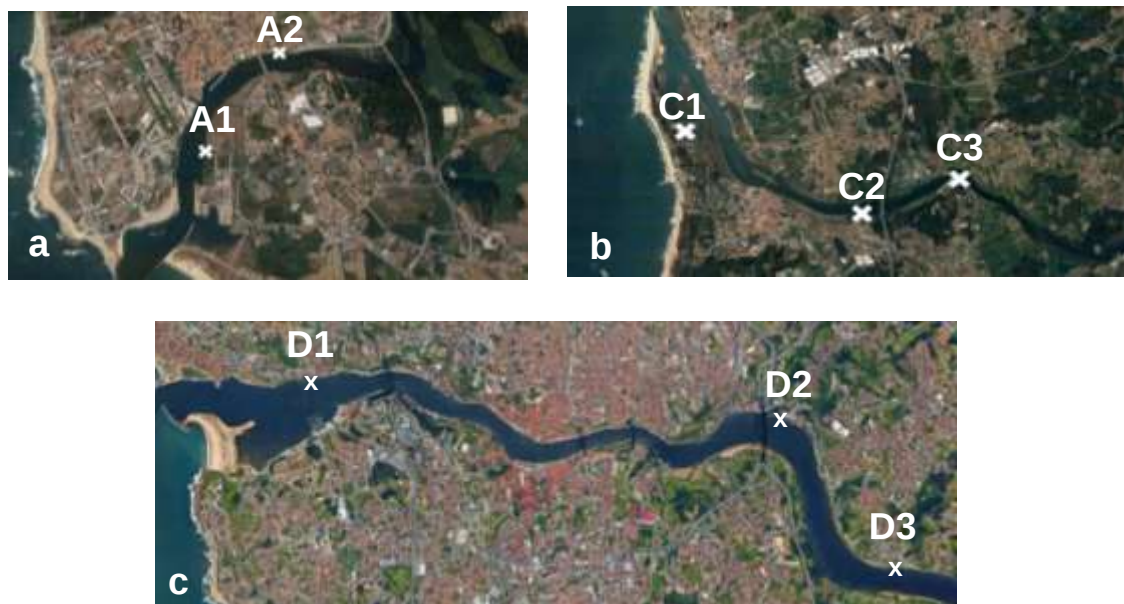


Figure 4 - Sampling sites (a - Ave River; b – Cávado River; c – Douro River). Source: Google Maps.

Table 1 – Geographical coordinates of the sampling sites (Datum – WGS 84).

	Sites	Latitude N	Longitude W
Douro	D1	41,14777	-8,65200
	D2	41,14377	-8,57952
	D3	41,12531	-8,55980
Ave	A1	41,34598	-8,74358
	A2	41,35158	-8,73794
Cávado	C1	41,52275	-8,78591
	C2	41,51099	-8,75720
	C3	41,51617	-8,74159

The sampling collection was performed seasonally in summer (Jul), fall (Nov), winter (Feb), and spring (May). The water samples were collected with syringes and 10 mL were added to 20 mL serum bottles (Figure 5a). Triplicate water samples were collected at each site. The serum bottles were previous sealed with butyl stopper and aluminium crimp, and purged 10 minutes in N₂ to remove the O₂. One litre of water was collected in a plastic bottle to be used in the sediment incubations. Sediment samples were collected using cut-off end syringes (Figure 5b, 5c). Fifteen sediment cores (5 cm depth) were collected at each site, and placed in a plastic bag. All samples were refrigerated in ice chests and transported to the laboratory for further processing. Water samples were analysed immediately upon arrival, and sediments samples were processed within 24h. Temperature, salinity, conductivity, dissolved oxygen, pH and turbidity were measured in situ using a YSI 6920 V2 multiprobe (figure 5d).



Figure 5 –Sampling collection (water collection (a), sediments collection (b, c)), and multiparametric sonde used to measure water variables in situ (d).

Experimental procedures

Sediment samples were characterized in terms of water and organic matter contents. Three replicates of sediment from each site were dried for 24 h (105°C), providing the percentage of water content (humidity), and then ignited in the muffle for 4 h (550°C), providing the percentage of organic matter.

To analyse the concentration of CH_4 and N_2O in the water, each serum bottle was vigorously shaken for 1 minute, 4 mL were collected to measure CH_4 concentration, and 8 mL of headspace was collected by displacement to measure N_2O concentration (Teixeira et al., 2010). CH_4 and N_2O levels were quantified in a Varian gas chromatograph (CP-3800). CH_4 levels were determined using a PoraPak T column and a Flame Ionization Detector (GC-FID) (Figure 6a), and N_2O levels using two HayeSep D columns and an Electron Capture Detector (GC-ECD) (Figure 6b). The GC was calibrated using a certified gas standard mixture of N_2O and CH_4 .

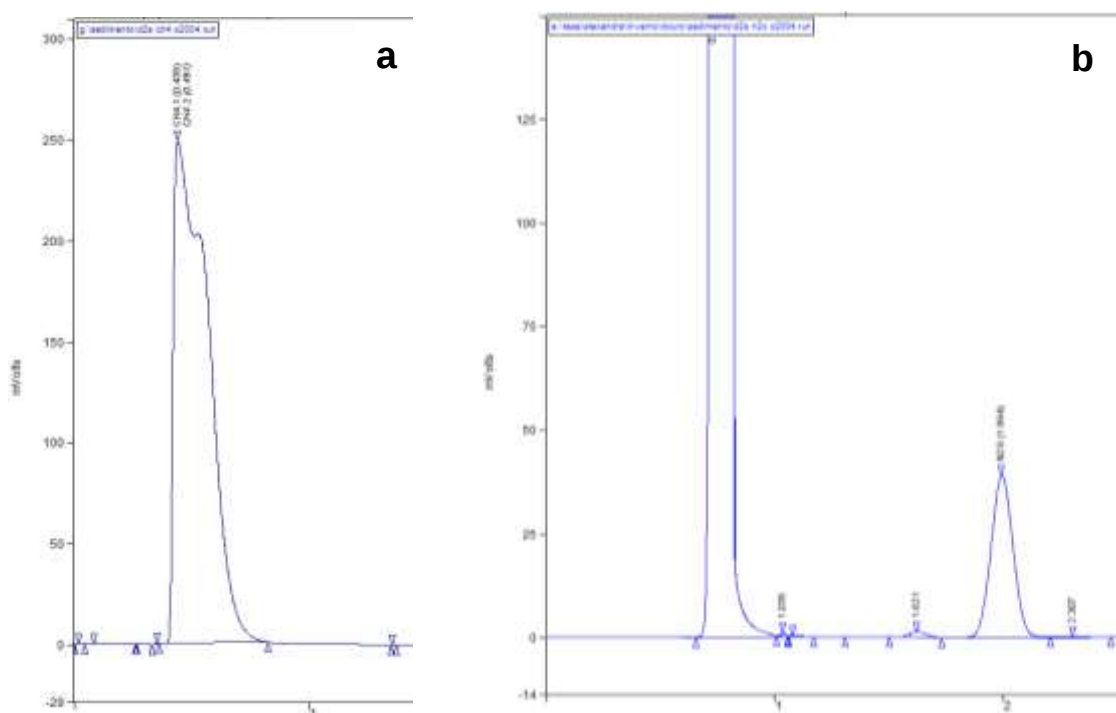


Figure 6 - Examples of chromatograms obtained by GC-FID (a) and GC-ECD (b).

The fluxes of CH_4 and N_2O from the sediments were estimated using slurry incubations. Slurries consisted of 10 mL of filtered (0.2 μm) estuarine water and 5 mL of homogenized sediment in 50 mL serum bottles sealed with butyl stopper and aluminium crimp. For each site, six serum bottles were prepared, three for incubation in the presence of oxygen (O_2), and three purged 20 min with N_2 , for incubation without O_2 . An extra set of time zero slurries were prepared and sacrificed immediately after closure/purging, to quantify initial CH_4 and N_2O levels. All remaining samples were incubated for 4h at constant stirring (100 rpm) (Figure 7a). At the end of the incubation, headspace samples were collected to quantify CH_4 and N_2O as described above (Figure 7b). Due to technical problems, N_2O could not be quantified in the spring samples.

