



Impact of heatwaves on net metabolism, nutrient and greenhouse gases fluxes at the sediment-water interphase of a shallow coastal ecosystems

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Abstract. Global average temperatures and the frequency of extreme climatic events, including heat waves, are increasing due to the anthropogenic rise in greenhouse gas (GHG) emissions. Shallow aquatic areas can potentially buffer some anthropogenic alterations, acting as net sinks for nutrients and GHGs such as CO₂, CH₄ and N₂O. However, these environments are also vulnerable to climate variations, with unknown consequences for their role as climate buffers. To investigate the impact of altered temperatures on the net exchange of GHGs and nutrients, we quantified variations in net metabolism, dissolved inorganic nitrogen and GHG fluxes in sediments from the Río San Pedro creek (Bay of Cádiz) exposed to a wide range of temperatures (10 to 42 °C) over 84 hours of laboratory-controlled incubation, using core incubations, oxygen microprofiles and microbial community analysis. Sediment respiration increased significantly with temperature, with evidence of inhibition after prolonged exposure to the highest temperatures (34–42 °C), typical of heatwave conditions. Nitrogen cycling was also affected, though not straightforwardly: moderate warming increased nitrification and net nitrite efflux, while dissimilatory processes (denitrification and DNRA) appeared enhanced at higher temperatures. Key nitrification gene abundances did not increase significantly, but functional predictions from 16S rRNA gene data using PICRUSt2 indicated enrichment of the predicted denitrification pathway at the highest temperature. Although sediments were a net source of CH₄ and N₂O throughout, their temperature responses diverged: N₂O release increased up to 26–34 °C then declined, and was higher during the first days, revealing a decoupling between production and consumption over time; methane release increased exponentially with temperature throughout. Increasing heatwave frequency and duration could therefore substantially raise net CO₂ and CH₄ release from shallow coastal sediments, while N₂O responses appear more complex and non-monotonic, with uncertain net feedback on global warming.



1 Introduction

Global warming is considered one of the most serious threats to ecosystems with temperatures of the Earth's surface increasing worldwide, being directly linked to the production and emission of anthropogenic Green House Gases (GHG) (Burkholz et al., 2020; Meehl et al., 2005; Perkins-Kirkpatrick and Lewis, 2020). This global warming is additionally increasing the intensity and frequency of episodes of extreme climatic conditions (ECC)(Schutte et al., 2019; Smith et al., 2023). The ecosystem response to the extreme temperature changes during heat waves may result in a feedback loop leading to an increase of naturally produced GHG during biogeochemical cycling, therefore reinforcing global warming (Burkholz et al., 2020).

Shallow coastal ecosystems like estuaries and wetlands play a vital role in providing essential ecosystem services. These areas are characterized by high biodiversity, support economically important species, and act as crucial buffers during the exchange of organic matter, nutrients, oxygen, and GHG between land and ocean. Additionally, they receive substantial inputs of organic carbon and nutrients from rivers and runoff, further emphasizing their importance as critical interfaces between land and sea (Froneman, 2005; Hines et al., 2013; Schutte et al., 2019). In these environments, the accumulated organic material in the sediment undergoes a sequence of microbial processes, where oxygen is rapidly consumed, leading to suboxic and anoxic conditions that further drive organic matter decomposition and nutrient recycling by anaerobic microbial metabolisms (Canfield et al., 1993; Schutte et al., 2019). These processes result in the production of CO₂ but might also generate or consume potent greenhouse gases like N₂O and CH₄. Shallow coastal environments might contribute significantly to global atmospheric emissions and play a crucial role in the global nitrogen cycle and the destruction of stratospheric ozone (Burgos et al., 2018b; Burkholz et al., 2020; Herbert, 1999).

Intertidal systems, are subjected to a wide range of physical forcings, such as daily and seasonal light cycles, immersion and emersion periods due to tidal changes, and temperature variations. Sediment temperatures can vary by several degrees under typical conditions, for example from 17 to 24 °C in northern systems, with larger fluctuations occurring in more thermally variable environments (Deldicq et al., 2021; Guarini et al., 1997). As a consequence, biogeochemical processes and fluxes of solutes, including GHG, can vary significantly during the day (Douglas et al., 2025; Gong et al., 2023; Lin et al., 2024).

Variations in temperature due to heatwaves can differentially modify physicochemical processes with opposite effects on solute solubility and diffusion. An increase in temperature can decrease, for example, the availability of oxygen while at the same time increase its diffusion rates thus affecting the rates and depth distribution of biogeochemical processes involved in carbon and nitrogen mineralization (Hicks et al., 2018; Rios-Yunes et al., 2023; Thamdrup et al., 1998). Temperature changes can also directly influence enzymatic processes in biogeochemical cycles, leading to varying impacts on metabolic rates and shifting the relative importance of different processes (Thamdrup et al., 1998; Weston and Joye, 2005). Typically, higher temperatures are expected to accelerate metabolic activities, but the relative effect can vary depending on the specific process and enzymes involved. For instance, elevated temperatures may enhance nitrification by increasing directly enzyme activity or by a higher availability of ammonium due to increased remineralization rates and simultaneously limit nitrification due to reduced oxygen concentrations (Brin et al., 2015; Rysgaard et al., 1994).



70 Monitoring and understanding the effects of rising temperatures on ecosystem components is essential for predicting ecosystem responses. However, knowledge about the impacts of extreme climatic events, such as heat waves, remains limited, and few studies have examined their impacts on intertidal communities (Easterling et al., 2000; Perkins-Kirkpatrick and Lewis, 2020; Stott et al., 2004). In the present study, we investigated the impact of maintained temperature variations maintained during a 4-day experiment in the absence of tides and in darkness to limit confounding factors. The aim was to assess how temperature changes affect sediment microbial community composition and metabolism. Sediment–water oxygen and nutrient exchange, greenhouse gas emissions, bacterial community composition and nitrogen-cycle functional potential were measured across an initial baseline and five temperature treatments, with 18 °C serving as the experimental control. Although the experimental conditions do not fully reproduce those in the field, this design provides insight into temperature-driven changes in sediment microbial communities and biogeochemical processes, as well as their capacity to remain stable or acclimate under substantial warming.

2 Material and Methods

2.1 Study site and sampling

Sediment cores (n = 24, id. 5.5 cm, 30 cm length) and *in situ* water were collected on May 2018 in a non-vegetated muddy area of the shallow Río San Pedro tidal creek (Cádiz, Spain) (36 ° 34 ' 37.5 "N, 6 ° 11 ' 14.3" W). The creek has an average depth of 3–6 meters and a semi-diurnal tidal regime. Water temperature varies between 10 and 23 °C in winter and summer, respectively (Ferrón et al., 2009a).

Cores were transported to the laboratory after collection and distributed among five 40 L tanks filled with aerated *in situ* water (36 ‰). Sediment cores were maintained for 4 days at 18 °C in darkness for acclimation. After this period, four sediment cores were used to measure initial conditions (time 0) as described below. Subsequently, temperature in each of the tanks was progressively modified to reach five different temperatures (10, 18, 26, 34, and 42 °C) by using electrical heaters or cooling units. The final temperatures were reached after approximately 6 hours. The effect of the temperature changes was measured after 12, 36, 60, and 84 hours.

For each temperature and day, three sediment cores were incubated to measure sediment-water fluxes of inorganic nitrogen nutrients (NO₃⁻, NO₂⁻, NH₄⁺), oxygen (O₂), methane (CH₄) and nitrous oxide (N₂O). One additional sediment core was used to measure oxygen microprofiles, as described below. Additional subsamples were taken for molecular analyses, including DNA extraction, 16S rRNA gene library sequencing to characterize the microbial community and quantification of genes involved in the nitrogen cycle to determine changes in its functional potential.



2.2 Sediment-water fluxes

100 Sediment cores (20 cm of sediment and 10 cm of water column) were equipped with magnetic stirrers suspended in the water column and driven by external motors to maintain turbulence in the water column. Once initial water samples were collected, sediment cores were sealed with rubber stoppers and incubated for 2–10 hours, depending on the temperature. At the end of the incubation period, sediment cores were opened outside the water tank for collecting final samples and cores returned to their corresponding tank.

105 Water samples (10 mL) for nutrient analyses were filtered (MF300 glass fiber filter, 0.7 μm nominal pore size, Fisherbrand) in 12 mL plastic tubes and stored frozen at -24°C until analysis. Nitrate and nitrite were analyzed following the procedure described in Garcia-Robledo et al. (2014), and ammonium according to Bower & Holm-Hansen (1980). Spectrophotometric measurements were performed on a MultiskanTM GO microplate reader (Thermo ScientificTM).

Oxygen fluxes for the determination of diffusive oxygen uptake (DOU) at the sediment-water interface were measured by O_2 vertical concentration profiles in darkness with oxygen selective microelectrodes (100 μm tip size, Unisense A/S, Denmark) controlled by a motorized micromanipulator (MS-232), connected to a computer using the Sensor Trace Pro software (Unisense®). The microelectrodes were calibrated in the water column with constant bubbling for the 100% oxygen saturation and in the anoxic zone of the sediment for the 0% saturation. From the profiles, the depth of maximum oxygen penetration (Z_{ox}) was identified and the oxygen fluxes were determined at the diffusive boundary layer by applying Fick's first law for molecular diffusion using the molecular diffusion coefficient D_0 , determined from the salinity and temperature conditions (Soetaert and Petzoldt, 2008), and the concentration gradient at the diffusive boundary layer (Jørgensen and Revsbech, 1985; Kühl et al., 1994; Revsbech, 1989).

115 Samples for the determination of N_2O and CH_4 concentrations were collected in 50 mL serum bottles and preserved with 120 μL of 37% formaldehyde. Concentrations of the dissolved gases were measured using a Bruker® gas chromatograph model 450 GC as described previously by Sierra (2017).

Sediment-water fluxes ($\text{mmol m}^{-2} \text{ h}^{-1}$) for nutrients, total oxygen uptake (TOU) and GHGs were calculated from the concentration changes over time in the overlying water of the cores during the incubation. Positive flux represents release from the sediment and negative flux uptake by the sediment.

125 2.3 Organic matter, DIC and nutrients profiles in the sediment

On the last day of the experiment, one core from each temperature treatment was sliced every cm down to 5 cm depth. Slices were transferred to 50 mL Falcon tubes and homogenized to collect about 1 g of the sediment slurry for DNA extraction as explained below. The rest was centrifuged at $4700 \times g$ for 20 min at 4°C in a SorvallTM LegendTM X1R centrifuge (Thermo ScientificTM) to extract the porewater (PW). Three mL were transferred to Exetainers® (LabCO, UK) and preserved with 50 μL of 32–37% formaldehyde for later analysis of Dissolved Inorganic Carbon (DIC) via the Gran titration method (Mehrbach



et al., 1973), and the remaining fraction was transferred to 6 mL plastic vials and kept frozen at -24 °C until later analysis for nutrients concentrations. Centrifuged sediment was frozen, freeze-dried and homogenized. Carbon and N content were analyzed on an elemental analyzer (Leco CHNS 932) at the Central Services of the University of A Coruña, Spain. Organic carbon (C_{org}) was determined as the difference between the total carbon measured in the freeze-dried sediment samples and inorganic carbon measured on a replicate previously combusted at 550 °C for 4 h. Total nitrogen was measured in the freeze-dried sediment samples.

Besides, dissolved inorganic carbon (DIC) and dissolved inorganic nitrogen (DIN) fluxes at the sediment-water interface were calculated from the DIC and DIN profiles, using Fick's first law equation applied to the upper 2 cm sediment layer and the diffusion coefficient with the sediment (D_s), estimated from D_0 and corrected for the sediment porosity, hereafter referred as steady state fluxes.

2.4 Apparent activation energy and Q_{10} calculation

The dependence of the measured microbial processes with temperature was quantified by the calculation of the apparent activation energy (E_a) and the Q_{10} value. For that purpose, the measured rates were adjusted to the Arrhenius equation, and E_a was calculated by eq. 1:

$$E_a = -R \cdot T \cdot \ln \left(\frac{\text{Rate}}{A} \right) \quad (1)$$

where, A is a constant, R is the ideal gases constant ($J K^{-1} mol^{-1}$) and T is the experimental temperature (°K). E_a was then used to calculate the Q_{10} , following eq. 2 (Glud, 2008; Thamdrup et al., 1998):

$$Q'_{10} = \exp \left(E_a \cdot 10K \cdot (R \cdot T \cdot (T + 10K))^{-1} \right) \quad (2)$$

In addition, we also calculated Q_{10} values as the difference in rates between two temperatures following eq. 3:

$$Q_{10} = \left(\frac{R_2}{R_1} \right)^{\frac{10}{(T_2 - T_1)}} \quad (3)$$

Apparent activation energies and Q_{10} values calculated for each oxygen-uptake variable and exposure time are provided in Table S1.

2.5 DNA extraction, PCR and sequencing of 16S rRNA

Before centrifuging the sediment to extract PW, a portion (~1g) was placed in 1.5 mL microcentrifuge tubes and frozen at -80 °C. DNA extraction was performed according to mechanical-chemical lysis protocol of Griffiths et al (2000). The quality and concentration of the extracted DNA was checked by spectrophotometry using a µdrop Multiskan GO (Thermo-Scientific) and confirmed by Qubit (Thermo-Scientific). The V4 region of the bacterial and archaeal 16S rRNA gene was amplified using primers 515F (5'-GTGYCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACNVGGGTWTCTAAT-3') (Walters et al., 2016). PCR was conducted using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) with the following cycling conditions:



95 °C for 5 minutes, followed by 30 cycles of 95 °C for 30 seconds, 53 °C for 40 seconds and 72 °C for 1 minute, and a final elongation step at 72 °C for 10 minutes. PCR products were checked by gel electrophoresis. Samples are multiplexed using unique dual indices and are pooled together in equal proportions based on their molecular weight and DNA concentrations. Pooled samples were purified using calibrated Ampure XP beads. Then the pooled and purified PCR product was used to
165 prepare an Illumina DNA library. Sequencing was performed at MR DNA (www.mrdnalab.com, Shallowater, TX, USA) on a MiSeq following the manufacturer's guidelines.

