Final Report

Brilliant Marine Research Idea 2025

This report should be submitted no later than 28 February 2026 via filantropie@vliz.be and consists of the following documents:

- A final report listing the work done and the problems encountered. This report will be made available online. If any of the tasks has not been completely finished, the report should clearly mention this, including a short explanation. max. 5 pages
- An overview of all expenditures including invoices.
- A set of five pictures (low resolution in this document). The five high resolution pictures should be delivered to VLIZ by email to karen.rappe@vliz.be. Pictures should be free from use - to upload on the VLIZ website and to use in VLIZ communications.

Keep in mind that VLIZ should be mentioned in the acknowledgements of publications following the results of this Brilliant Marine Research Idea.

1. General information

Title of the idea	Electric seafloors and the climate: Impact of cable bacteria on alkalinity dynamics in high $CaCO_3$ sediments
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Flemish University or University College	University of Antwerp

2. Brilliant Marine Research Idea – Report about the activities

Abstract

Cable bacteria are filamentous microorganisms that transport electrons over centimeter scale distances via electrogenic sulfide oxidation (e-SOx), spatially separating sulfide oxidation in anoxic sediments from oxygen or nitrate reduction near the sediment surface. This metabolism generates strong pH gradients and can enhance sedimentary alkalinity release, increasing the CO_2 uptake capacity of overlying waters. To date, this process has been investigated mostly in sediments with low calcium carbonate ($CaCO_3$) contents, leaving their role in carbonate-rich environments largely unexplored.

Here, we investigated the impact of cable bacteria on alkalinity dynamics in hypersaline (>40), carbonate-rich (>35 wt% $CaCO_3$) sediments from the Ghar El Melh Lagoon (NE Tunisia) during a 12-week microcosm incubation. Sediment-water fluxes (total alkalinity (TA), Dissolved Inorganic Carbon (DIC), O_2, ^{13}C-DIC and nutrients), porewater cations (Ca^{2+}, Fe^{2+} and Mn^{2+}) microsensor profiling (O_2, pH, H_2S, electric potential) to assess cable bacteria activity, and FISH analyses to quantify cable bacteria presence were combined to evaluate biogeochemical responses.

The presence and metabolic activity of cable bacteria were evident throughout the incubation. Total alkalinity (TA) fluxes increased during the first three weeks, peaking concurrently with maximum cable bacteria abundance and electrogenic activity. This peak resulted from two coupled processes: (1) proton consumption at the sediment-water interface during oxygen reduction, elevating pH and promoting alkalinity release, and (2) subsurface acidification from sulfide oxidation,

driving $CaCO_3$ dissolution and releasing Ca^{2+}, Fe^{2+}, and alkalinity. The generated alkalinity and associated dissolved cations consequently migrated upward along the redox gradient toward the sediment surface.

TA fluxes subsequently declined and became negative as a consolidated surface crust gradually formed on top of the sediment. This crust was composed of $CaCO_3$, iron oxides, and also NaCl, and developed through the accumulation, precipitation, and oxidation of Ca^{2+} and Fe^{2+} near the sediment-water interface, thus restricting sediment-water exchange and trapping alkalinity within the sediment. The presence of NaCl, not previously reported in cable bacteria-induced crusts, reflects the hypersaline conditions and expands the known mineralogical consequences of cable bacteria activity.
Our results demonstrate that in hypersaline, carbonate-rich sediments, cable bacteria promote short-term alkalinity release. However, the long-term export of alkalinity is limited through progressive mineral crust formation. These insights broaden the environmental context in which cable bacteria influence the alkalinity cycling in coastal environments.