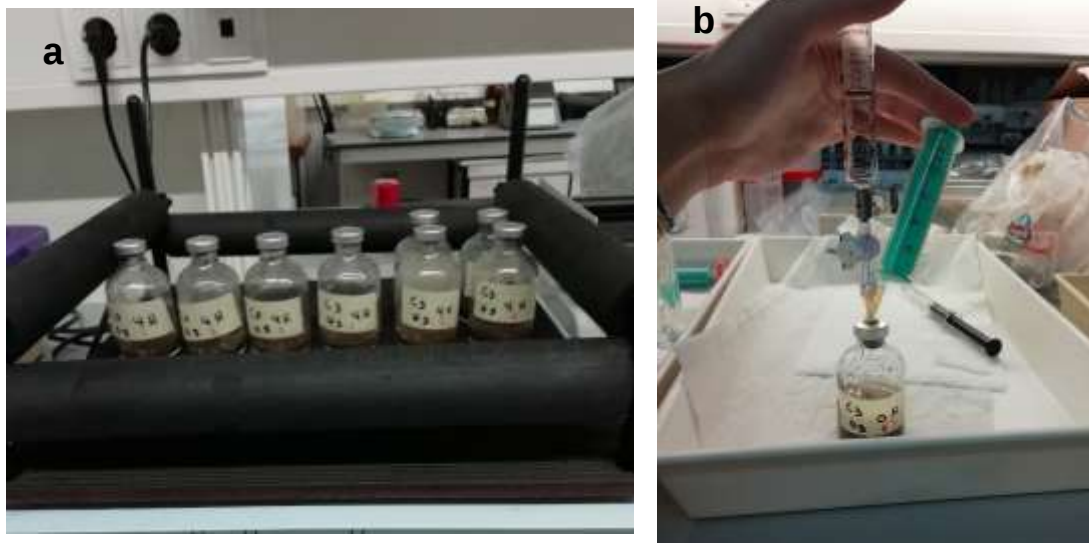


Figure 7 – Slurry incubations (a), and sampling of the headspace for N₂O measurement (b).

Data analysis

Data were tested for normality and homogeneity of variances. Linear correlation analysis was conducted to establish relationships between CH₄ and N₂O and environmental parameters measured. Statistical analysis was performed at the 95% confidence level ($p < 0.05$) using STATISTICA 13.0 (StatSoft, Inc., Palo Alto, CA, USA).

Results

Characterization of water and sediment samples

The water variables measured *in situ*, from Douro, Ave and Cávado estuaries, are presented in Figures 8, 9 and 10, respectively.

In Douro estuary, the variation of water temperature was according to the season, with higher values during the summer and lower in the winter. The salinity presented higher values in the lower estuary during the summer, site D1. The other two sites, D2 and D3, presented identical low salinity values during all seasons. Dissolved oxygen saturation ranged from 72.2 to 96.7 %, with the higher value found during the winter. Estuarine water pH varied between 6.98 and 7.66, being the higher value obtained in the summer. During the summer, maximum turbidity was found in the middle estuary, whereas in the upstream site values were always lower.

In Ave estuary, the temperature presented also a clear seasonal variation, with a similar temporal and spatial trend as in the Douro (Figure 9). The values obtained for salinity were higher in the summer, on the lower estuary, site A1. In this site, the salinity variation was higher than in site A2. Similar values of salinity were found for fall and spring in both sites. The highest value of dissolved oxygen saturation was obtained in the fall, with values ranging from 90.7 to 110.6 % during the four seasons. In this estuary, the water pH ranged from 6.04 to 7.57, the highest value being measured in the winter. In terms of turbidity, site A1 presented a seasonal variation, but not in site A2.

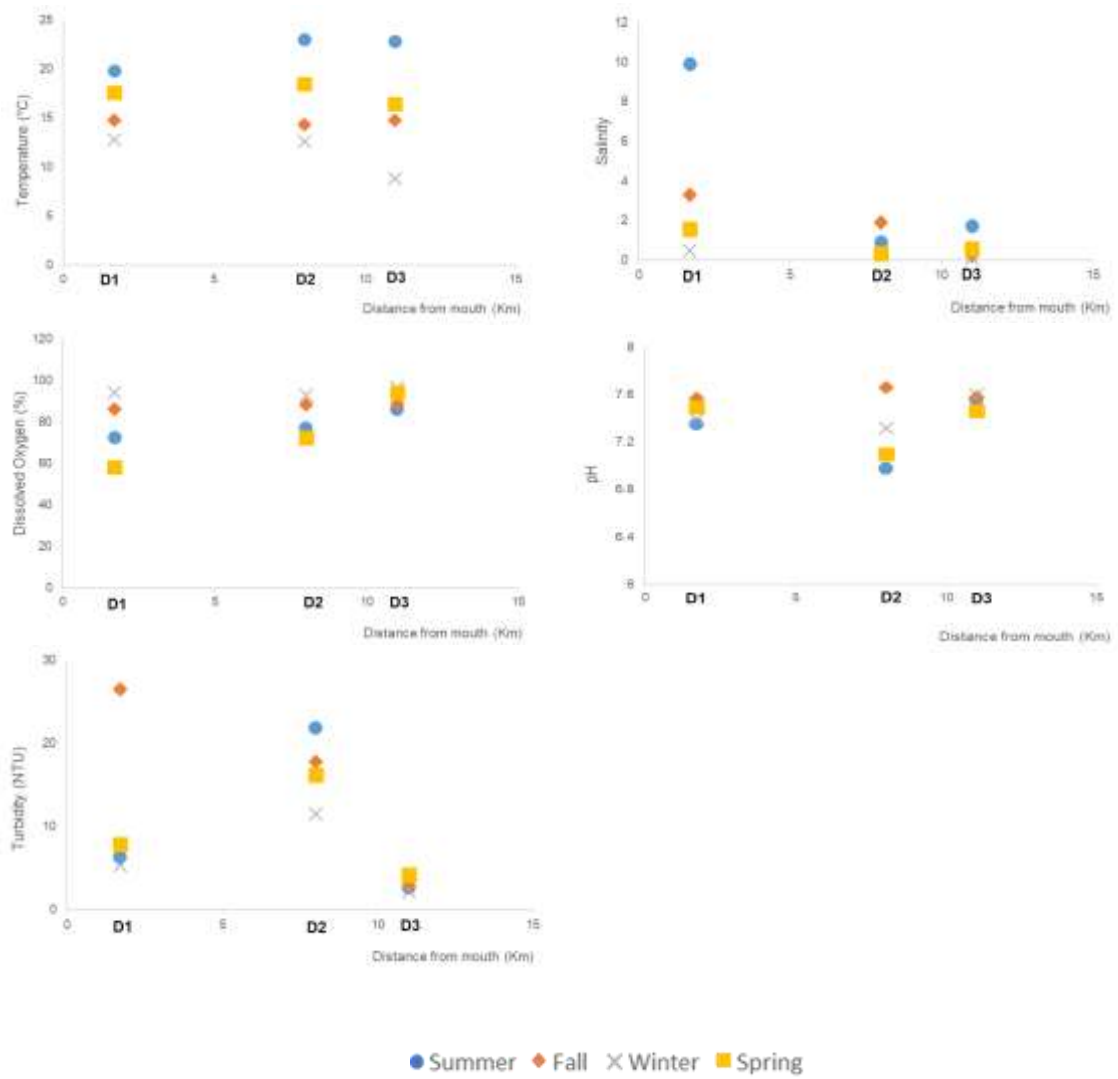


Figure 8 – Water variables measured in situ in Douro estuary though one year.