2.6 Bioinformatics analysis

Sequences (fastq files) were processed using DADA2 pipeline (Callahan et al., 2016) in R V.4.0 (R Core Team 2021). Forward
170 and reverse reads were trimmed to 250 and 220 bases, respectively. Filtering settings were maximum number of Ns (maxN) = 0, maximum number of expected errors (maxEE) = (1.1). The primer sequences were removed using trimLeft=c(20,20). Sequences were dereplicated and the DADA2 core sequence variant inference algorithm applied. mergePairs was used to merge sequences and ASV tables were constructed. Chimeric sequences were removed using removeBimeraDenovo default settings. ASVs were taxonomically assigned using assignTaxonomy at default settings against the SILVA version 138.2
175 database (Quast et al., 2012). Samples were rarefied to 150,000 reads per sample for Bacteria before further analysis and singleton ASVs removed, as well as ASVs appearing in less than 2 samples.

The ASV abundance table and representative 16S rRNA gene sequences were analysed using PICRUST2 v2.2.0 (Douglas et al., 2020) to estimate the functional potential of the bacterial and archaeal communities. PICRUST2 places each ASV within a reference phylogeny and uses the gene content of related sequenced genomes to predict which gene families are likely to be
180 present. ASV abundances were corrected for predicted variation in 16S rRNA gene copy number and combined with these gene-family predictions to generate a predicted metagenomic profile for each sample. Predicted genes were then summarised into metabolic pathways, which were compared among temperature treatments to identify potential shifts in community functional capacity. These predictions represent inferred genomic potential rather than direct measurements of gene abundance, expression or metabolic activity.

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2.7 Quantitative PCR

Gene abundance involved in the nitrogen cycle (nirK, nirS, nosZ, nxrB, nrfA, and amoA for Bacteria, Archaea and commamox) were quantified by qPCR (**Table S2**). All samples were analysed in duplicates. A 1:10 dilution was used to remove inhibitory substances (Tatti et al., 2016). Standard curves constructed by amplifying the gene of interest according to Smith et al., (2006)
190 and no-template controls (NTC) were included in triplicate in each assay. Quantitative PCR was performed using the SYBR Green GoTaq qPCR Master Mix (Promega), and the appropriate primers and cycling conditions in a 20 µl reaction (**Table S2**).



qPCR assays were conducted using a CFX Connect Real-Time PCR Detection System (BioRad). Successful amplification of the desired fragments was assessed by agarose gel electrophoresis, while specificity was checked by melt curve analysis.

195 2.8 Statistical analysis

The effects of day and temperature on sediment-water fluxes were analyzed using linear mixed-effects models (LMMs). Exposure time (12, 36, 60 and 84 h), temperature (10, 18, 26, 34 and 42 °C) and their interaction were included as categorical fixed effects, while sediment core was included as a random intercept to account for repeated measurements on the same cores. The significance of the fixed effects and their interaction was assessed using likelihood-ratio tests comparing nested models
200 fitted by maximum likelihood.

When the temperature × exposure-time interaction was significant, follow-up comparisons of estimated marginal means were performed for all pairwise temperature contrasts within each exposure time and all pairwise exposure-time contrasts within each temperature. Degrees of freedom were estimated using the Satterthwaite method, and p values were adjusted using Tukey's method within each family of comparisons. CH₄ fluxes were transformed as $\ln(\text{CH}_4 + 0.32)$ before analysis. Because
205 the temperature × exposure-time interaction was not significant for transformed CH₄ fluxes, temperature effects were assessed using an additive model, followed by Tukey-adjusted comparisons among temperatures averaged across exposure times. Likelihood-ratio test results are provided in Table S3, significant follow-up comparisons are reported in Tables S4 and S5, and all temperature comparisons for CH₄ are provided in Table S6.

When the dataset was insufficient for fitting a linear mixed model (LMM), Kruskal-Wallis tests or 2-way ANOVAs were
210 employed instead. The relationship between DIC and NH₄⁺ production rates after 84 h was assessed using linear regression forced through the origin. All analyses were performed using R version 4.3.1 (R Core Team, 2023), using the lme4, lmerTest and emmeans packages. Statistical significance was accepted at $p < 0.05$. Results are reported as mean ± standard error (SE) unless otherwise indicated.

All analyses were performed in R version 4.3.0 (R Core Team, 2021). Bacterial and archaeal datasets were analysed separately
215 using the phyloseq (McMurdie & Holmes, 2013) and microeco packages. Taxonomic composition was summarised as relative abundance at selected phylum, class and order levels and visualised using stacked bar plots and heatmaps. Significance in taxa across time and treatment was assessed using ANOVA and DESeq2 (Love et al., 2014). Differences in the relative abundance of selected taxonomic groups among the Initial, 10, 18, 26, 34 and 42 °C treatments were additionally assessed using Kruskal–Wallis tests followed by Dunn's post hoc comparisons.

220 Alpha diversity was quantified using observed amplicon sequence variant (ASV) richness and the Shannon diversity index (H'). Differences among treatments were assessed using Kruskal–Wallis tests followed by Dunn's post hoc comparisons, and individual replicate values were displayed alongside boxplots. Beta diversity was calculated using Bray–Curtis dissimilarities based on ASV abundance data. Differences in community composition were visualised using principal coordinates analysis (PCoA), with individual samples connected to the centroid of their respective treatment group. The effect of temperature



225 treatment on community composition was tested using permutational multivariate analysis of variance (PERMANOVA),
implemented using the `adonis2` function in the `vegan` package (Oksanen et al., 2001). Statistical significance was accepted at
 $p < 0.05$.

3 Results

230 3.1 Diffusive and total oxygen fluxes at the sediment-water interface

Temperature had a clear effect on the measured O_2 microprofiles (**Fig. 1**). At high temperatures, O_2 was quickly consumed by
the sediment, resulting in decreased oxygen penetration depth (z_{ox}) (~1.3 mm at 42 °C) while smoother, more deeply extended
 O_2 microprofiles were recorded at lower temperatures (~4 mm at 10 °C). Both diffusive and total sediment oxygen fluxes
(consumption rates) increased with temperature (**Fig. 2**). Diffusive oxygen fluxes increased approximately linearly with
235 temperature, over the measured range, at a rate of $34 \mu\text{mol } O_2 \text{ m}^{-2} \text{ h}^{-1} \text{ } ^\circ\text{C}^{-1}$. The rate was 1.6 times higher at 42 °C compared
to 10 °C after 12 h and 2.8 times after 60 h. After 84 h, however, DOU decreased at the two highest temperatures. Volumetric
DOU (DOU_{vol}), calculated by dividing the areal DOU rate by the oxic-layer thickness (m), showed more pronounced changes
as expected from the definition, given the decrease in z_{ox} with temperature (**Fig. 2b**). Total oxygen uptake (TOU) increased
with temperature as well, showing a maximum around 26–34 °C after 12 h (**Fig. 2c**). TOU at the highest temperatures of 34–
240 42 °C was double that at 10–18 °C; however, the increase did not follow a clear linear pattern as in DOU. Both DOU and TOU
showed significant temperature $\times$ exposure-time interactions (DOU: $\chi^2_{12} = 37.03$, $p < 0.001$; TOU: $\chi^2_{12} = 45.47$, $p < 0.001$;
LMM). Tukey-adjusted comparisons showed that DOU differed between 10 and 42 °C after 12 h, with broader temperature
differences from 36 h onwards; after 84 h, DOU was highest at 26 °C and differed from all other temperatures. For TOU, 10
°C differed from 26 and 34 °C after 12 h and from 42 °C after 36 h, whereas no temperature differences were detected after
245 60 or 84 h.

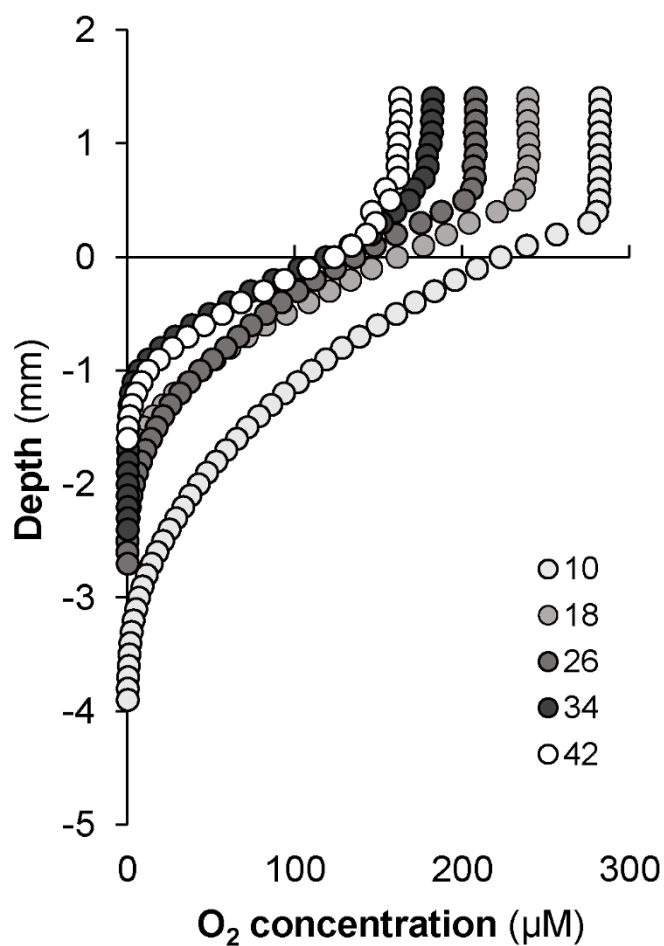


Figure 1. Average vertical microprofiles of dissolved oxygen (O_2) across the sediment–water interface in sediments exposed to the different temperatures tested (10–42 °C) for 24 h. Depth is referenced to the sediment–water interface (0 mm); profiles

250 were measured with O_2 microelectrodes and are averages of replicate measurements ($n=3$).

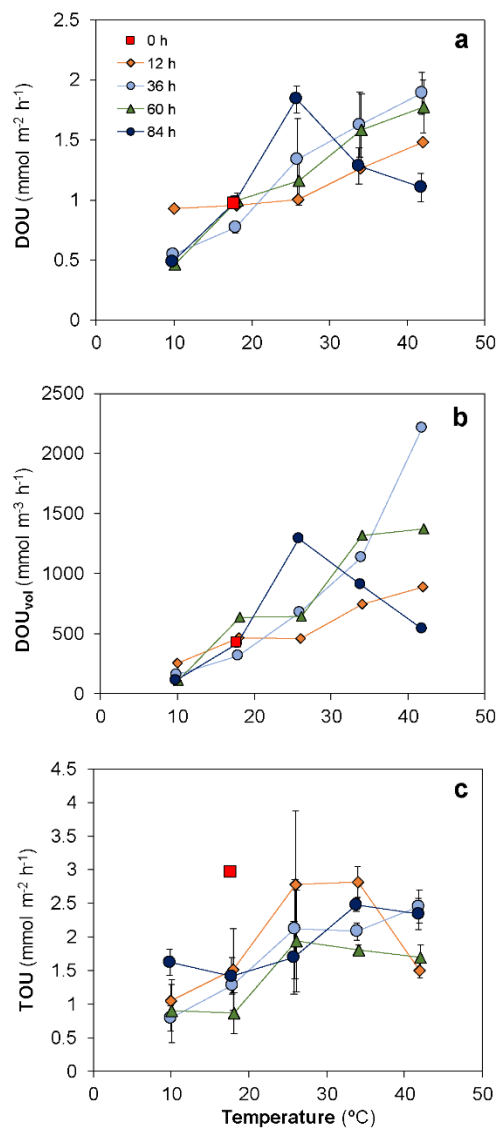


Figure 2. Diffusive oxygen uptake (DOU) expressed as areal rates (a) and as volumetric rates within the oxic layer, i.e. normalised to the oxygen penetration depth (b), together with total oxygen uptake (TOU) (c), in sediments exposed to the different temperatures (10–42 °C) over 4 days (84 h). DOU was derived from O₂ microelectrode profiles and TOU from whole-core incubations. Data are means ± standard error (SE); n = 3.



260 The apparent activation energy (E_a) calculated from areal ($\text{mmol O}_2 \text{ m}^{-2} \text{ h}^{-1}$) and volumetric ($\text{mmol O}_2 \text{ m}^{-3} \text{ h}^{-1}$) DOU rates showed an apparent increase over time (**Fig. 3**). However, no significant differences were observed throughout the experiment ($p\text{-value} = 0.3916$; Kruskal-Wallis test). Mean E_a (areal) increased from 10.5 kJ mol^{-1} after 12 hours to 58.2 kJ mol^{-1} after 84 h. (**Table 1**) Mean E_a (volumetric) were between 2.7 to 1.7 times bigger than areal rates, after 12 and 84 h, respectively. In contrast, when E_a was calculated for areal TOU rates its value decreased with incubation time from 34.7 to 12.3 kJ mol^{-1} . For comparison with other studies, Q_{10} values were calculated from E_a as well (Eq. 4) (**Table 2**). Q_{10} was 1.2 - 2.2 when calculated from E_a ($\text{DOU}_{\text{areal}}$), 1.5 – 4.0 from E_a (DOU_{vol}), and 1.6 and 1.2 from E_a (TOU) after 12 and 84 h, respectively.