Intro

Cable bacteria are long, multicellular, filamentous microorganisms with the extraordinary ability to conduct electricity over centimeter-scale distances in sediments. The shuttling of electrons results from their metabolism, known as electrogenic sulfide oxidation (e-SOx), which links sulfide (H_2S) oxidation in deeper, anoxic sediment layers to oxygen (O_2) or nitrate (NO_3^-) reduction in surface sediments[1,2]. This unique metabolism profoundly influences the cycling of key elements by physically separating redox reactions, creating a charge gradient, and altering sediment pH[3].
Importantly, initial studies suggest that cable bacteria can increase the ocean's capacity to take up CO_2 by modifying the release of alkalinity from sediments[4, 6]. Alkalinity, defined as the excess of proton acceptors (bases) over proton donors (acids)[7], is a key factor regulating CO_2 storage in seawater[8]. Higher alkalinity enhances the ability of seawater to absorb CO_2, directly influencing atmospheric CO_2 levels and, consequently, global climate.
Although their influence on elements such as sulfur and oxygen is well established, the role of cable bacteria in alkalinity generation has only recently gained attention[4, 6]. Two mechanisms drive this alkalinity release[4]: (1) e-SOx induces a pH decrease that promotes dissolution of calcium carbonate ($CaCO_3$) in the sediment, producing alkalinity; and (2) the spatial separation of O_2 reduction (proton consumption) and H_2S oxidation (proton production) leads to a transfer of alkalinity from deeper sediment layers to the sediment-water interface, a process known as the "alkalinity pump." Together, these processes buffer the release of dissolved inorganic carbon (DIC, ΣCO_2) at the sediment–water interface, mitigating the effects of benthic respiration on coastal ocean pH[4].
In Lake Grevelingen (the Netherlands), approximately 75% of alkalinity production in sediments inhabited by cable bacteria was attributed to net $CaCO_3$ dissolution, while the remaining 25% resulted from the alkalinity pump[4]. However, the $CaCO_3$ content of sediments strongly influences the impact of cable bacteria on sedimentary alkalinity release[5]. To date, sediments containing up to 22 wt% $CaCO_3$ have been investigated for this purpose[5], with one exception, Florida Bay, which had 88 % $CaCO_3$[9,10]. Therefore, further studies in sediments with higher $CaCO_3$ concentrations are required to better understand the overall impact of cable bacteria on alkalinity cycling.
Given the urgent need to remove CO_2 from the atmosphere, there is increasing interest in processes that enhance ocean alkalinity. This study therefore investigates the influence of cable bacteria on alkalinity production in the unique environment of the Ghar El Melh Lagoon. Located in northeastern Tunisia, the lagoon is characterized by summer salinities exceeding 40 (compared to 32–35 in the open ocean) and sedimentary $CaCO_3$ contents exceeding 35%[11]. These extreme environmental conditions, high $CaCO_3$ concentrations, elevated pH, and high salinity have not previously been examined in relation to cable bacteria. We hypothesize that under these conditions, cable bacteria may play a significant role in alkalinity production, particularly through enhanced $CaCO_3$ dissolution.
In this study, sediment-water fluxes were measured in microcosms containing incubated sediments from the lagoon. The growth of cable bacteria was monitored, sedimentary alkalinity release was quantified, and the relative contributions of the underlying processes were assessed.

This work substantially expands the range of environments investigated for the biogeochemical impact of cable bacteria, as the conditions in the Ghar El Melh Lagoon differ markedly from those typically examined in cable bacteria research.

References

1. Nielsen et al., (2010) *Electric currents couple spatially separated biogeochemical processes in marine sediment***.** Nature 463, 1071–1074. **2. Pfeffer et al., (2012)** *Filamentous bacteria transport electrons over centimetre distances.* Nature 491, 218–221. **3. Risgaard-Petersen et al., (2012)** *Sulfur, iron-, and calcium cycling associated with natural electric currents running through marine sediment*. Geochim. Cosmochim. Acta 92, 1–13. **4. Rao et al., (2016)** *The impact of electrogenic sulfide oxidation on elemental cycling and solute fluxes in coastal sediment*. Geochim. Cosmochim. Acta 172, 265–286. **5.Burdorf et al., (2024)** *Electrogenic sulfur oxidation by cable bacteria in two seasonally hypoxic coastal system.* Estuarine, Coastal and Shelf Science 297 (2024) 108615. **6. Seitaj et al., (2016)** *Sedimentary oxygen dynamics in a seasonally hypoxic basin*. Limnology and Oceanography 62, 2017, 452–473. **7. Zeebe and Wolf-Gladrow (2001)** *CO2 in seawater : equilibrium, kinetics, isotopes*. Elsevier, Amsterdam. **8. Berner (2004)** *The Phanerozoic Carbon Cycle: CO2 and O2*, Oxford University Press, USA. **9. Yin et al., (2022)** *Cable bacteria activity and impacts in Fe and Mn depleted carbonate sediments*. Marine Chemistry 246. **10. Scott et al., (1997)** *Progress report on sediment analyses at selected faunal monitoring sites in north-central and northeastern Florida Bay*. Open-File Report 97-534. **11. Oueslati et al., (2017)** *Sulfide influence on metal behavior in a polluted southern Mediterranean lagoon*: implications for management.

Materials and Methods

Sampling and microcosm experimental setup

Surface sediment was collected in April 2025 using a sediment grab deployed from a small boat. Sediment was sieved to <1.4 mm to remove coarse particles that could damage microsensors used to detect the geochemical signature of cable bacteria, and subsequently homogenized.

The homogenized sediment was incubated in microcosms (30 × 19 cm chambers) for twelve flux sessions (= twelve weeks) to follow cable bacteria growth and to quantify sediment-water solute fluxes. Four replicate microcosms were monitored over these twelve weekly flux sessions.