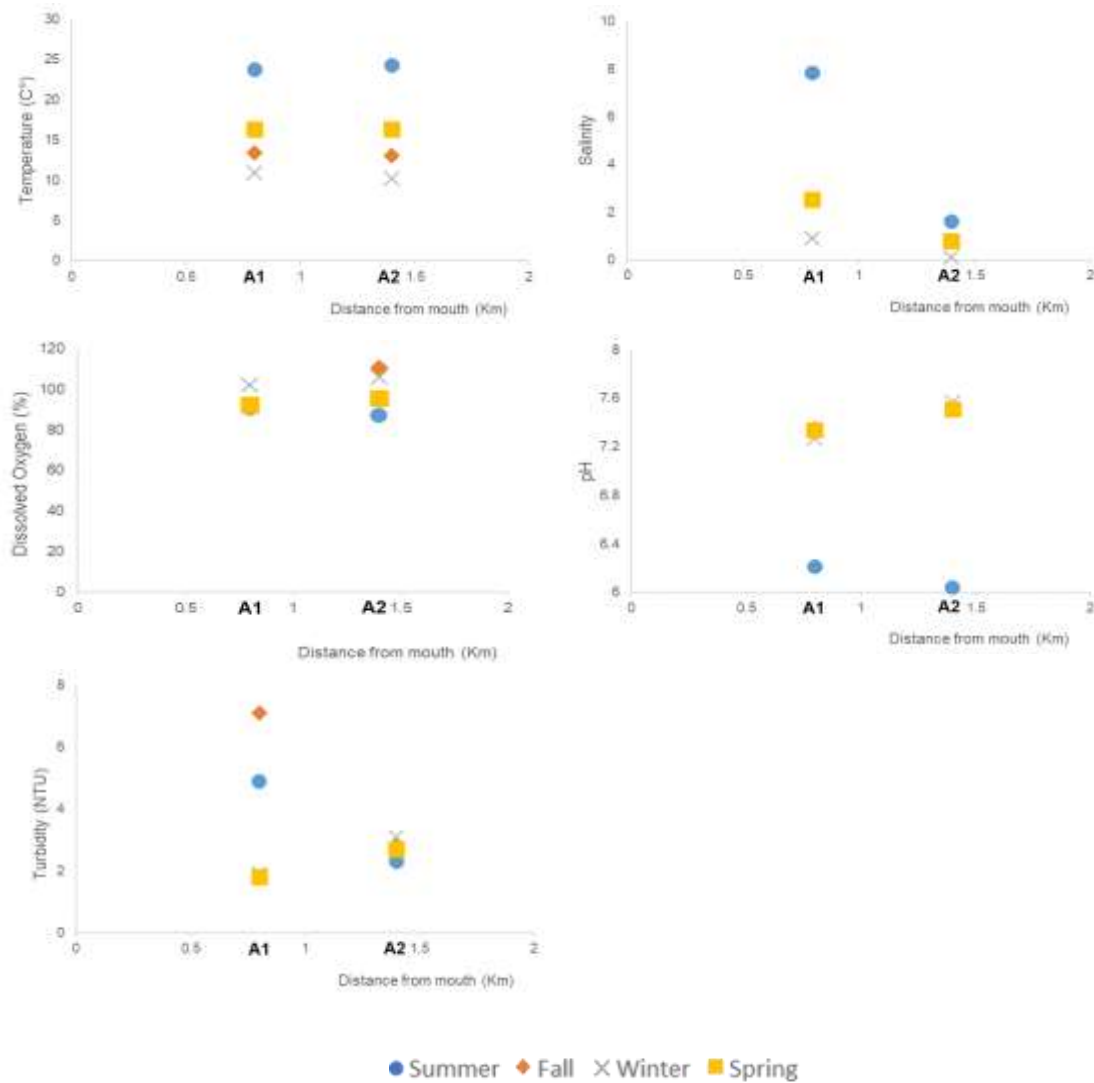


Figure 9 – Water variables measured in situ, in Ave estuary through one year.

In Cávado estuary, the water temperature fluctuated according to the season, as in the other estuaries (Figure 10). The values obtained for salinity in site C1, in the lower estuary, during the summer were the higher obtained in all the study. The sites C2 and C3 presented a lower variation. The dissolved oxygen saturation ranged between 79 to 142.6 %. The abnormally high value obtained in the spring might be related to the presence of phytoplankton. In terms of pH, the values range from 6.57 to 8.18, this last one obtained in the spring. The values of turbidity presented a noticeable variation within the sites and the estuary.

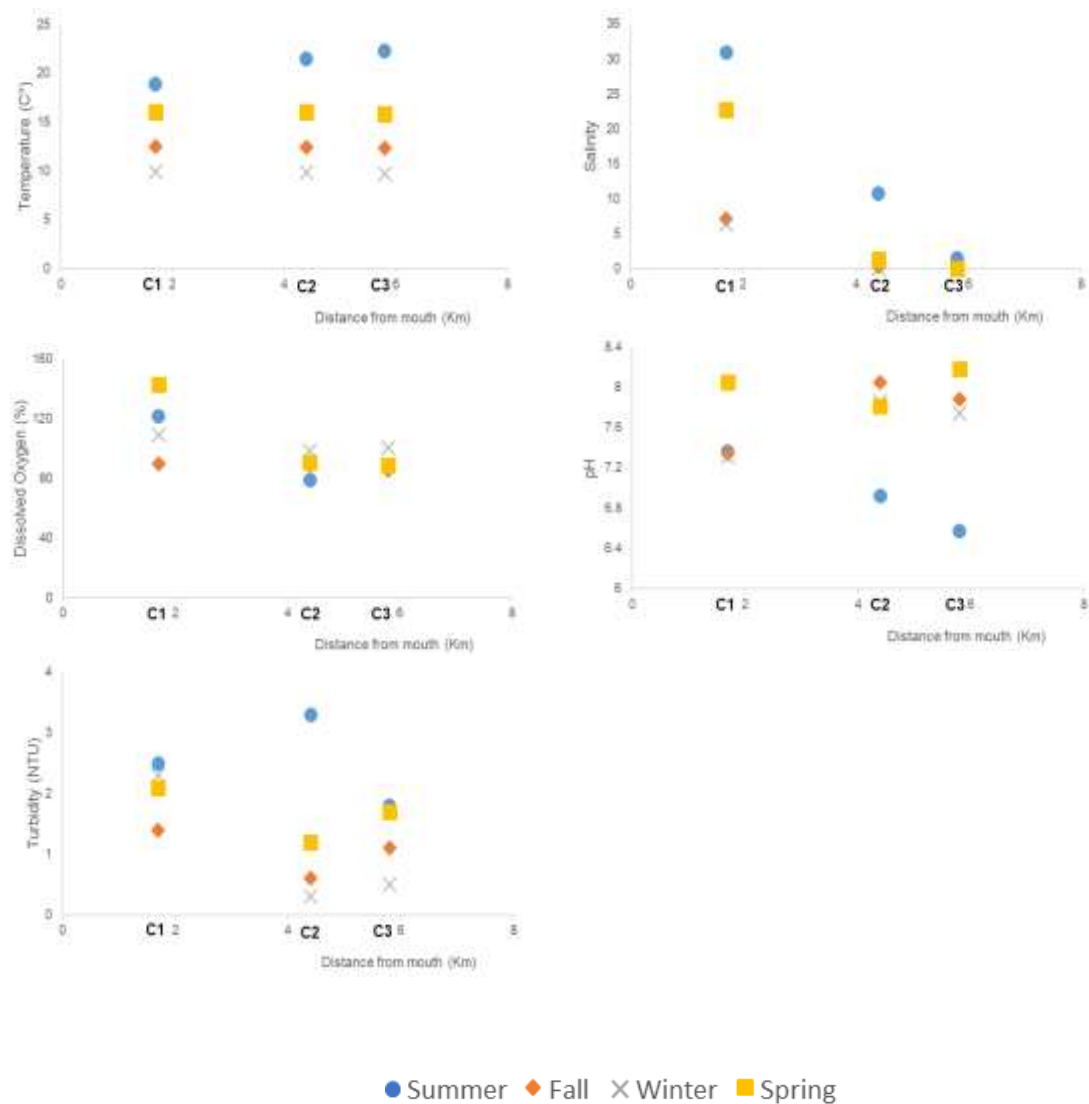


Figure 10 – Water variables measured in situ, in Cávado estuary through one year.