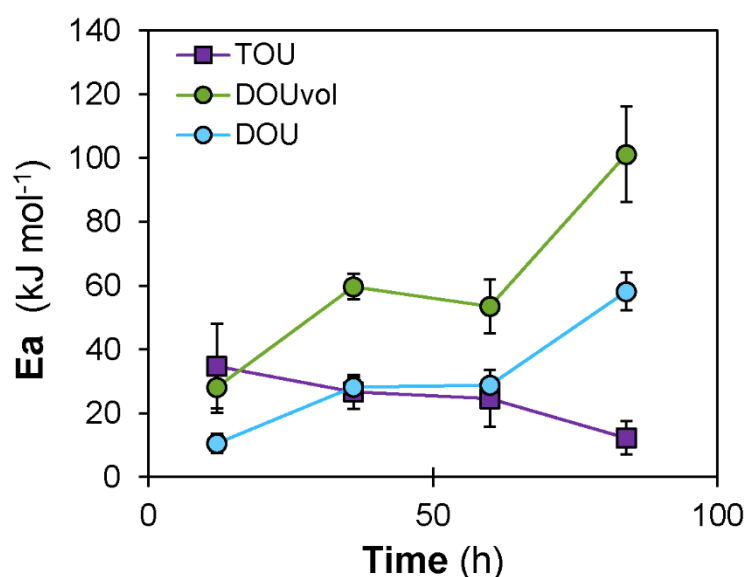


Figure 3. Apparent activation energy (E_a , kJ mol^{-1}) of sediment oxygen consumption, calculated from areal diffusive oxygen uptake (DOU; $\text{mmol O}_2 \text{ m}^{-2} \text{ h}^{-1}$), volumetric DOU within the oxic layer (DOU_{VOL} ; $\text{mmol O}_2 \text{ m}^{-3} \text{ h}^{-1}$) and total oxygen uptake (TOU; $\text{mmol O}_2 \text{ m}^{-2} \text{ h}^{-1}$). E_a and its standard error (SE) were derived from the slope and associated SE of the linear Arrhenius regression (Eq. 2)



Table 1. Apparent activation energy (E_a , kJ mol^{-1}) and temperature coefficient (Q_{10}) of sediment oxygen uptake at each sampling time, calculated from areal diffusive oxygen uptake ($\text{mmol O}_2 \text{ m}^{-2} \text{ h}^{-1}$) and from volumetric uptake within the oxic layer ($\text{mmol O}_2 \text{ m}^{-3} \text{ h}^{-1}$). E_a was obtained from Eq. 2 and Q_{10} from Eq. 3, referenced to 18 °C; values are mean $\pm$ SE. When Q_{10} was instead computed over successive temperature intervals (Eq. 5), the overall mean was 1.33 ± 0.14 for areal rates and 2.47 ± 0.52 for volumetric rates ($n = 16$).

<i>Hour s</i>	<i>Areal rates</i>		<i>Volumetric rates</i>	
	<i>E_a (kJ mol^{-1})</i>	<i>Q_{10}</i>	<i>E_a (kJ mol^{-1})</i>	<i>Q_{10}</i>
12	10.50 ± 2.99	1.61 ± 0.83	28.11 ± 7.9	1.47 ± 0.90
36	28.17 ± 3.27	1.44 ± 0.93	59.69 ± 4.10	2.27 ± 0.95
60	28.84 ± 4.78	1.40 ± 0.88	53.49 ± 8.53	2.08 ± 0.89
84	58.19 ± 5.94	1.18 ± 0.93	101.10^*	4.01^*

*Calculated with the linear part of the curve (between 10-26 °C)

Table 2. Compilation of published temperature-dependence data (apparent activation energy, E_{as} and/or temperature coefficient, Q_{10}) for benthic respiration in different sediment types, compiled for comparison with the present study. Used technique: Whole core incubation, (WCI); Incubation chambers (IC); Winkler method (WM); Microsensors (M).

<i>Ecosystem</i>	<i>Site</i>	<i>T (°C)</i>	<i>Used technique</i>	<i>Ea (kJ mol⁻¹)</i>	<i>Exp. duration (h)</i>	<i>Rates range</i>	<i>Light</i>	<i>Type</i>	<i>Reference</i>
<i>West Lawrenceto wn marsh</i>	Canada	10-20	<i>WCI</i>	1.0-2.2	96-120	0.5-15 mm ⁻³ cm ⁻² h ⁻¹	Light	Intertidal high and low sediment	<i>(Duff & Teal, 1965)</i>
		2-10		1.9-4.1		3-100 mm ⁻³ cm ⁻² h ⁻¹			
<i>Sapelo marshes</i>	Georgia	10-20	<i>WCI</i>	1.7-1.9	96-120	0.7-18 mm ⁻³ cm ⁻² h ⁻¹	Light	Spartina marshes muddy sediment	
		2-10		1.3-4.8		0.7-9 mm ⁻³ cm ⁻² h ⁻¹			
<i>Norsminde Fjord</i>	Denmark	2-20	<i>WCI</i>	2.7	4	36.6-43.0 mmol m ⁻² d ⁻¹	Dark	Homogenized sediment	<i>(B. Jørgensen & Sørensen, 1985)</i>
<i>Nova Scotia</i>	Canada	6-14	<i>WCI</i>	6.5		0-18 mg O ₂ m ⁻² h ⁻¹	Dark	Sandflat upper intertidal sediment	<i>(Grant, 1986)</i>
				2		13-90 mg O ₂ m ⁻² h ⁻¹	Light		
<i>A-horizon soils</i>		4-28	<i>IC</i>	1.7-1.9	24h (50-120 days)	0.12-1.6 umol CO ₂ g ⁻¹ d ⁻¹	Dark	Ultisol forest surface	<i>(Winkler et al., 1996)</i>
<i>Aarhus Bay sediment</i>	Denmark	4-14	<i>WCI + M</i>	1.9-3.1	4-11	0-11 umol cm ⁻³ d ⁻¹	Light	Homogenized sediment	<i>(Thamdrup, Hansen, & Jørgensen, 1998)</i>



<i>Menai Strait</i>	North Wales	7.6-15	WM	4.0-9.3	24	25 $\mu\text{mol O}_2 \text{ dm}^{-3} \text{ d}^{-1}$	Dark	<i>Phaeocystis</i> bloom	(Lefèvre et al., 1994)
<i>Svalbard fjords</i>	Norway	0-10	WCI + WM	1.7-2.1	1-10	0.13-1.50 $\mu\text{mol g d.w.}^{-1} \text{ h}^{-1}$		Fine brown silt sediment	(Thamdrup & Fleischer, 1998a)
<i>Weser estuary</i>	Germany	0-10	WM	2.5-4.9	2-6	0-8 $\mu\text{mol g d.w.}^{-1} \text{ h}^{-1}$		Fine silt cover by diatoms	
<i>Hornsund</i>	Norway	-2-45	WCI + WM	1.84	6	15 $\mu\text{mol O}_2 \text{ cm}^{-3} \text{ d}^{-1}$		Fine-grained sediment	(Arnosti et al., 1998a)
<i>Storfjorden</i>	Norway		WM	1.77	5	4.6 $\mu\text{mol O}_2 \text{ cm}^{-3} \text{ d}^{-1}$	Dark	Soft and clayey sediment	
<i>Ebro delta</i>	Spain	15-30	M + IC	2.35	1-1.5	196-630 $\text{nmol O}_2 \text{ cm}^{-3} \text{ min}^{-1}$	Light	Intertidal/saltmarsh	(Epping & Kuhl, 2000)
<i>Niva Bay</i>	Denmark	5-15	WCI + M	2.2-5.2	24	0-0.05 $\text{nmol O}_2 \text{ cm}^{-2} \text{ s}^{-1}$	12:12	Subtidal sediment	(Hancke & Glud, 2004b)
<i>Trondheims fjord</i>	Norway			1.7-2.6	34-62	0-0.08 $\text{nmol O}_2 \text{ cm}^{-2} \text{ s}^{-1}$	Dark/Light	from semi-enclosed bays	
<i>Adventfjord</i>	Norway			2.4-3.2	55-75	0-0.03 $\text{nmol O}_2 \text{ cm}^{-2} \text{ s}^{-1}$			
<i>Cadiz Bay intertidal sediment</i>	Spain	10-42	WCI + M	1.15 - 2.22	84	0.5-3 $\text{mmol O}_2 \text{ m}^{-2} \text{ h}^{-1}$	Dark	Intertidal creek non-vegetated sediment	This study



3.2 Dissolved inorganic carbon and nitrogen profiles

DIC and NH_4^+ porewater profiles were affected by temperature variations, at higher temperatures. Initial concentration of DIC (18 °C, 0h) was constant with depth around 4.01 ± 0.13 mM (**Fig. 4a**). At the end of the experiment, sediment cores exposed to temperatures above 18 °C showed a clear accumulation of DIC inside the sediment with a peak at 2–3 cm reaching 9.6 mM at 42° C. As a result, integrated DIC concentration with depth increased linearly with temperature (**Fig. S2**).

Porewater NH_4^+ increased progressively with depth from 12.3 μM to 102.0 μM at 5 cm at the beginning of the experiment (**Fig. 4b**). After 84 h, concentrations increased in all the treatments. At the three lowest temperatures, values reached 350 μM whereas a much higher build up with depth was observed at 34 and 42 °C, reaching maximum values of 670 and 1050 μM , respectively. Integrated NH_4^+ concentration with depth did not change up to 26 °C but increased linearly with temperature up to 42 °C (**Fig. S2a**).

In contrast to DIC and NH_4^+ , porewater profiles of NO_2^- and NO_3^- in the sediment indicated generally net consumption (**Fig. 4c,d**). NO_2^- and NO_3^- concentrations were very low and very little NO_3^- was measured below the first centimeter. NO_3^- concentrations were higher at lower temperatures both in the water column and sediment. (**Fig. 4c,d**). Integrated NO_2^- and NO_3^- concentrations did not show a trend with temperature; a minimum at 26° C was recorded for both nutrients (**Fig. S2b**).

The ratio between integrated DIC and integrated total dissolved inorganic nitrogen (NH_4^+ (directly from remineralization) + NO_2^- + NO_3^- (produced by nitrification of that NH_4^+)) (DIC:DIN) decreased considerably from initial conditions to those measured after 84 h. DIC:DIN showed its lowest value of 8.3 at 42° C and its highest of 25 at 26° C (**Fig. S2c**).

3.3 DIC and DIN fluxes at the sediment-water interface

The steady state fluxes at different temperatures indicated an important increase of DIC and NH_4^+ efflux with temperature after 84 h, increasing from values of 60.0 and 10.7 $\mu\text{mol m}^{-2} \text{h}^{-1}$ at 18° C to 861.3 and 257.7 $\mu\text{mol m}^{-2} \text{h}^{-1}$ at 42° C for DIC and NH_4^+ , respectively (**Fig. 5a**). DIC and NH_4^+ production rates showed a strong linear relationship ($\text{DIC} = 3.2 \cdot \text{NH}_4^+$, $r^2 = 0.97$, $p < 0.05$). NO_3^- flux in the upper sediment layer indicated consumption and a decreasing trend with temperature; rates at least three orders of magnitude lower than those of DIC and NH_4^+ production rates. (**Fig. 5b**).



Core incubations indicated that increasing temperature produced a clear increase in NH_4^+ consumption, resulting in an increasing influx to the sediment (**Fig. 6a**). The rate of flux change with temperature was mostly linear and was highest after 12 h of exposure ($-22.7 \pm 5.8 \mu\text{mol m}^{-2} \text{h}^{-1} \text{ } ^\circ\text{C}^{-1}$), decreasing progressively with time to reach values of $-12.6 \pm 3.2 \mu\text{mol m}^{-2} \text{h}^{-1} \text{ } ^\circ\text{C}^{-1}$ after 84h. NH_4^+ fluxes showed a significant interaction between temperature and exposure time ($\chi^2_{12} = 26.71$, $p = 0.009$; LMM). The magnitude of the flux decreased progressively with time, even shifting to positive fluxes for the lowest temperatures after 36 h. Tukey-adjusted comparisons showed the clearest temperature differences after 12 and 36 h: fluxes at 34°C differed from those at 10, 18 and 26°C at both times, while 42°C differed from 10 and 26°C after 12 h and from 10 and 18°C after 36 h. After 84 h, only 10 and 42°C differed significantly.

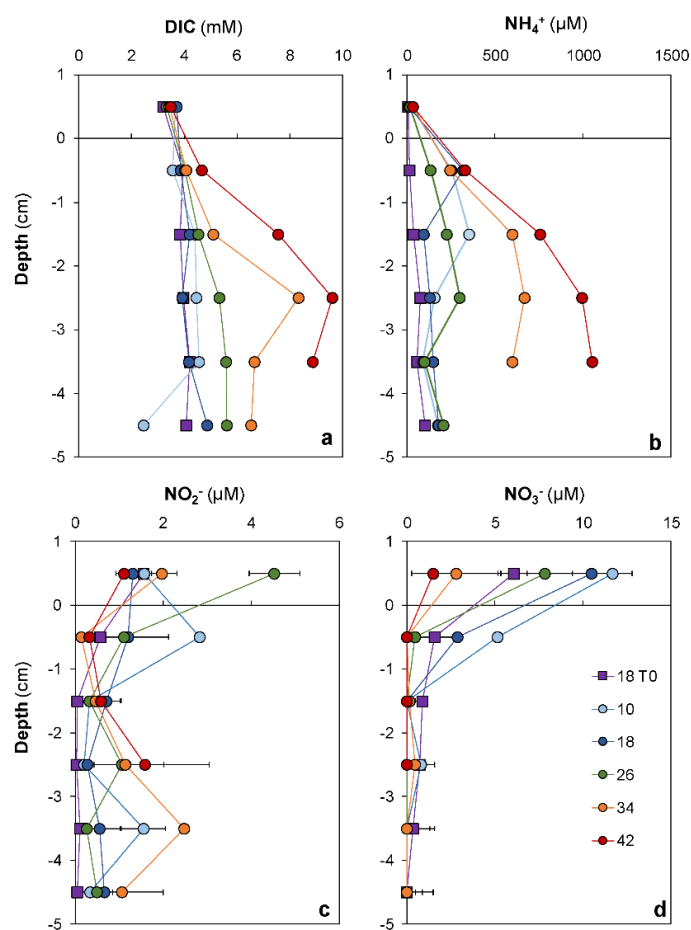


Figure 4. Porewater depth profiles of (a) dissolved inorganic carbon (DIC), (b) ammonium (NH_4^+), (c) nitrite (NO_2^-) and (d) nitrate (NO_3^-) in sediment cores before the experiment (initial, 18 °C; open squares) and after 84 h of exposure to each temperature (10–42 °C; circles). Data are means $\pm$ SE ($n = 3$).

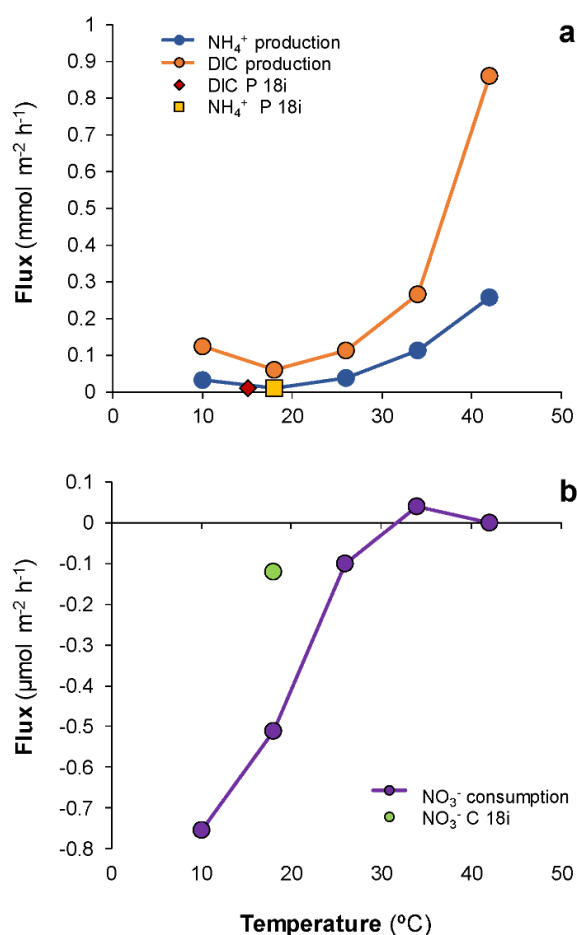


Figure 5. Sediment–water fluxes of (a) DIC and NH₄⁺ production and (b) NO₃⁻ consumption as a function of temperature, estimated from the steady-state porewater gradients in the upper 2 cm of sediment. DIC, dissolved inorganic carbon. '18i' denotes the initial condition; all other points correspond to 84 h of exposure. Positive values indicate net production/release from the sediment.



NO_2^- was mainly exported to the water column from the sediment, with positive fluxes measured over the course of the experiment at temperatures below 42 °C (**Fig. 6b**). Temperature increased the net NO_2^- efflux, showing highest positive values at 34 °C after 12h and at 26 °C for the rest of the experiment; however, fluxes were mostly negative at 42 °C. Nitrite fluxes showed a significant interaction between temperature and exposure time ($\chi^2_{12} = 26.34$, $p = 0.010$; LMM). Tukey-adjusted comparisons confirmed that the pattern depended on exposure time: after 12 h, fluxes at 26 and 34 °C differed from those at 10 and 42 °C; after 36 and 60 h, the 26 °C treatment differed from all other temperatures; and after 84 h, 42 °C differed from all lower temperatures. NO_3^- fluxes did not show a clear pattern with temperature or time. NO_3^- flux differences over time were smaller at the lowest, mostly showing efflux, and highest temperatures, mostly showing influx. In contrast, they varied from +100 to -110 $\mu\text{mol m}^{-2} \text{h}^{-1}$ with no clear temporal trend at 26 °C (**Fig. 6c**). NO_3^- fluxes showed a significant interaction between temperature and exposure time ($\chi^2_{12} = 58.43$, $p < 0.001$; LMM). No temperature pairwise comparison was significant after 12 h. At 36, 60 and 84 h, the interaction was driven mainly by the 26 °C treatment, which differed from 10, 34 and 42 °C after 36 h, from all other temperatures after 60 h, and from 10, 18 and 42 °C after 84 h (Tukey-adjusted comparisons).

The sensitivity of NH_4^+ and NO_2^- fluxes to temperature was estimated by E_a and Q_{10} . E_a for NH_4^+ increased with time throughout the experiment from 31.0 ± 7.4 to $82.0 \pm 31.4 \text{ kJ mol}^{-1}$, corresponding to a Q_{10} of 1.5 and 3.1, after 12 and 84 h respectively. In contrast, E_a for NO_2^- fluxes decreased considerably after 84h (**Table S2**).

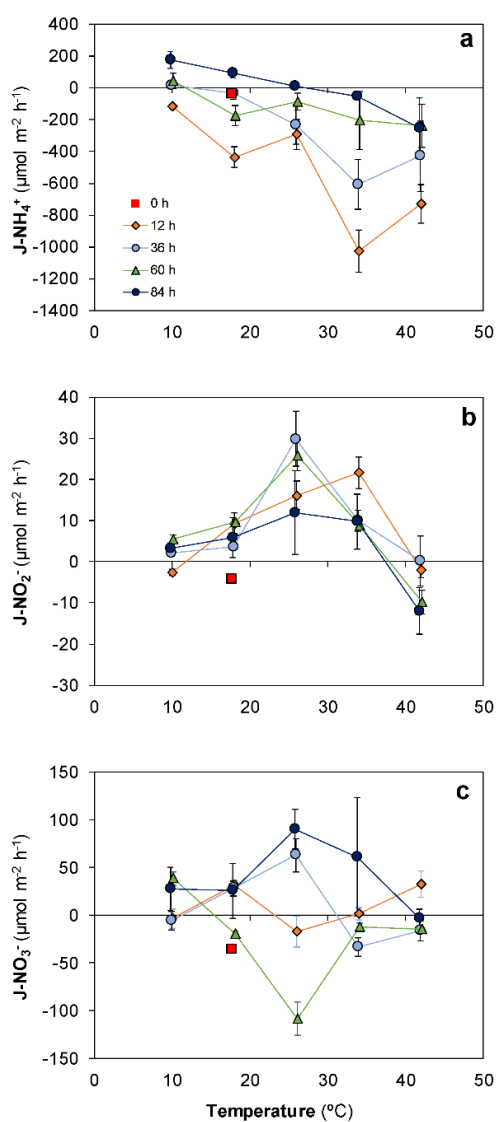


Figure 6. Sediment–water fluxes of dissolved inorganic nitrogen measured in whole-core incubations: NH₄⁺ (a), NO₂⁻ (b) and NO₃⁻ (c), in sediments exposed the different temperatures tested (10–42 °C) over 84 h relative to the in situ temperature of 18 °C recorded at time 0. Positive values indicate efflux from the sediment to the overlying water. Data are means $\pm$ SE (n = 3).



3.4 N₂O and CH₄ fluxes

A release of N₂O and CH₄ from the sediment was recorded throughout the experiment (**Fig. 7**). N₂O fluxes showed a significant interaction between temperature and exposure time ($\chi^2_{12} = 32.92$, $p < 0.001$; LMM). (**Fig. 7a**). At 12 h, N₂O flux increased exponentially with temperature. Net release decreased progressively with time i.e. the peak at 42 °C was observed at 12 h, at 34 °C at 36 h and at 26 °C at 60 h. Although differences were not clear statistically, highest values at the lowest temperatures (10 and 18 °C) were observed after 84 h. The values of E_a and Q_{10} for N₂O fluxes were lower than other solutes, 13.2 kJ mol⁻¹ and 1.2 respectively, and tended to decrease over time (**Table S2**). Tukey-adjusted comparisons showed that, after 12 h, N₂O flux at 42 °C differed from all lower temperatures. Within the 42 °C treatment, the 12 h flux differed from those after 36, 60 and 84 h.

CH₄ fluxes increased exponentially with temperature during the entire experiment (**Fig. 7b**). Temperature had a significant overall effect on CH₄ fluxes ($\chi^2_4 = 43.43$, $p < 0.001$; LMM), whereas the temperature $\times$ exposure-time interaction was not significant ($\chi^2_{12} = 16.53$, $p = 0.168$). Tukey-adjusted comparisons averaged across exposure times showed no differences among 10, 18 and 26 °C; fluxes at 34 °C were higher than at these three temperatures, and fluxes at 42 °C were higher than at all other temperatures (all adjusted $p \leq 0.018$). At the lowest temperatures (10–18 °C), fluxes varied slightly and ranged from 0.39 to 1.30 nmol m⁻² h⁻¹. In contrast, at 34 °C and 42 °C, the release of CH₄ increased one and two orders of magnitude, respectively. E_a and Q_{10} values for CH₄ production by the sediment were among the highest measured in this study, 85.5±10.9 kJ mol⁻¹ and 3.3 ± 0.5, respectively (**Table S2**).

3.5 Organic carbon and total nitrogen content

Initial concentration of organic carbon (C_{org}) and total nitrogen content (N_{total}) in the upper 5 cm were 20.8 ± 3.2 and 1.4 ± 0.3 mg g dw⁻¹, respectively, resulting in a C:N (mol:mol) of 18.5 ± 5.4 (**Fig. S1**). After 84h, we observed no clear changes in the vertical profiles of C_{org} at the higher temperature range (26–42 °C), whereas we observed a decrease in the upper sediment layer (up to 4 cm) at 10 and 18 °C. The final N_{total} concentration in the upper 0–3 cm sediment layer was lower than the initial one after 84 h of temperature exposure in all assayed temperatures. The



different response of C_{org} and N_{total} contents to temperature resulted in an increase in C:N ratio in the upper 2 cm at 26–42 °C with respect to the initial conditions at 18 °C.

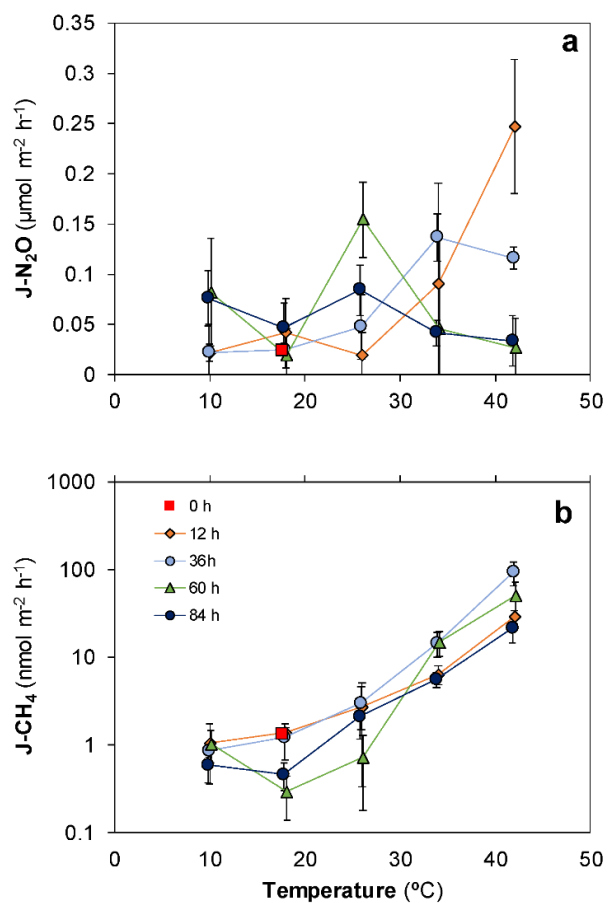


Figure 7. Sediment–water fluxes of the greenhouse gases (a) nitrous oxide (N_2O) and (b) methane (CH_4) measured in whole-core incubations of sediments exposed to the different temperatures tested (10–42 $^{\circ}\text{C}$) over 84 h relative to the in situ temperature of 18 $^{\circ}\text{C}$ recorded at time 0. CH_4 fluxes are shown on a $\log_{10}$ scale. Data are means $\pm$ SE ($n = 3$).



3.6 Quantitative PCR

The gene abundance of the different enzymes involved in the nitrogen cycle showed a general pattern of higher content at the middle temperatures of 18 and 26 °C (**Fig 8**). The abundance of the different genes varied several orders of magnitude with *nirS* and *nrfA* showing the highest abundances and Arch *amoA* and *nxrB* the lowest. Differences at the end of the experiment between treatments did not show any clear differences ($p>0.05$) with the exception of *nxrB*, where 10 and 42 °C were different from the initial and 18 °C. Normalizing the abundances to total 16S rDNA copy numbers resulted in relatively similar patterns with highest numbers at 18 to 26 °C with the exception of Arch *amoA* and *nosZ* that showed the highest ratio at 42 °C (**Fig. S3**). For Bac *amoA*, CoAmox, *nxrB*, and *nrfA*, the initial ratio was clearly different than the rest.

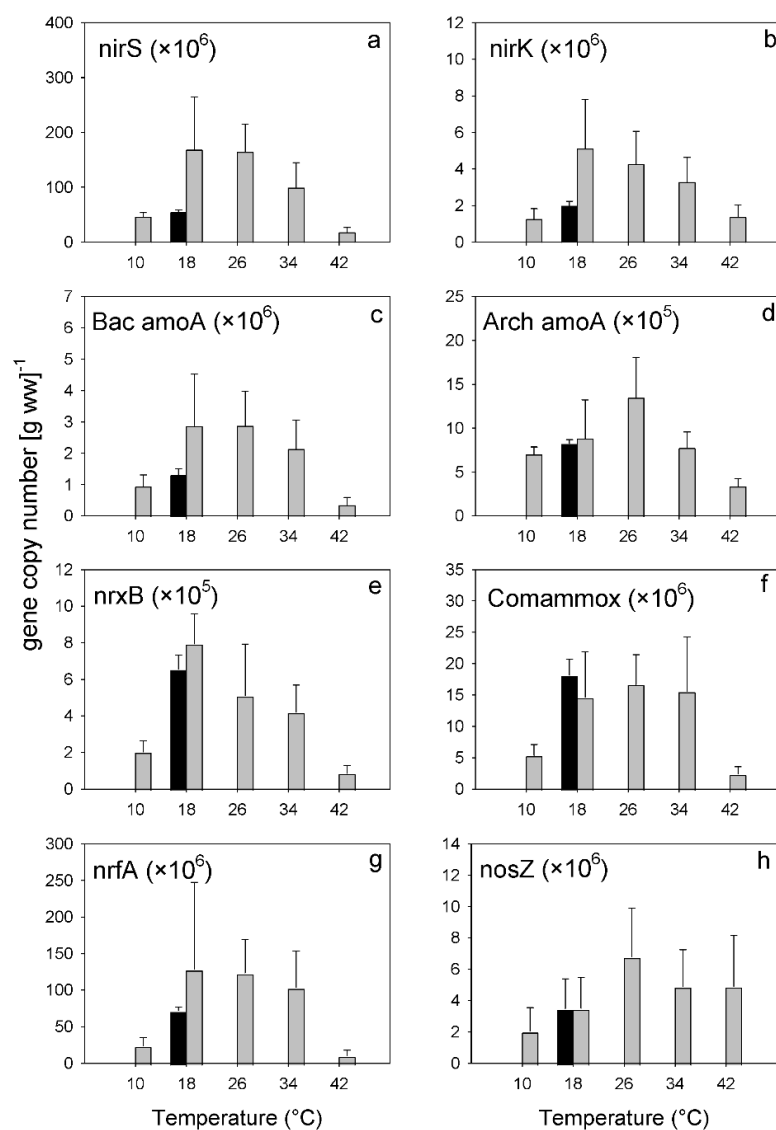


Figure 8. Abundance of nitrogen-cycling functional genes in the 0–1 cm sediment layer across the five temperatures (10, 18, 26, 34 and 42 °C): (a) nirS and (b) nirK (nitrite reductases; denitrification), (c) bacterial and (d) archaeal amoA (ammonia monooxygenase; ammonia oxidation), (e) nrxB (nitrite oxidoreductase; nitrite oxidation), (f) comammox amoA (complete



ammonia oxidisers), (g) *nrfA* (marker of dissimilatory nitrate reduction to ammonium, DNRA) and (h) *nosZ* (nitrous oxide reductase). Within each panel, left bars show absolute gene copy numbers and right bars show copies normalised to the 16S rRNA gene. Black bars, initial conditions (0 h); grey bars, final conditions (84 h). Data are means $\pm$ SE ($n = 3$).