The sediment water and organic matter contents are presented in Table 2.

The highest percentage of humidity was 57.1 % obtained in Cávado estuary, C2, during the winter. In the Douro estuary, the higher percentage of humidity was obtained in the site D2 during the winter, 32.8 %. In the Ave estuary, the higher percentage of humidity was 53.2 % obtained during the fall on site A2. The higher percentage of organic matter was 12.11 % obtained during the fall in Ave estuary, A2. In the Douro estuary, the higher percentage was obtained in the fall, on site D2, 4.63 %. The Cávado estuary presented a higher percentage of organic matter in the winter, on site C2 with 9.03 %. As expected, all three estuaries, the correlation between the humidity and organic matter was high, with coefficients of 0.92, 0.91 and 0.96, for Douro, Ave and Cávado estuaries, respectively.

Table 2 – Values of water and organic matter contents in sediments (values represent averages $\pm$ sd of 3 replicates).

		Water (%)	Organic matter (%)			Water (%)	Organic matter (%)
Summer	D1	28.7 $\pm$ 0.78	4.22 $\pm$ 0.41	Winter	D1	20.1 $\pm$ 0.25	1.73 $\pm$ 0.05
	D2	20.4 $\pm$ 0.52	1.34 $\pm$ 0.04		D2	32.8 $\pm$ 2.67	3.97 $\pm$ 0.64
	D3	23.1 $\pm$ 0.60	1.83 $\pm$ 0.13		D3	29.0 $\pm$ 0.95	2.94 $\pm$ 0.22
	A1	15.2 $\pm$ 0.46	0.73 $\pm$ 0.11		A1	12.8 $\pm$ 1.56	0.74 $\pm$ 0.18
	A2	28.0 $\pm$ 1.19	3.48 $\pm$ 0.26		A2	39.1 $\pm$ 0.76	6.64 $\pm$ 0.13
	C1	17.8 $\pm$ 0.16	1.46 $\pm$ 0.38		C1	19.4 $\pm$ 0.36	0.86 $\pm$ 0.03
	C2	27.5 $\pm$ 0.79	1.52 $\pm$ 0.12		C2	57.1 $\pm$ 1.27	9.03 $\pm$ 0.64
	C3	29.6 $\pm$ 0.97	1.86 $\pm$ 0.26		C3	19.4 $\pm$ 1.29	1.18 $\pm$ 0.04
Fall	D1	30.7 $\pm$ 0.67	4.63 $\pm$ 0.36	Spring	D1	20.3 $\pm$ 0.56	1.87 $\pm$ 0.09
	D2	30.4 $\pm$ 0.86	4.30 $\pm$ 0.22		D2	21.1 $\pm$ 0.82	1.94 $\pm$ 0.28
	D3	22.8 $\pm$ 0.36	1.56 $\pm$ 0.22		D3	22.3 $\pm$ 0.51	2.35 $\pm$ 0.15
	A1	17.3 $\pm$ 0.64	5.59 $\pm$ 0.00		A1	16.0 $\pm$ 0.59	1.07 $\pm$ 0.67
	A2	53.2 $\pm$ 0.79	12.11 $\pm$ 0.54		A2	50.7 $\pm$ 0.74	9.39 $\pm$ 0.19
	C1	17.4 $\pm$ 0.57	1.35 $\pm$ 0.14		C1	17.9 $\pm$ 0.62	1.26 $\pm$ 0.03
	C2	44.0 $\pm$ 0.66	5.74 $\pm$ 0.21		C2	52.3 $\pm$ 0.29	8.59 $\pm$ 0.23
	C3	19.3 $\pm$ 0.50	0.98 $\pm$ 0.02		C3	13.3 $\pm$ 0.48	0.58 $\pm$ 0.03

Dissolved CH₄ and N₂O in estuarine water

In Table 3, the results obtained for dissolved CH₄ and N₂O in the water are summarized.

The highest value of dissolved CH₄ was obtained in D2 during spring (1.149 nmol L⁻¹). The highest value of dissolved N₂O was also obtained in D2 but during the winter (0.104 nmol L⁻¹). In the Ave estuary, the higher value of dissolved CH₄ was 0.056 nmol L⁻¹, obtained during spring at site A1. The higher value of dissolved N₂O was obtained during the summer at site A2 (0.011 nmol L⁻¹). In Cávado estuary, the higher value of dissolved

CH₄ was 0.068 nmol L⁻¹, obtained during fall at site C3. The higher value of dissolved N₂O was obtained at site C1 during the winter (0.010 nmol L⁻¹).

Douro estuary presented higher concentrations for both dissolved CH₄ and N₂O. The Ave and Cávado estuaries presented similarly lower concentrations.

Table 3 – Dissolved CH₄ and N₂O in sample water. The values presented are the average $\pm$ sd . (n.d. – not determined due to technical problems)

		CH ₄ (nmol L ⁻¹)	N ₂ O (nmol L ⁻¹)			CH ₄ (nmol L ⁻¹)	N ₂ O (nmol L ⁻¹)
Summer	D1	0.091 $\pm$ 0.004	0.011 $\pm$ 0.001	Winter	D1	0.055 $\pm$ 0.000	0.014 $\pm$ 0.001
	D2	0.472 $\pm$ 0.029	0.021 $\pm$ 0.001		D2	0.891 $\pm$ 0.582	0.104 $\pm$ 0.017
	D3	0.006 $\pm$ 0.001	0.007 $\pm$ 0.000		D3	0.015 $\pm$ 0.003	0.004 $\pm$ 0.000
	A1	0.021 $\pm$ 0.018	0.010 $\pm$ 0.001		A1	0.046 $\pm$ 0.001	0.010 $\pm$ 0.002
	A2	0.017 $\pm$ 0.003	0.011 $\pm$ 0.000		A2	0.031 $\pm$ 0.001	0.008 $\pm$ 0.000
	C1	0.010 $\pm$ 0.000	0.004 $\pm$ 0.001		C1	0.011 $\pm$ 0.001	0.010 $\pm$ 0.001
	C2	0.025 $\pm$ 0.001	0.007 $\pm$ 0.000		C2	0.024 $\pm$ 0.001	0.008 $\pm$ 0.001
	C3	0.026 $\pm$ 0.004	0.008 $\pm$ 0.000		C3	0.024 $\pm$ 0.001	0.009 $\pm$ 0.000
Fall	D1	0.073 $\pm$ 0.044	0.005 $\pm$ 0.001	Spring	D1	0.128 $\pm$ 0.010	n.d.
	D2	0.666 $\pm$ 0.158	0.028 $\pm$ 0.004		D2	1.149 $\pm$ 0.346	n.d.
	D3	0.029 $\pm$ 0.000	0.017 $\pm$ 0.002		D3	0.071 $\pm$ 0.002	n.d.
	A1	0.028 $\pm$ 0.004	0.009 $\pm$ 0.001		A1	0.056 $\pm$ 0.015	n.d.
	A2	0.040 $\pm$ 0.000	0.007 $\pm$ 0.001		A2	0.045 $\pm$ 0.015	n.d.
	C1	0.028 $\pm$ 0.003	0.006 $\pm$ 0.000		C1	0.029 $\pm$ 0.003	n.d.
	C2	0.040 $\pm$ 0.007	0.009 $\pm$ 0.000		C2	0.049 $\pm$ 0.003	n.d.
	C3	0.068 $\pm$ 0.057	0.009 $\pm$ 0.001		C3	0.031 $\pm$ 0.009	n.d.

Production and consumption of CH₄ in sediments

In the Douro estuary, the site with the higher values of CH₄ production was D2 (middle estuary) in both incubations (Figure 11). Under aerobic conditions, the higher value was obtained in the fall, 13.37 nmol h⁻¹ g⁻¹ wet sed. The higher production rate, under anaerobic conditions, was 26.48 nmol h⁻¹ g⁻¹ wet sed, and was obtained in the winter.

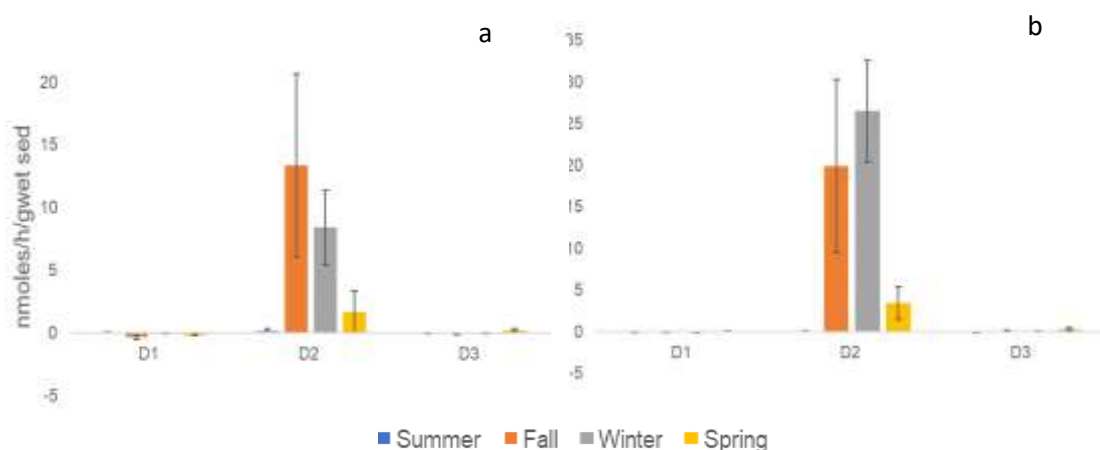


Figure 11 - Methane production in Douro estuary sediments in aerobic (a), and anaerobic (b) conditions.

In the Ave estuary, site A2 presented the higher values of both production and consumption of CH_4 (figure 12). The highest consumption value was $51.7 \text{ nmol h}^{-1} \text{ g}^{-1}$ wet sed, and was obtained under aerobic conditions, in the spring. Under anaerobic conditions, the highest value of production was $1.98 \text{ nmol h}^{-1} \text{ g}^{-1}$ wet sed in the winter.

In the Cávado estuary, the site with the higher production and consumption of CH_4 was the C2 (Figure 13). Under aerobic condition, in the winter, there was a consumption of $0.27 \pm 0.081 \text{ nmol h}^{-1} \text{ g}^{-1}$ wet sed. Also, in the winter, but under anaerobic conditions, the higher value of production was $0.12 \pm 0.103 \text{ nmol h}^{-1} \text{ g}^{-1}$ wet sed.

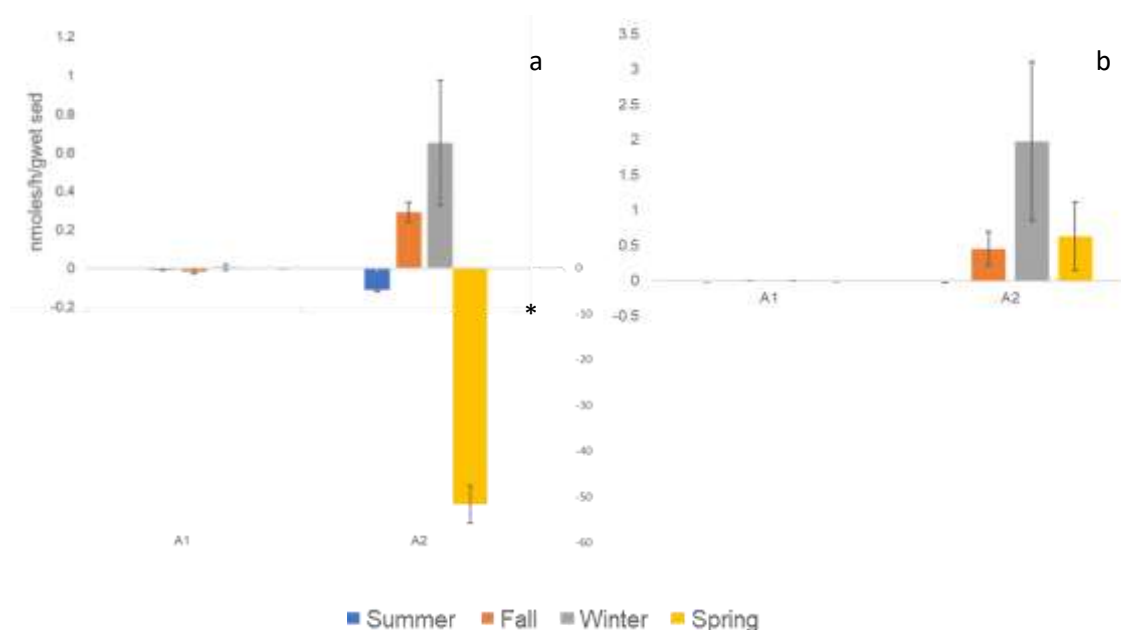


Figure 12 - Methane production in Ave estuary sediments in aerobic (a), and anaerobic (b) conditions. * Value reported in the right vertical axis.

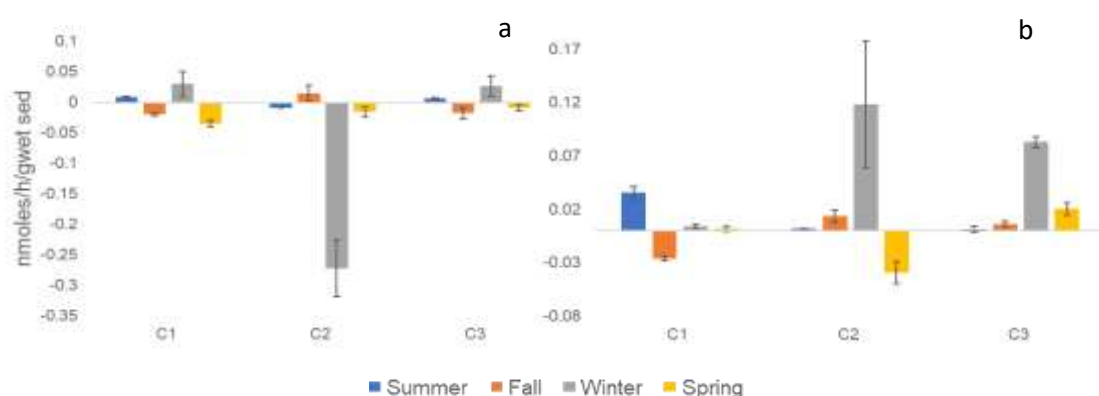


Figure 13 - Methane production in Cávado estuary sediments in aerobic (a), and anaerobic (b) conditions.

Sediments from the Douro estuary, under aerobic and anaerobic conditions fostered CH₄ production, while in Ave and Cávado middle stretches, under aerobic conditions, acted as CH₄ sinks, in the spring and winter, respectively. However, during the winter and under anaerobic conditions, Ave and Cávado middle and upper estuaries were active places of CH₄ production. Overall, looking at the CH₄ emission potential from the studied estuaries, Douro estuary presented higher production rates, followed by Ave estuary, and finally Cávado estuary with the lowest rates.