3.7 Taxonomic responses of Archaea and Bacteria to heat treatments

The archaeal dataset contained 846 ASVs represented by 30,288 sequence reads, with Archaea accounting for, on average, less than 0.6% of the total reads. The bacterial dataset contained 7,983 ASVs represented by 5,796,536 reads. The most abundant bacterial phyla, defined as those with a mean relative abundance greater than 3%, were Pseudomonadota, accounting for an average of 47.9% of reads per sample, followed by Thermodesulfobacteriota (10.3%), Bacteroidota (9.3%), Chloroflexota (4.8%), Planctomycetota (3.9%), Gemmatimonadota (3.9%), Verrucomicrobiota (3.6%) and Myxococcota (3.5%) (**Fig. 9a**).

There were few differences in the relative abundance of the dominant phyla among the Initial, 10, 18, 26 and 34 °C treatments (Kruskal–Wallis tests, $p > 0.05$). However, comparison of the Initial and 42 °C treatments identified significant increases in Chloroflexota, particularly Anaerolineales, and Bacillota, particularly Peptostreptococcales–Tissierellales (Kruskal–Wallis tests, $p < 0.05$; Dunn’s post hoc tests, $p < 0.05$). Myxococcota and Verrucomicrobiota decreased significantly at 42 °C (Kruskal–Wallis tests, $p < 0.05$; Dunn’s post hoc tests, $p < 0.05$).

The low abundance of Archaea in some samples limited more detailed analysis. Nevertheless, a clear increase in Methanosarcinales was observed between the Initial condition and the 42 °C treatment (**Fig. 9b**). SILVA 138.2 places Methanosarcinales within the class Methanosarcinia. Within Pseudomonadota (**Fig. S4**), the Alphaproteobacteria order Rhodospirillales increased approximately 18-fold, from a mean relative abundance of 0.1% in the Initial samples to 1.8% in the 42 °C treatment (Dunn’s test, $p < 0.05$). Caulobacteriales increased approximately tenfold, from 0.1% in the Initial samples to 1.1% at 42 °C (Dunn’s test, $p < 0.05$). Within Gammaproteobacteria, Pseudomonadales increased from a mean relative abundance of 10% in the Initial samples to 13% at 42 °C (Dunn’s test, $p < 0.05$). Steroidobacteriales increased in the 18 and 26 °C treatments but subsequently decreased at 42 °C (Kruskal–Wallis test, $p < 0.05$; Dunn’s post hoc test, $p < 0.01$).

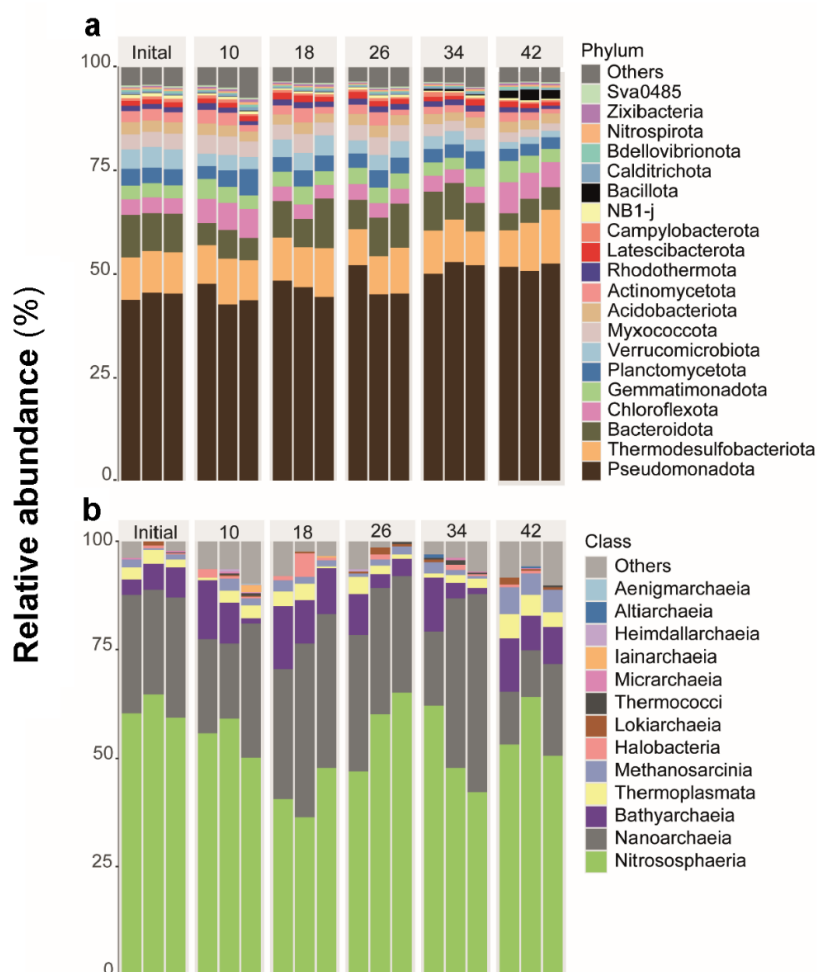


Figure 9. Relative abundance of microbial taxa at the sediment surface across temperature treatments (initial, 10, 18, 26, 34 and 42 °C), under initial conditions and after 84 h. Stacked bars show mean proportions of (a) bacterial phyla and (b) archaeal classes. Only taxa accounting for $\geq 1\%$ of the community in at least one sample are shown individually; remaining groups are pooled as 'Other'.



3 8. Richness, diversity and community responses of Bacteria to heat treatments

ASV richness varied considerably among replicates and showed an overall decline with increasing temperature, with approximately a twofold difference between the highest and lowest treatment means; however, differences among treatments were not statistically significant (**Fig. 10a**). Shannon diversity also declined with increasing temperature. An overall difference among treatments was detected using a Kruskal–Wallis test ($p < 0.01$), with Dunn’s post hoc comparisons indicating significantly lower diversity at 34 and 42 °C than under the Initial condition ($p < 0.05$; **Fig. 10b**). Community composition based on Bray–Curtis dissimilarities differed significantly only between the Initial condition and the 42 °C treatment in pairwise PERMANOVA tests ($p < 0.05$; **Fig. 11**).

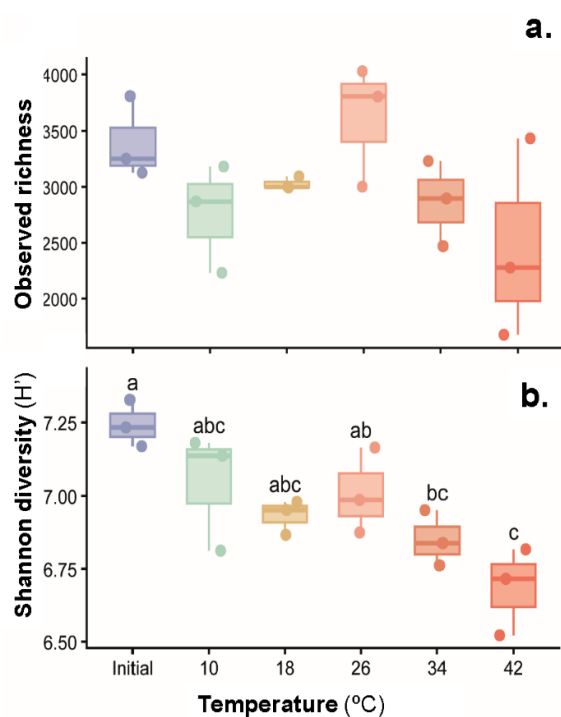


Figure 10. Microbial α -diversity at the sediment surface across temperature treatments (initial, 10, 18, 26, 34 and 42 $^{\circ}\text{C}$), under initial conditions and after 84 h. Points represent individual replicates ($n=3$ per treatment), boxes show the interquartile range and median, and whiskers indicate the data range. Different letters in panel (b) indicate significant differences among treatments based on Dunn's post hoc test following a Kruskal–Wallis test ($P<0.05$).

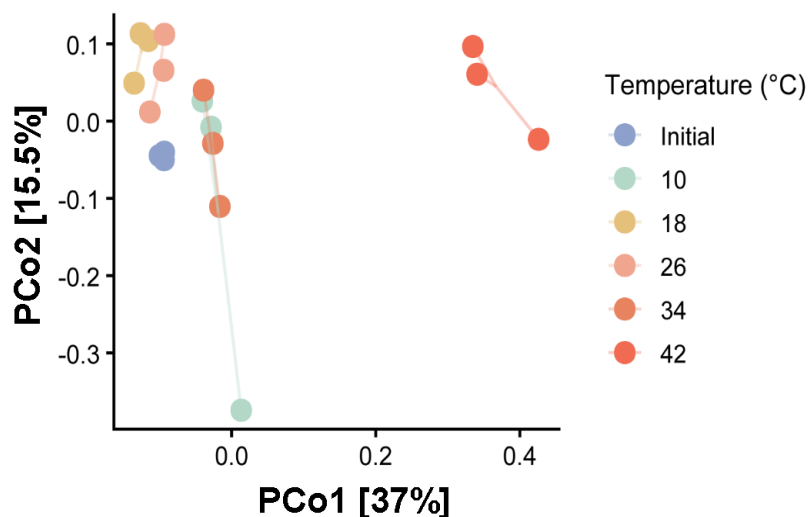


Figure 11. Principal coordinates analysis (PCoA) of microbial community composition at the sediment surface across temperature treatments. Each point is a replicate sample (n=3 per treatment) coloured by temperature — the initial community and the communities after 84 h of exposure at 10, 18, 26, 34 and 42 °C — and thin lines connect replicates to their group centroid. The ordination is based on Bray–Curtis dissimilarities among amplicon sequence variants (ASVs); axes PCo1 and PCo2 explain 37 % and 15.5 % of the total community variation, respectively.



3. 9. Reconstructed functional analysis using PiCrust2

ASV abundance profiles and representative sequences were used to predict the functional potential of the microbial communities. Principal component analysis of predicted MetaCyc pathway abundances showed clear separation of the Initial, 34 °C and 42 °C treatments, indicating shifts in predicted community function at the highest temperatures. Direct comparison of the Initial and 42 °C treatments identified significant differences in several pathways (**Fig. S5**). The predicted denitrification pathway was relatively enriched at 42 °C, together with pathways associated with formaldehyde metabolism, branched-chain amino-acid degradation, β -alanine biosynthesis, protocatechuate and gallate degradation, and carotenoid biosynthesis. In contrast, pathways associated with methylphosphonate degradation, 2,3-butanediol biosynthesis, isopropanol biosynthesis and degradation of chitin derivatives were relatively enriched under Initial conditions. No clear differences were detected in pathways associated with methanogenesis or methane oxidation. Principal component analysis of predicted MetaCyc pathway profiles showed clear differences among temperature treatments, with the strongest separation observed between the initial and 42 °C (Fig S6) samples.

4 Discussion

Previous studies have shown that warming increases respiration and oxygen consumption in marine sediments, although responses vary with sediment type, temperature range and exposure duration (Thamdrup et al., 1998; Wieland & Kühl, 2000; Hancke & Glud, 2004a; Weston & Joye, 2005). More recent experiments have examined heatwave effects on benthic metabolism, nutrient cycling or greenhouse-gas exchange, but these components have rarely been assessed together (Simone et al., 2024; Göbeler et al., 2025; Douglas et al., 2025). By combining microsensor measurements, whole-core fluxes, porewater profiles, greenhouse-gas exchange, functional-gene quantification and microbial community analyses, the present study provides evidence of a largely resistant sediment microbial community. Although warming progressively increased sediment metabolism, microbial composition and several biogeochemical functions remained comparatively stable across most of the temperature range, with pronounced restructuring occurring mainly at 42 °C. This indicates resistance to moderate warming but a threshold response under extreme heat exposure.



4. 1. Effect of temperature increase on total sediment metabolism

Sediment metabolism was strongly affected by temperature enhancing heterotrophic microbial metabolism and organic matter degradation rates (Hancke and Glud, 2004). Oxygen consumption rates (DOU, TOU areal and DOU volumetric rates) increased linearly with temperature, as did sediment porewater DIC and NH_4^+ . The increase in total metabolism observed here is consistent with earlier studies on temperature effects on benthic communities (Hancke and Glud, 2004; Wieland and Kühl, 2000). Despite the variability in exposure times in the different experiments and across the wide range of temperatures all resulted in a sustained rise in sediment O_2 demand. However, the effect varies several folds between different temperatures.

The impact of heat waves on sediment oxygen consumption is complex, as temperature influences several interdependent physicochemical and biological processes (Hancke and Glud, 2004; Jørgensen and Revsbech, 1985; Wieland and Kühl, 2000). Higher temperatures reduce O_2 solubility, being the concentration in equilibrium with the atmosphere is 0.58 times lower at 42° with respect to 10°C . In addition, an increase in T would reduce the apparent diffusive boundary layer (DBL) thickness. The apparent DBL, estimated from eq. 1 in Jørgensen and Revsbech (1985), at 42° was 0.69 times the value at 10°C . Finally, higher T increases the molecular diffusion coefficient; D_0 is 2.27 times higher at 42° vs. 10°C . While the reduced DBL thickness and increased diffusion could elevate DOU, the lower oxygen solubility may offset this effect. Accurately assessing these temperature-dependent changes is challenging due to the simultaneous interplay of these variables (Jørgensen and Revsbech, 1985; Millero and Poisson, 1981; Wieland and Kühl, 2000). Our calculations, following Rasmussen and Jørgensen (1992) (Eq 2 and 17, therein), to obtain the concentration of O_2 at the sediment surface and the oxygen flux, respectively, suggest that sediment oxygen demand (DOU) increases with temperature by 20% (1.2 times) from 10°C to 42°C , driven by physicochemical only temperature-dependent effects. This is a minor effect compared to the 178% increase observed on average (2.78-fold) in DOU across the same temperature range during the experiment. In addition, factors like wind and tidal-induced turbulence likely play a more important role than the physicochemical changes in O_2 solubility and diffusivity in the control of oxygen fluxes *in situ*.