Production and consumption N₂O in sediments

In the Douro estuary, the higher values of production and consumption of N₂O were obtained in the winter in site D2 (Figure 14). Under aerobic conditions, positive fluxes (production) were measured for all sediments, with the higher rates being 0.061 nmol h⁻¹ g⁻¹ wet sed. Inversely, in incubations without oxygen, negative fluxes (consumption) was observed for most sediments. The higher consumption rate obtained, was 0.065 nmol h⁻¹ g⁻¹ wet sed. As referred previously, the values of N₂O from spring were not available due to technical problems.

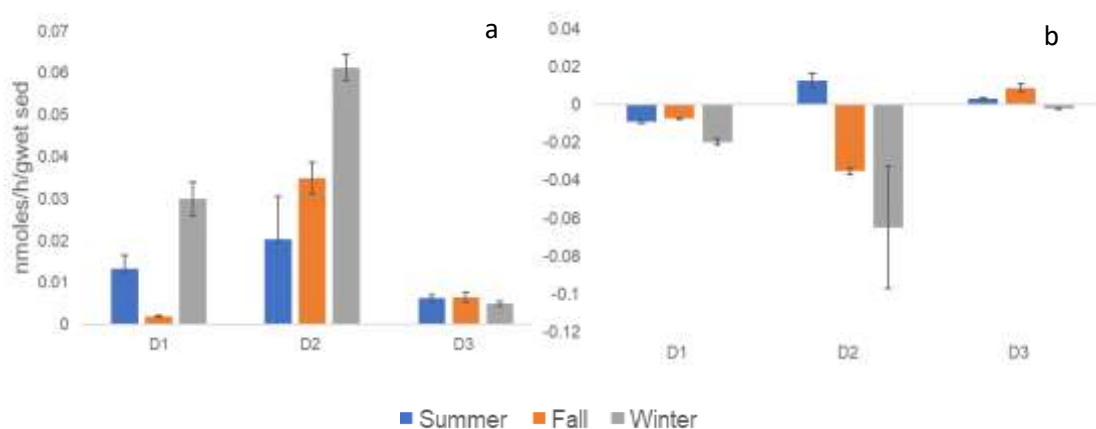


Figure 14 – Nitrous oxide production in Douro estuary sediments in aerobic (a), and anaerobic (b) conditions.

In the Ave estuary, site A1 presented the higher production values of N₂O (Figure 15). In aerobic conditions, the value was 0.10 nmol h⁻¹ g⁻¹ wet sed. The value in anaerobic condition was 0.05 nmol h⁻¹ g⁻¹ wet sed. In both incubations, the higher values were obtained in the summer.

For Cávado estuary, the higher production and consumption values were both obtained in site C2, and in the fall (Figure 16). Under aerobic conditions, the higher production value was 0.007 nmol h⁻¹ g⁻¹ wet sed. The higher value of consumption was obtained under anaerobic conditions, 0.005 nmol h⁻¹ g⁻¹ wet sed.

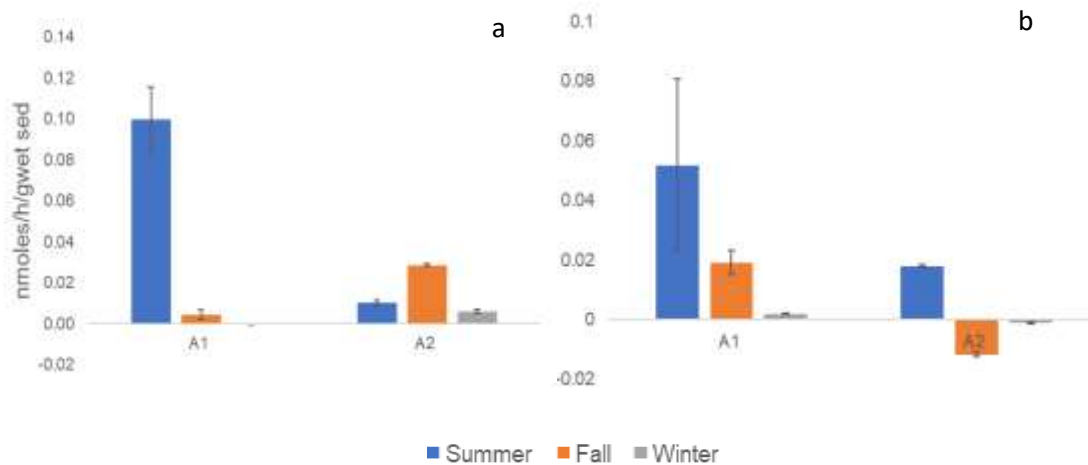


Figure 15 – Nitrous oxide production in Ave estuary sediments in aerobic (a), and anaerobic (b) conditions.

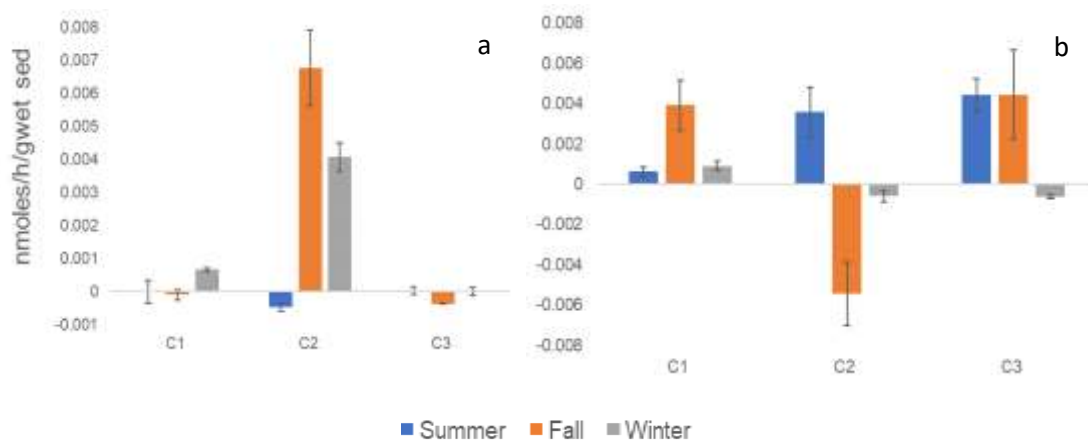


Figure 16 – Nitrous oxide production in Cávado estuary sediments in aerobic (a), and anaerobic (b) conditions.

Overall, in what N_2O emissions are concerned, Ave estuary presented the higher production potential followed by Douro estuary and, finally, Cávado estuary with lower rates.

CH_4 and N_2O fluxes per unit area

The obtained results from the sediments were tentatively compared with other studies by estimating the fluxes per unit area using the surface area of sediments in incubation

bottles. The Douro estuary, under anaerobic conditions, reached $57.0 \mu\text{mol h}^{-1} \text{m}^{-2}$ of CH_4 production in winter survey, while Ave and Cávado estuaries presented maximum values one to two orders of magnitude lower ($5.9 \mu\text{mol h}^{-1} \text{m}^{-2}$, $0.32 \mu\text{mol h}^{-1} \text{m}^{-2}$, respectively). Under aerobic conditions, the obtained results were in the same line with the Douro estuary, reaching $37.9 \mu\text{mol h}^{-1} \text{m}^{-2}$, in the fall, while the Ave and Cávado estuaries presented values of $1.9 \mu\text{mol h}^{-1} \text{m}^{-2}$ and $0.07 \mu\text{mol h}^{-1} \text{m}^{-2}$, respectively in the winter. The emissions of N_2O from the sediments in the Douro estuary, under anaerobic conditions, reached $27.8 \text{ nmol h}^{-1} \text{m}^{-2}$. Under aerobic conditions, the emissions of N_2O reached $135.8 \text{ nmol h}^{-1} \text{m}^{-2}$. In the Ave estuary, the emissions of N_2O reached $153.9 \text{ nmol h}^{-1} \text{m}^{-2}$, and $201.4 \text{ nmol h}^{-1} \text{m}^{-2}$ under anaerobic and aerobic conditions, respectively. Under anaerobic conditions, in the Cávado estuary, the emissions reached $15.5 \text{ nmol h}^{-1} \text{m}^{-2}$. Under aerobic conditions, the N_2O emission reached $11.3 \text{ nmol h}^{-1} \text{m}^{-2}$.

Correlations between CH_4 and N_2O and environmental variables

The correlation coefficients between dissolved CH_4 and N_2O and measured environmental variables for each studied estuary are presented in Table 4.

In the water from the Douro estuary, the dissolved CH_4 concentration was correlated with the dissolved N_2O concentrations ($R=0.84$). In the Ave estuary, the dissolved CH_4 concentration was positively related with dissolved oxygen saturation, and pH, presenting R values of 0.75 and 0.86, respectively. The dissolved N_2O concentration in Ave estuary was inversely related with the pH ($R=-0.91$). In the Cávado estuary, the dissolved CH_4 concentration was inversely correlated with the oxygen saturation ($R=-0.59$), and the dissolved N_2O concentration showed an inverse relation with salinity ($R=-0.84$).

The correlation coefficients between CH_4 and N_2O fluxes estimated in sediments from Douro, Ave, Cávado, and environmental values are presented in Tables 5, 6 and 7.

In the Douro estuary, the anaerobic CH_4 production, presented a positive relation with sediment water content, $R=0.62$ (Table 5). Under aerobic conditions, it correlated with turbidity, ($R=0.78$), and again with the percentage of water in the sediment ($R=0.60$). CH_4 production presented a positive relation with N_2O production under aerobic conditions ($R=0.72$), while under anaerobic conditions an opposite trend was found ($R=-0.91$).

Table 4 – Correlation coefficients between dissolved CH₄ and N₂O, and environmental variables measured in Douro, Ave and Cávado estuaries, (significant correlations, $p < 0.05$, are presented in red) (T – Temperature; Sal – Salinity; OD Sat – Dissolved oxygen saturation; Turb – Turbidity; Sed water – Sediments water content; Sed OM – Sediments Organic matter).

	Douro		Ave		Cávado	
	CH ₄	N ₂ O	CH ₄	N ₂ O	CH ₄	N ₂ O
N ₂ O	0.8403 p=0.005	1	-0.6466 p=0.165	1	0.3611 p=0.340	1
T	0.0520 p=0.873	-0.2140 p=0.580	-0.5334 p=0.121	0.7937 p=0.059	-0.1025 p=0.751	-0.5270 p=0.145
Sal	-0.2291 p=0.474	-0.2484 p=0.519	0.4595 p=0.252	0.4082 p=0.422	-0.5053 p=0.094	-0.8379 p=0.005
OD Sat	-0.1617 p=0.616	0.2401 p=0.534	0.7465 p=0.033	-0.8020 p=0.055	-0.5859 p=0.045	-0.3362 p=0.376
pH	-0.5406 p=0.070	-0.2692 p=0.484	0.8614 p=0.006	-0.9095 p=0.012	0.3153 p=0.318	0.2977 p=0.437
Turb	0.3584 p=0.253	0.0542 p=0.890	-0.2439 p=0.560	-0.2179 p=0.678	-0.3126 p=0.322	-0.4098 p=0.273
Sed Water	0.1474 p=0.648	0.4338 p=0.243	0.1314 p=0.756	-0.5883 p=0.219	-0.0375 p=0.908	0.2048 p=0.597
Sed OM	0.1229 p=0.704	0.2492 p=0.518	0.0693 p=0.870	-0.7693 p=0.074	-0.0296 p=0.927	0.1102 p=0.778

Table 5 – Correlation between environmental variables and sediments in Douro estuary under aerobic and anaerobic conditions (only variables with significant correlations ($p < 0.05$) are presented which coefficients are highlighted in red).

	Aerobic incubations		Anaerobic incubations	
	CH ₄	N ₂ O	CH ₄	N ₂ O
N ₂ O	0.7202 p=0.029	1	-0.9025 p=0.001	1
Turb	0.7838 p=0.003	0.2880 p=0.452	0.5286 p=0.077	-0.2912 p=0.447
Sed Water	0.5988 p=0.040	0.2905 p=0.448	0.6214 p=0.031	-0.6353 p=0.066

In the Ave estuary, only the production of N₂O in sediments (Table 6) presented a significant ($p<0.05$) correlation with salinity, under both anaerobic and aerobic conditions, $R=0.94$ and $R=0.91$, respectively.

Table 6 – Correlation between environmental variables and sediments in Ave estuary under aerobic and anaerobic conditions (only variables with significant correlations ($p<0.05$) are presented which coefficients are highlighted in red).

	Aerobic incubations		Anaerobic incubations	
	CH ₄	N ₂ O	CH ₄	N ₂ O
N ₂ O	-0.1805 $p=0.732$	1	-0.4437 $p=0.378$	1
Sal	0.1743 $p=0.680$	0.9074 $p=0.012$	-0.3458 $p=0.401$	0.9359 $p=0.006$

In the Cávado estuary, the production of N₂O, under aerobic conditions, presented a positive and significant ($p<0.05$) relation with sediment characteristics, water ($R=0.81$) and the organic matter ($R=0.83$) contents (Table 7).

Table 7 – Correlation between environmental variables and sediments from Cávado estuary under aerobic and anaerobic conditions (only variables with significant correlations ($p<0.05$) are presented which coefficients are highlighted in red)

	Aerobic incubations		Anaerobic incubations	
	CH ₄	N ₂ O	CH ₄	N ₂ O
N ₂ O	-0.3708 $p=0.326$	1	-0.4410 $p=0.235$	1
Sed water	0.4043 $p = 0.192$	0.8081 $p=0.008$	0.5104 $p=0.090$	-0.5170 $p=0.154$
Sed OM	-0.5359 $p=0.073$	0.8256 $p=0.006$	0.4852 $p=0.110$	-0.5761 $p=0.105$

Discussion

Background

The increase of greenhouse gases emissions, through anthropogenic activities, have an important role in the greenhouse effect (Houghton, 2009). Greenhouse gases, such as CO₂, CH₄, and N₂O owe their importance based on two factors: their atmosphere concentration, and their ability of absorbing infrared radiation (Lashof et al., 1990). CO₂ is considered to be the most important greenhouse gas present in the atmosphere (IPCC, 2007b). Although its concentration in the atmosphere is the highest of the three above mentioned gases, its absorption capacity is the lowest. With a lower concentration in the atmosphere CH₄ and N₂O have, however, absorption capacities 34 and 298 times higher than CO₂, making them the two most important greenhouse gases in the atmosphere (IPCC, 2014a). Greenhouse gases have two sources of emissions: natural and anthropogenic. Natural sources of CH₄ are wetlands, methanogenic activity in soil and sediments, animal digestive processes such as termites and ruminants, and natural transfer from the soil to the air. Anthropogenic sources are burning of fossil fuel, agriculture, burning or destruction of biomass (Hu et al., 2019). In the N₂O case, the natural sources are soils and oceans. The anthropogenic sources are agriculture, wastewater management and fossil combustion (Houghton, 2009).

Estuaries are important ecosystems that provide essential ecological functions such as decomposition and nutrient cycling (Levin et al., 2001). The consequence of global warming can cause serious problems in estuaries and coastal zones. The rising of sea levels can cause more erosion and an increase of saltwater intrusion in the estuaries, causing the lost decrease in freshwater availability (Houghton, 2009).

Dissolved methane emissions to the atmosphere has been studied previously. Studies have been focused on water systems like lakes (Sanches et al., 2019), estuaries (Gwenaël et al., 2002; Middelburg et al., 2002; Upstill-Goddard et al., 2000), coastal waters (Bange et al., 1998), and salt marshes (Ferrón et al., 2007). In these studies, the higher value was obtained in a river in Brazil (8000 nmol L⁻¹). Emissions of CH₄ from sediments have been studied in rice paddy fields (Zhou et al., 2018), in grassland regions (Tang et al., 2019), in mangroves (Allen et al., 2011), in estuarine intertidal sediments (Li et al., 2019), and manure of dairy cows (Hu et al., 2018). The last environment provided the highest CH₄ production (720 μmol h⁻¹ m⁻²).