Although both DOU and TOU increased with temperature, the effect was clearer for DOU, showing a clear progressive increase in DOU with temperature and time in the first 60 h, indicating greater temperature sensitivity, likely due to microbial metabolic adjustments or changes in community composition. Following the increase in rates, a decrease was clearly



observed at the two highest temperatures that could indicate inhibition of microbial respiration from prolonged heat exposure. Previous studies have shown that warming alters sediment metabolism and microbial community structure, although responses vary with temperature, exposure duration and sediment characteristics (Hicks et al., 2018; Seidel et al., 2023a, b). By combining microsensor measurements, whole-core fluxes, porewater profiles, greenhouse-gas exchange, functional-gene quantification and microbial community analysis, the present study provides evidence of a metabolically responsive but compositionally resistant sediment community. Oxygen consumption and nutrient cycling responded across the temperature gradient, whereas microbial diversity and community composition remained comparatively stable from the initial conditions to 34 °C. Pronounced restructuring occurred primarily at 42 °C, with reduced diversity, a decline in Pseudomonadota and enrichment of Chloroflexi, particularly Anaerolineales, and Firmicutes, particularly Peptococcales. This indicates resistance to moderate warming but a threshold response when temperatures exceeded the range normally experienced by the community. The decrease in oxygen consumption rates was then most likely due to the depletion of labile organic substrates (Arnosti et al., 1998; Glud, 2008; Hansen and Kristensen, 1998; Papaspyrou et al., 2004), as the community seemed to adjust to the temperature changes. TOU rates, influenced by both diffusive and advective fluxes, showed a less clear temperature dependence, likely due to differences in the abundance and type of micro and meiofauna affecting the overall temperature changes. Consequently, TOU did not show a clear decrease at the highest temperatures, but it was maximum, remaining highest at the highest temperatures (34–42 °C). The contribution of DOU to TOU increased from about 30% at 18 °C to 60–80% across the temperature range, suggesting a potential negative effects to the infauna under higher temperature or at least a higher contribution of microorganisms to the O₂ fluxes at higher temperature. Thus, despite the temperature-dependent shift observed here, the overall magnitude of the DOU contribution remained within the broad range previously reported for intertidal and subtidal sediments (Musat et al., 2006; Rabouille et al., 2003).

The sensitivity of DOU to temperature, measured by Ea and Q₁₀, increased over time. However, an increase in temperature sensitivity is the opposite of classical thermal acclimation, and more likely reflects changes from an early flux under physical O₂-transport control with the enzyme-limited biological signal strengthening over time. After 12 to 84 hours of exposure, mean Ea (areal) for DOU increased from 10.5 to 58.2 kJ mol⁻¹ (Q₁₀ from 1.15 to 2.22), whereas mean Ea (volumetric) was 2.7 to 1.7 times higher, respectively. These findings, which align with previous research using O₂ in short-term incubation experiments with different tidal and subtidal sediment



communities from different geographic locations, including experiments with sediment slurries, suggest that exposure duration influences the response of sediment oxygen demand to warming. In contrast to DOU, E_a for TOU rates decreased from 34.7 to 12.3 kJ mol⁻¹ over time (Q_{10} from 1.61 to 1.18). These values are lower than those reported in short-term studies at higher latitudes for whole core incubations but at lower temperature ranges (Q_{10} =2.4 - 3.3, Hancke & Glud (2004)). This suggests that the effect of temperature increase seems to be higher at lower temperatures. The decline in E_a for TOU likely reflects the harmful effects on infauna due to prolonged exposure to increased temperatures and the reduced oxygen availability. This suggests that the duration and intensity of temperatures increase, such as heat waves, can significantly affect both sediment biochemistry and trophic webs.

4 2. Nitrogen cycling processes

The effect of temperature on the nitrogen cycle varied both in time and with temperature. NH_4^+ fluxes revealed a linear change with temperature whose intensity, however, changed over time. Initially, after 12 hours, the sediment acted as a net sink for NH_4^+ , with uptake rates being 7.5 times higher at 34 - 42 °C with respect to 10 °C. Since the experiment was conducted in the dark to exclude the effect of assimilation by benthic microalgae, this demand likely stemmed from a stimulation of the nitrification, anammox and bacterial assimilation near the sediment-water interface (Dalsgaard and Thamdrup, 2002; Rosales Villa et al., 2019; Schutte et al., 2019). Deeper anaerobic remineralisation accumulated NH_4^+ below the shrinking oxic zone acting as another source contributing to the bacterial demand. In fact, production rates of NH_4^+ calculated from the steady profiles at the end of the experiment increased exponentially with temperature. The $\text{DIC}:\text{NH}_4^+$ production ratio of 3.2 was well below both the Redfield remineralisation C:N of 6.6 and the bulk sediment C:N of 18.5 (section 3.5), indicating preferential mineralisation of labile, nitrogen-rich organic matter rather than the refractory bulk pool; the higher integrated porewater $\text{DIC}:\text{DIN}$ (8.3–25; Fig. S2c) is reconcilable as a flux-versus-standing-stock difference (Anderson and Sarmiento, 1994; Redfield, 1958). Regardless, these production rates were similar to values previously reported for Cadiz Bay sediments (Burgos et al., 2018a) and other temperate regions (Berg et al., 1998; Hansen and Kristensen, 1998; Leote and Epping, 2015; Papaspyrou et al., 2007). Although, the sediment was a net source of NH_4^+ at lower temperatures, at 34 °C and 42 °C, the sediment continued to uptake NH_4^+ from the water column over time, albeit at lower rates, suggesting a stimulation of nitrification rates.



Although NO_3^- profiles indicated a consumption of this solute in the sediment, increasing with temperature, suggesting temperature-stimulated denitrification (Canion et al., 2014), they did not have the sufficient resolution to discern the fine depth changes occurring at the few upper mm. In fact, NO_2^- and NO_3^- fluxes were mostly positive, supporting an increase in nitrification rates. NO_2^- rates exhibited a more consistent pattern with NO_2^- efflux increasing with temperature up to 34 °C, but an influx at 42 °C, whereas those of NO_3^- varied unpredictably with fluxes alternating between consumption and release within a day, most notable at 26 °C. The exception was the fluxes at 42 °C that like NO_2^- were negative although very close to undetectable levels.

Nitrifying microorganisms exhibit broad thermal tolerance, with optimal activity typically between 25 and 35 °C in temperate coastal sediments (Herbert, 1999). Yet, whole-core measurements often record lower nitrification rates in summer than in winter, an effect attributed to the thermal reduction of the oxic layer, and thus O_2 availability-alongside intensified competition for NH_4^+ by fast-growing heterotrophs (Hansen et al., 1981). In our study, sediment O_2 demand increased markedly with temperature, causing oxygen penetration depth to shrink from roughly 4 mm to 1.3 mm. Such narrowing of the oxic zone shifts the balance toward anaerobic processes and can uncouple nitrification–denitrification coupling, ultimately dampening the net stimulation of nitrification at the highest temperatures (Thamdrup and Fleischer, 1998). However, despite pronounced functional shifts, we observed no significant change in the absolute abundance of key nitrification genes (bacterial and archaeal *amoA*, *nxrB*), except for a relative enrichment of archaeal *amoA* at 42 °C. This decoupling between gene abundance and process rates likely reflects the inherently slow growth and high activation energies of nitrifiers, whose biomass responds more slowly to temperature changes than enzyme-mediated reaction rates (Reay et al., 1999). Concurrently, the net fluxes of NO_2^- and NO_3^- revealed non-linear responses: effluxes rose with warming up to 34 °C but shifted toward a slight uptake at 42 °C. Such behavior suggests that dissimilatory nitrate reduction processes (denitrification and DNRA) increasingly outpace downstream NO_2^- oxidation at extreme temperatures. Indeed, DNRA is known to intensify under warming and low- O_2 conditions, competing effectively with denitrification (Dawson and Murphy, 1972; Nizzoli et al., 2010). Furthermore, temperature-mediated enhancements of denitrification and anammox rates have been reported in saline sediments, with differing thermal optima among pathways (Brin et al., 2017). By the end of the experiment (84 h), the cumulative effect of heat stress drove a shift in NO_2^- dynamics that underscores the dual importance of heat-wave intensity and duration in situ (Tan et al., 2020). PICRUST2 predictions were consistent with a shift in community functional potential at 42 °C,



including an increased predicted relative abundance of pathways associated with denitrification, acetate production (PWY-7254) and ectoine biosynthesis (P101-PWY). The enrichment of ectoine biosynthesis may indicate an increased potential for cellular stress protection, although these predictions do not demonstrate changes in gene abundance, expression or process rates. Incorporating the effects of episodic thermal anomalies into predictive models will therefore be important for forecasting nutrient fluxes and greenhouse-gas emissions from intertidal and coastal wetland sediments under future climate scenarios.

4 3. Production of greenhouse gases

Estuaries can act as either sources or sinks for N_2O depending on conditions and timing (Lamontagne et al., 2003). In the San Pedro River, N_2O fluxes ranged from -0.11 and $0.38 \mu\text{mol m}^{-2} \text{h}^{-1}$ ($-2.64 - 9.12 \mu\text{mol m}^{-2} \text{d}^{-1}$), these net emission rates aligns with previously reported values in the area (Ferrón et al., 2009b). Our results did not show a consistent, sustained effect of temperature, despite that increased temperatures may enhance denitrification by expanding anoxic conditions, potentially leading to greater N_2O production. However, this might trigger contrary effects, for instance, the thinner oxic zone might limit nitrification, reducing NO_x^- availability for N_2O production. PiCrust2 functional predictions suggest a higher relative abundance of *nosZ*, which might be consuming the N_2O produced in previous steps. *nosZ* encodes nitrous oxide reductase, which catalyses the terminal step of denitrification ($\text{N}_2\text{O} \rightarrow \text{N}_2$) and is the only known biological sink for N_2O (Dalsgaard and Thamdrup, 2002; Sanford et al., 2012; Zumft, 1997); its predicted enrichment at 42°C would therefore favour net N_2O consumption rather than accumulation. In parallel, both DNRA and anammox route reactive nitrogen away from N_2O — DNRA returns NO_3^- to NH_4^+ , and anammox combines NO_2^- with NH_4^+ directly into N_2 with no N_2O intermediate (Thamdrup and Dalsgaard, 2002). However, because anammox in temperate marine sediments peaks near 15°C and declines above $\sim 37^\circ\text{C}$ (Thamdrup and Dalsgaard, 2002), DNRA and complete denitrification are the more likely N_2O -suppressing routes at the highest temperatures. As the net N_2O flux integrates production by incomplete denitrification and nitrifier pathways against these consumption and bypass routes, their opposing temperature responses can offset one another, which would account for the muted net signal despite any pronounced shifts in the underlying nitrogen cycle.

Methane (CH_4) in coastal systems primarily originates from estuarine inputs or benthic production linked to organic matter mineralization. Its production mainly results from methanogenesis, which



involves anaerobic processes such as acetate fermentation or CO₂ reduction (Faber et al., 2012; Ferrón et al., 2009a) although recent discoveries also revealed the relevance of other processes going on in the sediment surface and even at oxic marine environments (Repeta et al., 2016; Xiao et al., 2017, 2018). Methane concentration in the water column is influenced by both aerobic and anaerobic oxidation processes. While methane oxidation can continue under hypoxic-anoxic conditions as oxygen levels decrease, its dynamics are complex due to the interplay between physicochemical processes and biological aerobic and anaerobic processes (Abril et al., 2002; Bastviken et al., 2008; Gudas et al., 2010). Higher temperatures enhance the production of methane carried out by microorganisms and increase the diffusive flux from the deeper parts of the sediment to the water column. However, the methane that diffuses upwards is also consumed by microbial oxidation processes, also favored at higher temperatures (Abril and Iversen, 2002; Herbert, 1999; Stocker, 2014). Both production of CH₄ and emission from the sediment show a remarkable temperature dependence for wide range of ecosystems and microbial communities. Nevertheless, a relative increase of the emissions at higher temperatures it is commonly observed, then suggesting a decoupling between production and consumption processes (Yvon-Durocher et al., 2014). In our experiment, we also observed that methane released from the sediment increased with temperature, reaching a peak of 93.4 nmol m⁻² h⁻¹ (2.24 μmol m⁻² d⁻¹) at 42 °C. The range of fluxes measured are higher than previous data reported in the Río San Pedro, but they are similar to those reported in coastal basins of the Mexico's Western Margin (Abril et al., 2002; Bastviken et al., 2008; Ferrón et al., 2009a; Sansone et al., 2004).

The taxonomic data showed that warming to 42 °C notably shifted the key methanogenic players. Methanosarcinales, archaeal methanogens that tolerate higher temperatures and rely on acetate, became significantly more abundant than at the experiment's outset (Biddle et al., 2012; Dellagnezze et al., 2023; Lever and Teske, 2015). At the same time, Rhodospirillales, a group of purple non-sulfur bacteria that ferment organic substrates into acetate, also increased ($p < 0.01$). This tandem rise suggests a simple but effective partnership: Rhodospirillales produce acetate, which Methanosarcinales then convert to methane. In contrast, the broader PiCrust2 bacterial community showed no clear change in methanogenic or methanotrophic genes, indicating that these two groups are likely to drive the temperature-induced spike in CH₄ production.

The results obtained during the laboratory simulation of a short-term heatwave on intertidal sediments from the Río San Pedro creek revealed a quick and significant impact on the biogeochemical processes and microbial community structure. The increase in oxygen consumption, coupled with relevant changes in nitrogen cycling pathways towards denitrification



at higher temperatures, revealed the vulnerability of shallow coastal areas to short-term alterations of temperatures caused by extreme climate events. The rise in the methane efflux at high temperatures, driven by alterations in the archaeal and bacterial communities involved in methanogenesis, highlights the potential for increased greenhouse gas emissions from coastal sediments under future climate scenarios. Although we have used a simplified system for simulating the impacts of heatwaves, further research is needed to better understand the impacts of the increased intensity and duration of heatwave events in the response of shallow coastal ecosystems to global warming.