Previous studies on the emissions of nitrous oxide to the atmosphere from aquatic systems and sediments include estuaries (Bange et al., 1996; de Wilde et al., 2000), coastal waters (Bange et al., 1996), and marginal seas (Ferrón et al., 2007; Hashimoto et al., 1999). As in the case of N_2O , estuaries with 338 nmol L^{-1} were the most productive. In the case of sediment emissions, studies were performed in rice paddy fields (Zhou et al., 2018), in mangroves (Allen et al., 2011), and in estuaries intertidal sediments (Li et al., 2019; Middelburg et al., 1995). The highest values were obtained in mangroves ($4587 \text{ nmol h}^{-1} \text{ m}^{-2}$).

Dissolved CH_4 and N_2O in estuarine water

The highest methane levels in estuarine water were $0.056 \text{ nmol L}^{-1}$, $0.068 \text{ nmol L}^{-1}$ and $1.149 \text{ nmol L}^{-1}$, in Ave, Cávado and Douro estuaries, respectively. Throughout the seasons, the higher concentrations were always obtained at site D2, in the Douro estuary, located at the mouth of Tinto river. The obtained values might be related to the presence of effluent from two WWTPs, and subsequently an increase of organic matter availability. Methane production is usually related to microbial organic matter decomposition (Wuebbles et al., 2002). No seasonal trend was observed for measured CH_4 concentrations for the studied estuaries. Additionally, no common relation was found between CH_4 levels and the measured environmental variables, with dissolved oxygen being positively related to CH_4 in Ave estuary, while negatively related in Cávado estuary.

Previous studies report values for dissolved CH_4 in European estuaries ranging from 111 to 600 nmol L^{-1} , and in rivers from 5 to 8000 nmol L^{-1} (Middelburg et al., 2002). Ferrón et al. (2007) compiled values for dissolved CH_4 from 2.1 to 4201 nmol L^{-1} in estuaries. For coastal waters and marginal seas concentrations ranged from 2.0 to 1360 nmol L^{-1} (Ferrón et al., 2007). When comparing the results obtained in this study to the available data from the literature, it is clear that the three estuaries studied presented low levels of dissolved CH_4 , i.e. the productivity was smaller.

Dissolved N_2O levels reached $0.010 \text{ nmol L}^{-1}$, $0.011 \text{ nmol L}^{-1}$, and $0.104 \text{ nmol L}^{-1}$, corresponding to maximum concentrations obtained in Cávado, Ave, and Douro estuaries. Similarly, to dissolved CH_4 , the highest values of dissolved N_2O were obtained in Douro, at site D2. As in the case of CH_4 , the values obtained of dissolved N_2O did not present a seasonal trend as in the case of CH_4 . The only estuary where dissolved N_2O presented a relation with the studied environmental variables was the Cávado, with an

inverse relation with the salinity, in the vein of the trend reported also for other estuaries (Bange, 1992). In Douro estuary, a significant relation ($p < 0.05$) was obtained between the dissolved N_2O and CH_4 , denoting a possible common source for substrate.

Values of dissolved N_2O ranging from 10 to 338 nmol L^{-1} have been found in estuaries (de Wilde et al., 2000), representing 2-3 orders of magnitude higher than in the most productive studied estuary (Douro). Therefore, the tree estuaries studied presented lower dissolved N_2O concentrations than other studied environments.

CH_4 and N_2O fluxes from estuarine sediments

Fluxes of CH_4 and N_2O were measured in sediment slurries during incubations with and without oxygen in order to ascertain eventual sinks and sources. In the presence of oxygen, negative fluxes (sinks) of CH_4 were observed in sediments from two locations: Ave estuary site A2 (spring), and Cávado estuary site C2 (winter). Anaerobic conditions stimulated CH_4 production in most sediments. The higher CH_4 production values, recorded in site D2, were obtained in fine-grained sediments rich in organic matter, environment, suitable for methanogens (Wuebbles et al., 2002). Methane production in the Douro and Ave estuaries were higher than previously reported in Australian estuaries ($2.9 \mu\text{mol h}^{-1} \text{m}^{-2}$) (Allen et al., 2011), but lower than reported from estuaries in China ($570.4 \mu\text{mol h}^{-1} \text{m}^{-2}$ and $74.2 \mu\text{mol h}^{-1} \text{m}^{-2}$) (Hu et al., 2019; Li et al., 2019). Douro estuary presented a relation between CH_4 production and water column turbidity. Also, in Douro estuary, a positive relation was found between CH_4 and sediment water content. Higher percentages of water were found in fine grained sediments associated with higher organic matter contents. Nonetheless, no significant relation was found between organic matter and CH_4 production.

Positive fluxes of N_2O were generally found in incubations in the presence of O_2 . Under aerobic conditions, N_2O is likely to be produced during the nitrification process (Bange, 2000). In incubations without O_2 , N_2O was consumed in most slurries from Douro estuary. In sediments from Ave and Cávado estuaries, anaerobic production of N_2O was found, especially in downstream locations. Under anaerobic conditions, denitrification was probably the main responsible for N_2O production (Bange, 2000). Consumption of N_2O was generally observed in the colder seasons, with higher values obtained in the site D2. A possible effect of temperature in the production of N_2O was observed in Ave estuary with higher production rates of N_2O obtained in the summer. Still, the measured

N₂O values were lower than previously reported in estuaries from Australia, China, and Belgium (Allen et al., 2011; Li et al., 2019; Middelburg et al., 1995). In Douro estuary, a relation was found between CH₄ and N₂O production, a positive relation under aerobic conditions, and an inverse relation under anaerobic conditions. Ave estuary presented a positive relation with salinity, under both aerobic and anaerobic conditions. A relation between N₂O emission and salinity was also found in other estuarine systems (Franklin et al., 2017; Li et al., 2019). Cávado N₂O production presented a positive relation, under aerobic conditions, with sediments water and sediment organic matter.

In spite of the above mentioned, no consistent relation was found between CH₄ and N₂O fluxes and environmental variables measured, indicating that the sites on this study represented unique ecosystems, with various process and interactions occurring simultaneously (Welti et al., 2016). Indeed, the different range of emissions of both CH₄ and N₂O from the three estuaries reflected the specificity of environmental conditions. The selected sites and estuaries present different characteristics between them and anthropogenic pressures. Site, D2, in the Douro estuary is a clear example, with effluents from two polluted tributaries (rivers Tinto and Torto), two wastewater treatment plants, and a recreational port, all within the same area. Coincidentally, the highest dissolved gases concentrations and potential emission rates from sediment were found for this location. Nonetheless, the potential emission fluxes of both CH₄ and N₂O from these estuaries were lower than reported for other estuaries around the world. Therefore, the contribution to global warming is expected to be of smaller magnitude under current conditions, then in other places.

This study provided knowledge about the situation of the three estuaries from Northern Portugal. However, more studies in different areas within the estuary are necessary to better understand their contribution to the emission of greenhouse gases. Studies in freshwater tributaries are necessary to better understand their contribution to the emission of greenhouse gas. Increasing the magnitude of the studies allows a better understanding of the real emissions rates that occur in our estuaries.

Conclusions

Although the three estuaries are located within the same geographic area, they presented substantially different results in what N₂O and CH₄ emissions are concerned.

Even though the three estuaries are heavily urbanized and modified, the values obtained were lower than reported in other estuarine systems worldwide, although higher values were found in the most human impacted locations. Measures to address and limit the problem are important to prevent negative impacts in the future.

Since estuaries are ecologically important, further studies are necessary to better understand the anthropogenic impact of N₂O and CH₄ emissions, and the need for preventive and remediation measures.

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