5 Conclusions

Taken together, our results show that short-term warming of the magnitude reached during heatwaves markedly alters the benthic metabolism, nitrogen cycling and greenhouse-gas exchange of these shallow tidal-flat sediments. The strong thermal stimulation of sediment respiration, followed by a decrease above $\sim 34^{\circ}\text{C}$ and by a pronounced compression of the oxic zone, is consistent with the temperature sensitivity of benthic metabolism reported for other temperate intertidal systems (Papaspyrou et al., 2007; Thamdrup and Fleischer, 1998) and for this same estuarine system under ambient conditions (Burgos et al., 2018a; Ferrón et al., 2009b). As oxygen penetration shrinks, the balance of nitrogen transformations shifts from nitrification toward denitrification and DNRA, in line with the temperature-stimulated dissimilatory nitrogen loss described elsewhere (Brin et al., 2017; Canion et al., 2014), while the exponential rise in methane efflux is comparable to the high CH_4 emission potential of organic-rich coastal sediments (Abril et al., 2002; Sansone et al., 2004). The broader ecological implication is that intertidal flats, often regarded as buffers that sequester carbon and nutrients, can shift toward net sources of CO_2 , CH_4 and, transiently, N_2O once thermal thresholds typical of heatwaves are exceeded, and that this transition is driven by restructuring of the microbial community rather than a simple change in reaction rates. As such extremes become more frequent and prolonged, the capacity of these systems to act as coastal climate buffers may weaken, generating positive feedbacks on regional carbon and nitrogen budgets that warrant longer-term, field-based assessment.



Data availability

The 16S rRNA gene sequences generated in this study are available at the European Nucleotide Archive under the project accession PRJEB125159. The experimental data supporting the findings of this study are available in the Zenodo database 10.5281/zenodo.22758431.

Author contributions

EGR, SP, and AC conceived and designed the study and provided financial support; VPR and EGR performed the incubations, microsensor and flux measurements; SP, JDT, CJS and FC contributed to the molecular and bioinformatics analyses; VPR carried out the chemical analysis. AS and VPR performed the greenhouse flux analyses. VPR, AC and EGR performed biogeochemical data analysis. VPR, AC, and SP prepared the manuscript with contributions from all co-authors.

Competing interests

The authors declare that they have no competing interests.

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References

Abril, G. and Iversen, N.: Methane dynamics in a shallow non-tidal estuary (Randers Fjord, Denmark), *Mar. Ecol. Prog. Ser.*, 230, 171–181, <https://doi.org/10.3354/meps230171>, 2002.

Abril, G., Nogueira, M., Etcheber, H., Cabeçadas, G., Lemaire, E., and Brogueira, M. J.: Behaviour of Organic Carbon in Nine Contrasting European Estuaries, *Estuarine, Coastal and Shelf Science*, 54, 241–262, <https://doi.org/10.1006/ecss.2001.0844>, 2002.

Anderson, L. A. and Sarmiento, J. L.: Redfield ratios of remineralization determined by nutrient data analysis, *Global Biogeochemical Cycles*, 8, 65–80, <https://doi.org/10.1029/93GB03318>, 1994.

Arnosti, C., Jørgensen, B., Sagemann, J., and Thamdrup, B.: Temperature dependence of microbial degradation of organic matter in marine sediments: polysaccharide hydrolysis, oxygen consumption, and sulfate reduction, *Mar. Ecol. Prog. Ser.*, 165, 59–70, <https://doi.org/10.3354/meps165059>, 1998.

Bastviken, D., Cole, J. J., Pace, M. L., and Van De Bogert, M. C.: Fates of methane from different lake habitats: Connecting whole-lake budgets and CH₄ emissions, *J. Geophys. Res.*, 113, 2007JG000608, <https://doi.org/10.1029/2007JG000608>, 2008.

Berg, P., Risgaard-Petersen, N., and Rysgaard, S.: Interpretation of measured concentration profiles in sediment pore water, *Limnology & Oceanography*, 43, 1500–1510, <https://doi.org/10.4319/lo.1998.43.7.1500>, 1998.

Biddle, J. F., Cardman, Z., Mendlovitz, H., Albert, D. B., Lloyd, K. G., Boetius, A., and Teske, A.: Anaerobic oxidation of methane at different temperature regimes in Guaymas Basin hydrothermal sediments, *The ISME Journal*, 6, 1018–1031, <https://doi.org/10.1038/ismej.2011.164>, 2012.

Bower, C. E. and Holm-Hansen, T.: A Salicylate–Hypochlorite Method for Determining Ammonia in Seawater, *Can. J. Fish. Aquat. Sci.*, 37, 794–798, <https://doi.org/10.1139/f80-106>, 1980.

Brin, L. D., Giblin, A. E., and Rich, J. J.: Effects of experimental warming and carbon addition on nitrate reduction and respiration in coastal sediments, *Biogeochemistry*, 125, 81–95, <https://doi.org/10.1007/s10533-015-0113-4>, 2015.

Brin, L. D., Giblin, A. E., and Rich, J. J.: Similar temperature responses suggest future climate warming will not alter partitioning between denitrification and anammox in temperate marine sediments, *Global Change Biology*, 23, 331–340, <https://doi.org/10.1111/gcb.13370>, 2017.



Burgos, M., Ortega, T., and Forja, J.: Carbon Dioxide and Methane Dynamics in Three Coastal Systems of Cadiz Bay (SW Spain), *Estuaries and Coasts*, 41, 1069–1088, <https://doi.org/10.1007/s12237-017-0330-2>, 2018a.

Burgos, M., Ortega, T., Bohórquez, J., Corzo, A., Rabouille, C., and Forja, J. M.: Seasonal variation of early diagenesis and greenhouse gas production in coastal sediments of Cadiz Bay: Influence of anthropogenic activities, *Estuarine, Coastal and Shelf Science*, 200, 99–115, <https://doi.org/10.1016/j.ecss.2017.10.016>, 2018b.

Burkholz, C., Garcias-Bonet, N., and Duarte, C. M.: Warming enhances carbon dioxide and methane fluxes from Red Sea seagrass (<i>Halophila stipulacea</i>) sediments, *Biogeosciences*, 17, 1717–1730, <https://doi.org/10.5194/bg-17-1717-2020>, 2020.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P.: DADA2: High-resolution sample inference from Illumina amplicon data, *Nat Methods*, 13, 581–583, <https://doi.org/10.1038/nmeth.3869>, 2016.

Canfield, D. E., Jørgensen, B. B., Fossing, H., Glud, R., Gundersen, J., Ramsing, N. B., Thamdrup, B., Hansen, J. W., Nielsen, L. P., and Hall, P. O. J.: Pathways of organic carbon oxidation in three continental margin sediments, *Marine Geology*, 113, 27–40, [https://doi.org/10.1016/0025-3227\(93\)90147-N](https://doi.org/10.1016/0025-3227(93)90147-N), 1993.

Canion, A., Kostka, J. E., Gihring, T. M., Huettel, M., Van Beusekom, J. E. E., Gao, H., Lavik, G., and Kuypers, M. M. M.: Temperature response of denitrification and anammox reveals the adaptation of microbial communities to in situ temperatures in permeable marine sediments that span 50° in latitude, *Biogeosciences*, 11, 309–320, <https://doi.org/10.5194/bg-11-309-2014>, 2014.

Dalsgaard, T. and Thamdrup, B.: Factors Controlling Anaerobic Ammonium Oxidation with Nitrite in Marine Sediments, *Appl Environ Microbiol*, 68, 3802–3808, <https://doi.org/10.1128/AEM.68.8.3802-3808.2002>, 2002.

Dawson, R. N. and Murphy, K. L.: The temperature dependency of biological denitrification, *Water Research*, 6, 71–83, [https://doi.org/10.1016/0043-1354\(72\)90174-1](https://doi.org/10.1016/0043-1354(72)90174-1), 1972.

Deldicq, N., Langlet, D., Delaeter, C., Beaugrand, G., Seuront, L., and Bouchet, V. M. P.: Effects of temperature on the behaviour and metabolism of an intertidal foraminifera and consequences for benthic ecosystem functioning, *Sci Rep*, 11, 4013, <https://doi.org/10.1038/s41598-021-83311-z>, 2021.

Dellagnezze, B. M., Bovio-Winkler, P., Lavergne, C., Menoni, D. A., Mosquillo, F., Cabrol, L., Barret, M., and Etchebehere, C.: Acetoclastic archaea adaptation under increasing temperature in lake sediments and wetland soils from Alaska, *Polar Biol*, 46, 259–275, <https://doi.org/10.1007/s00300-023-03120-0>, 2023.

Douglas, E. J., Lam-Gordillo, O., Hailes, S. F., Lohrer, A. M., and Cummings, V. J.: Simulated heatwave alters intertidal estuary greenhouse gas fluxes, *Nat Commun*, 16, 10507, <https://doi.org/10.1038/s41467-025-65519-z>, 2025.



Douglas, G. M., Maffei, V. J., Zaneveld, J. R., Yurgel, S. N., Brown, J. R., Taylor, C. M., Huttenhower, C., and Langille, M. G. I.: PICRUSt2 for prediction of metagenome functions, *Nat Biotechnol*, 38, 685–688, <https://doi.org/10.1038/s41587-020-0548-6>, 2020.

Easterling, D. R., Meehl, G. A., Parmesan, C., Changnon, S. A., Karl, T. R., and Mearns, L. O.: Climate Extremes: Observations, Modeling, and Impacts, *Science*, 289, 2068–2074, <https://doi.org/10.1126/science.289.5487.2068>, 2000.

Faber, P. A., Kessler, A. J., Bull, J. K., McKelvie, I. D., Meysman, F. J. R., and Cook, P. L. M.: The role of alkalinity generation in controlling the fluxes of CO₂ during exposure and inundation on tidal flats, *Biogeosciences*, 9, 4087–4097, <https://doi.org/10.5194/bg-9-4087-2012>, 2012.

Ferrón, Ortega, and Forja: Benthic fluxes in a tidal salt marsh creek affected by fish farm activities: Río San Pedro (Bay of Cádiz, SW Spain), *Marine Chemistry*, 113, 50–62, <https://doi.org/10.1016/j.marchem.2008.12.002>, 2009a.

Ferrón, Alonso-Pérez, Anfuso, E., Murillo, F., Ortega, T., Castro, C., and Forja, J.: Benthic nutrient recycling on the northeastern shelf of the Gulf of Cádiz (SW Iberian Peninsula), *Mar. Ecol. Prog. Ser.*, 390, 79–95, <https://doi.org/10.3354/meps08199>, 2009b.

Froneman, P. W.: Biogeochemistry of Marine Systems, *African Journal of Aquatic Science*, 30, 91–91, <https://doi.org/10.2989/16085910509503840>, 2005.

García-Robledo, E., Corzo, A., and Papaspyrou, S.: A fast and direct spectrophotometric method for the sequential determination of nitrate and nitrite at low concentrations in small volumes, *Marine Chemistry*, 162, 30–36, <https://doi.org/10.1016/j.marchem.2014.03.002>, 2014.

Glud, R. N.: Oxygen dynamics of marine sediments, *Marine Biology Research*, 4, 243–289, <https://doi.org/10.1080/17451000801888726>, 2008.

Gong, J.-C., Li, B.-H., Hu, J.-W., Ding, X.-J., Liu, C.-Y., and Yang, G.-P.: Tidal effects on carbon dioxide emission dynamics in intertidal wetland sediments, *Environmental Research*, 238, 117110, <https://doi.org/10.1016/j.envres.2023.117110>, 2023.

Guarini, J., Blanchard, G., Gros, P., and Harrison, S.: Modelling the mud surface temperature on intertidal flats to investigate the spatio-temporal dynamics of the benthic microalgal photosynthetic capacity, *Mar. Ecol. Prog. Ser.*, 153, 25–36, <https://doi.org/10.3354/meps153025>, 1997.

Gudas, C., Bastviken, D., Steger, K., Premke, K., Sobek, S., and Tranvik, L. J.: Erratum: Temperature-controlled organic carbon mineralization in lake sediments, *Nature*, 466, 1134–1134, <https://doi.org/10.1038/nature09383>, 2010.

Hancke, K. and Glud, R.: Temperature effects on respiration and photosynthesis in three diatom-dominated benthic communities, *Aquat. Microb. Ecol.*, 37, 265–281, <https://doi.org/10.3354/ame037265>, 2004.



Hansen, Henriksen, K., and Blackburn, T.: Seasonal distribution of nitrifying bacteria and rates of nitrification in coastal marine sediments, *Microb Ecol*, 7, 297–304, <https://doi.org/10.1007/BF02341424>, 1981.

Hansen, K. and Kristensen, E.: The impact of the polychaete *Nereis diversicolor* and enrichment with macroalgal (*Chaetomorpha linum*) detritus on benthic metabolism and nutrient dynamics in organic-poor and organic-rich sediment, *Journal of Experimental Marine Biology and Ecology*, 231, 201–223, [https://doi.org/10.1016/S0022-0981\(98\)00070-7](https://doi.org/10.1016/S0022-0981(98)00070-7), 1998.

Herbert, R. A.: Nitrogen cycling in coastal marine ecosystems, *FEMS Microbiology Reviews*, 1999.

Hicks, N., Liu, X., Gregory, R., Kenny, J., Lucaci, A., Lenzi, L., Paterson, D. M., and Duncan, K. R.: Temperature Driven Changes in Benthic Bacterial Diversity Influences Biogeochemical Cycling in Coastal Sediments, *Front. Microbiol.*, 9, 1730, <https://doi.org/10.3389/fmicb.2018.01730>, 2018.

Hines, J., Hammrich, A., Steiner, D., and Gessner, M. O.: A field facility to simulate climate warming and increased nutrient supply in shallow aquatic ecosystems, *Oecologia*, 173, 1169–1178, <https://doi.org/10.1007/s00442-013-2720-x>, 2013.

Jørgensen, B. B. and Revsbech, N. P.: Diffusive boundary layers and the oxygen uptake of sediments and detritus1, *Limnology & Oceanography*, 30, 111–122, <https://doi.org/10.4319/lo.1985.30.1.0111>, 1985.

Kühl, M., Lassen, C., and Jørgensen, B.: Light penetration and light intensity in sandy marine sediments measured with irradiance and scalar irradiance fiber-optic microprobes, *Mar. Ecol. Prog. Ser.*, 105, 139–148, <https://doi.org/10.3354/meps105139>, 1994.

Lamontagne, M., Duran, R., and Valiela, I.: Nitrous oxide sources and sinks in coastal aquifers and coupled estuarine receiving waters, *The Science of The Total Environment*, 309, 139–149, [https://doi.org/10.1016/S0048-9697\(02\)00614-9](https://doi.org/10.1016/S0048-9697(02)00614-9), 2003.

Leote, C. and Epping, E. H. G.: Sediment–water exchange of nutrients in the Marsdiep basin, western Wadden Sea: Phosphorus limitation induced by a controlled release?, *Continental Shelf Research*, 92, 44–58, <https://doi.org/10.1016/j.csr.2014.11.007>, 2015.

Lever, M. A. and Teske, A. P.: Diversity of Methane-Cycling Archaea in Hydrothermal Sediment Investigated by General and Group-Specific PCR Primers, *Appl Environ Microbiol*, 81, 1426–1441, <https://doi.org/10.1128/AEM.03588-14>, 2015.

Lin, C.-W., Lin, W.-J., Ho, C.-W., Kao, Y.-C., Yong, Z.-J., and Lin, H.-J.: Flushing emissions of methane and carbon dioxide from mangrove soils during tidal cycles, *Science of The Total Environment*, 919, 170768, <https://doi.org/10.1016/j.scitotenv.2024.170768>, 2024.

Meehl, G. A., Washington, W. M., Collins, W. D., Arblaster, J. M., Hu, A., Buja, L. E., Strand, W. G., and Teng, H.: How Much More Global Warming and Sea Level Rise?, *Science*, 307, 1769–1772, <https://doi.org/10.1126/science.1106663>, 2005.



Mehrbach, C., Culberson, C. H., Hawley, J. E., and Pytkowicz, R. M.: Measurement of the Apparent Dissociation Constants of Carbonic Acid in Seawater At Atmospheric Pressure, *Limnology and Oceanography*, 18, 897–907, <https://doi.org/10.4319/lo.1973.18.6.0897>, 1973.

Millero, F. J. and Poisson, A.: International one-atmosphere equation of state of seawater, *Deep Sea Research Part A. Oceanographic Research Papers*, 28, 625–629, [https://doi.org/10.1016/0198-0149\(81\)90122-9](https://doi.org/10.1016/0198-0149(81)90122-9), 1981.

Musat, N., Werner, U., Knittel, K., Kolb, S., Dodenhof, T., Van Beusekom, J. E. E., De Beer, D., Dubilier, N., and Amann, R.: Microbial community structure of sandy intertidal sediments in the North Sea, Sylt-Rømø Basin, Wadden Sea, *Systematic and Applied Microbiology*, 29, 333–348, <https://doi.org/10.1016/j.syapm.2005.12.006>, 2006.

Nizzoli, D., Carraro, E., Nigro, V., and Viaroli, P.: Effect of organic enrichment and thermal regime on denitrification and dissimilatory nitrate reduction to ammonium (DNRA) in hypolimnetic sediments of two lowland lakes, *Water Research*, 44, 2715–2724, <https://doi.org/10.1016/j.watres.2010.02.002>, 2010.

Papaspyrou, S., Thessalou-Legaki, M., and Kristensen, E.: Impact of *Pestarella tyrrhena* on benthic metabolism in sediment microcosms enriched with seagrass and macroalgal detritus, *Mar. Ecol. Prog. Ser.*, 281, 165–179, <https://doi.org/10.3354/meps281165>, 2004.

Papaspyrou, S., Kristensen, E., and Christensen, B.: *Arenicola marina* (Polychaeta) and organic matter mineralisation in sandy marine sediments: In situ and microcosm comparison, *Estuarine, Coastal and Shelf Science*, 72, 213–222, <https://doi.org/10.1016/j.ecss.2006.10.020>, 2007.

Perkins-Kirkpatrick, S. E. and Lewis, S. C.: Increasing trends in regional heatwaves, *Nat Commun*, 11, 3357, <https://doi.org/10.1038/s41467-020-16970-7>, 2020.

Quast, C., Priesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., and Glöckner, F. O.: The SILVA ribosomal RNA gene database project: improved data processing and web-based tools, *Nucleic Acids Research*, 41, D590–D596, <https://doi.org/10.1093/nar/gks1219>, 2012.

Rabouille, C., Denis, L., Dedieu, K., Stora, G., Lansard, B., and Grenz, C.: Oxygen demand in coastal marine sediments: comparing in situ microelectrodes and laboratory core incubations, *Journal of Experimental Marine Biology and Ecology*, 285–286, 49–69, [https://doi.org/10.1016/S0022-0981\(02\)00519-1](https://doi.org/10.1016/S0022-0981(02)00519-1), 2003.

Rasmussen, H. and Jorgensen, B.: Microelectrode studies of seasonal oxygen uptake in a coastal sediment: role of molecular diffusion, *Mar. Ecol. Prog. Ser.*, 81, 289–303, <https://doi.org/10.3354/meps081289>, 1992.

Reay, D. S., Nedwell, D. B., Priddle, J., and Ellis-Evans, J. C.: Temperature Dependence of Inorganic Nitrogen Uptake: Reduced Affinity for Nitrate at Suboptimal Temperatures in Both Algae and Bacteria, *Appl Environ Microbiol*, 65, 2577–2584, <https://doi.org/10.1128/AEM.65.6.2577-2584.1999>, 1999.



Redfield, A. C.: The biological control of chemical factors in the environment, *American Scientist*, 46, 205–221, 1958.

Repeta, D. J., Ferrón, S., Sosa, O. A., Johnson, C. G., Repeta, L. D., Acker, M., DeLong, E. F., and Karl, D. M.: Marine methane paradox explained by bacterial degradation of dissolved organic matter, *Nature Geosci*, 9, 884–887, <https://doi.org/10.1038/ngeo2837>, 2016.

Revsbech, N. P.: An oxygen microsensor with a guard cathode, *Limnology & Oceanography*, 34, 474–478, <https://doi.org/10.4319/lo.1989.34.2.0474>, 1989.

Rios-Yunes, D., Tiano, J. C., Van Oevelen, D., Van Dalen, J., and Soetaert, K.: Annual biogeochemical cycling in intertidal sediments of a restored estuary reveals dependence of N, P, C and Si cycles to temperature and water column properties, *Estuarine, Coastal and Shelf Science*, 282, 108227, <https://doi.org/10.1016/j.ecss.2023.108227>, 2023.

Rosales Villa, A. R., Jickells, T. D., Sivy, D. B., Parker, E. R., and Thamdrup, B.: Benthic nitrogen cycling in the North Sea, *Continental Shelf Research*, 185, 31–36, <https://doi.org/10.1016/j.csr.2018.05.005>, 2019.

Rysgaard, S., Risgaard-Petersen, N., Niels Peter, S., Kim, J., and Lars Peter, N.: Oxygen regulation of nitrification and denitrification in sediments, *Limnology & Oceanography*, 39, 1643–1652, <https://doi.org/10.4319/lo.1994.39.7.1643>, 1994.

Sanford, R. A., Wagner, D. D., Wu, Q., Chee-Sanford, J. C., Thomas, S. H., Cruz-García, C., Rodríguez, G., Massol-Deyá, A., Krishnani, K. K., Ritalahti, K. M., Nissen, S., Konstantinidis, K. T., and Löffler, F. E.: Unexpected nondenitrifier nitrous oxide reductase gene diversity and abundance in soils, *Proc. Natl. Acad. Sci. U.S.A.*, 109, 19709–19714, <https://doi.org/10.1073/pnas.1211238109>, 2012.

Sansone, F. J., Graham, A. W., and Berelson, W. M.: Methane along the western Mexican margin, *Limnology & Oceanography*, 49, 2242–2255, <https://doi.org/10.4319/lo.2004.49.6.2242>, 2004.

Schutte, C. A., Ahmerkamp, S., Wu, C. S., Seidel, M., De Beer, D., Cook, P. L. M., and Joye, S. B.: Biogeochemical Dynamics of Coastal Tidal Flats, in: *Coastal Wetlands*, Elsevier, 407–440, <https://doi.org/10.1016/B978-0-444-63893-9.00012-5>, 2019.

Seidel, L., Broman, E., Nilsson, E., Ståhle, M., Ketzer, M., Pérez-Martínez, C., Turner, S., Hylander, S., Pinhassi, J., Forsman, A., and Dopson, M.: Climate change-related warming reduces thermal sensitivity and modifies metabolic activity of coastal benthic bacterial communities, *The ISME Journal*, 17, 855–869, <https://doi.org/10.1038/s41396-023-01395-z>, 2023a.

Seidel, L., Sachpazidou, V., Ketzer, M., Hylander, S., Forsman, A., and Dopson, M.: Long-term warming modulates diversity, vertical structuring of microbial communities, and sulfate reduction in coastal Baltic Sea sediments, *Front. Microbiol.*, 14, 1099445, <https://doi.org/10.3389/fmicb.2023.1099445>, 2023b.

Sierra, A., Jiménez-López, D., Ortega, T., Ponce, R., Bellanco, M. J., Sánchez-Leal, R., Gómez-Parra, A., and Forja, J.: Spatial and seasonal variability of CH₄ in the eastern Gulf of Cadiz (SW



Iberian Peninsula), *Science of The Total Environment*, 590–591, 695–707, <https://doi.org/10.1016/j.scitotenv.2017.03.030>, 2017.

Smith, C. J., Nedwell, D. B., Dong, L. F., and Osborn, A. M.: Evaluation of quantitative polymerase chain reaction-based approaches for determining gene copy and gene transcript numbers in environmental samples, *Environmental Microbiology*, 8, 804–815, <https://doi.org/10.1111/j.1462-2920.2005.00963.x>, 2006.

Smith, K. E., Burrows, M. T., Hobday, A. J., King, N. G., Moore, P. J., Sen Gupta, A., Thomsen, M. S., Wernberg, T., and Smale, D. A.: Biological Impacts of Marine Heatwaves, *Annu. Rev. Mar. Sci.*, 15, 119–145, <https://doi.org/10.1146/annurev-marine-032122-121437>, 2023.

Soetaert, K. and Petzoldt, T.: marelac: Tools for Aquatic Sciences, <https://doi.org/10.32614/CRAN.package.marelac>, 2008.

Stocker, T. F.: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, *Environ. Res. Lett.*, 9, 13pp, 2014.

Stott, P. A., Stone, D. A., and Allen, M. R.: Human contribution to the European heatwave of 2003, *Nature*, 432, 610–614, <https://doi.org/10.1038/nature03089>, 2004.

Tan, E., Zou, W., Zheng, Z., Yan, X., Du, M., Hsu, T.-C., Tian, L., Middelburg, J. J., Trull, T. W., and Kao, S.: Warming stimulates sediment denitrification at the expense of anaerobic ammonium oxidation, *Nat. Clim. Chang.*, 10, 349–355, <https://doi.org/10.1038/s41558-020-0723-2>, 2020.

Tatti, E., McKew, B. A., Whitby, C., and Smith, C. J.: Simultaneous DNA-RNA Extraction from Coastal Sediments and Quantification of 16S rRNA Genes and Transcripts by Real-time PCR, *JoVE*, 54067, <https://doi.org/10.3791/54067>, 2016.

Thamdrup, B. and Dalsgaard, T.: Production of N₂ through Anaerobic Ammonium Oxidation Coupled to Nitrate Reduction in Marine Sediments, *Appl Environ Microbiol*, 68, 1312–1318, <https://doi.org/10.1128/AEM.68.3.1312-1318.2002>, 2002.

Thamdrup, B. and Fleischer, S.: Temperature dependence of oxygen respiration, nitrogen mineralization, and nitrification in Arctic sediments, *Aquat. Microb. Ecol.*, 15, 191–199, <https://doi.org/10.3354/ame015191>, 1998.

Thamdrup, B., Hansen, J. W., and Jørgensen, B. B.: Temperature dependence of aerobic respiration in a coastal sediment, *FEMS Microbiology Ecology*, 25, 189–200, <https://doi.org/10.1111/j.1574-6941.1998.tb00472.x>, 1998.

Walters, W., Hyde, E. R., Berg-Lyons, D., Ackermann, G., Humphrey, G., Parada, A., Gilbert, J. A., Jansson, J. K., Caporaso, J. G., Fuhrman, J. A., Apprill, A., and Knight, R.: Improved Bacterial 16S rRNA Gene (V4 and V4-5) and Fungal Internal Transcribed Spacer Marker Gene Primers for Microbial Community Surveys, *mSystems*, 1, e00009-15, <https://doi.org/10.1128/mSystems.00009-15>, 2016.



Weston, N. B. and Joye, S. B.: Temperature-driven decoupling of key phases of organic matter degradation in marine sediments, *Proc. Natl. Acad. Sci. U.S.A.*, 102, 17036–17040, <https://doi.org/10.1073/pnas.0508798102>, 2005.

Wieland, A. and Kühl, M.: Irradiance and temperature regulation of oxygenic photosynthesis and O₂ consumption in a hypersaline cyanobacterial mat (Solar Lake, Egypt), *Marine Biology*, 137, 71–85, <https://doi.org/10.1007/s002270000331>, 2000.

Xiao, Beulig, F., Kjeldsen, K. U., Jørgensen, B. B., and Risgaard-Petersen, N.: Concurrent Methane Production and Oxidation in Surface Sediment from Aarhus Bay, Denmark, *Front. Microbiol.*, 8, 1198, <https://doi.org/10.3389/fmicb.2017.01198>, 2017.

Xiao, Beulig, F., Røy, H., Jørgensen, B. B., and Risgaard-Petersen, N.: Methylophilic methanogenesis fuels cryptic methane cycling in marine surface sediment, *Limnology & Oceanography*, 63, 1519–1527, <https://doi.org/10.1002/lno.10788>, 2018.

Yvon-Durocher, G., Allen, A. P., Bastviken, D., Conrad, R., Gudas, C., St-Pierre, A., Thanh-Duc, N., and Del Giorgio, P. A.: Methane fluxes show consistent temperature dependence across microbial to ecosystem scales, *Nature*, 507, 488–491, <https://doi.org/10.1038/nature13164>, 2014.

Zumft, W. G.: Cell biology and molecular basis of denitrification, *Microbiol Mol Biol Rev*, 61, 533–616, <https://doi.org/10.1128/mmlr.61.4.533-616.1997>, 1997.