Each flux session consisted of two phases

(1) Closed incubation (lid on) to determine fluxes of O_2, dissolved inorganic carbon (DIC), and ^{13}C-DIC (used as a tracer for $CaCO_3$ dissolution).

(2) Open incubation (lid off) to measure fluxes of NO_3^-, NH_4^+, PO_4^{3-}, total alkalinity (TA), and alkalinity-relevant ions (Fe^{2+}, Mn^{2+}, Ca^{2+}).

Data from these incubations helps identify the sources of alkalinity.

Porewater samples were collected at weeks 3, 6, and 12 using Rhizon samplers inserted at 1 cm depth intervals. Samples were taken for TA, Fe^{2+}, Mn^{2+}, Ca^{2+} and PO_4^{3-} analysis.

Cable bacteria activity and abundance

Weekly microsensor profiles of O_2, pH, H_2S, and electric potential (EP) were recorded to detect the characteristic geochemical fingerprint of electrogenic sulfide oxidation induced by cable bacteria (cable bacteria activity).

Sediment 0.5 mL scoops were collected using a 3 mL syringe at weeks 3, 6, and 12 for quantification and identification of cable bacteria biomass using **fluorescence in situ hybridization (FISH).**

Crust mineralogy

At the end of the experiment, scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM–EDX) was used to determine the mineralogical composition of the crust.

Results/Conclusions

Temporal evolution of alkalinity

Total alkalinity (TA) fluxes increased during the first three weeks of incubation and reached a clear maximum in week three. This peak coincided with the highest cable bacteria abundance and activity. After week three, TA fluxes progressively decreased and became negative by week eight, indicating net alkalinity retention in the sediment.

Mechanism: e-SOx and Alkalinity Release

Cable bacteria created a spatial separation between two half-reactions, contributing 2 main sources of alkalinity release:

(1) At the sediment–water interface: oxygen reduction consumed protons, increasing pH. This pH rise directly enhanced alkalinity release to the overlying water.

(2) A few centimeters below: sulfide oxidation produced protons, causing strong acidification.

The acidification at depth dissolved $CaCO_3$, generating alkalinity, and releasing Ca^{2+} and Fe^{2+} under these acidic conditions. The dissolved Ca^{2+}, Fe^{2+}, and alkalinity migrated upward. When cable bacteria activity was at its maximum, dissolution and upward transport were strongest, explaining the peak in alkalinity release observed in week three.

Crust formation and decline in alkalinity flux

After the activity peak, continued upward migration of Ca^{2+} and Fe^{2+} led to precipitation near the sediment surface, where pH was elevated. This formed a consolidated crust composed of: $CaCO_3$, Iron oxides and NaCl

As this crust developed, it reduced exchange between sediment and overlying water. Alkalinity became trapped within the sediment instead of being released. This explains the progressive decrease in TA fluxes and the shift to negative values.

Novel finding: salt (NaCl) in the crust

An important new result is the presence of NaCl in the crust. Previous studies of cable bacteria–induced crusts reported $CaCO_3$ and iron oxides but not salt.

The incorporation of NaCl is likely related to the high sediment salinity (41). Such elevated salinity conditions have not been previously studied in the context of cable bacteria–driven crust formation, making this a novel mineralogical observation.

Conclusions

Cable bacteria strongly control alkalinity dynamics in the carbonate-rich, high-salinity sediments of this south mediterranean lagoon.

Their activity initially enhances alkalinity release by inducing subsurface acidification and $CaCO_3$ dissolution a few centimeters deeper in the sediment. However, continued upward transport of dissolved species leads to mineral precipitation and crust formation at the sediment surface. This crust ultimately traps alkalinity and reverses the flux.

Thus, in hypersaline carbonate-rich sediments, cable bacteria promote short-term alkalinity export but may limit long-term alkalinity release through crust formation. The identification of NaCl in the crust extends current understanding of cable bacteria–driven geochemical processes to hypersaline environments.

3. Overview of the expenditures

Describe in detail how the requested fund was spent within the implementation period (1 March 2025 and 28 February 2026). Be as specific as possible.

Experiment equipment and shipping

- Rhizon samplers (for pore water extraction in the microcosm core setup): 3 bags (10 samplers per bag)	277 € + 58,17 € VAT
- Rhizon connectors (to connect Rhizons to the cores)	130 €
- DHL transport Rhizon connectors	39.16 €
- DHL shippement between Tunisia and Antwerp	453.98 €
Total	**958,31€**

Lab analysis

- ICP-OES (Ca^{2+}, Fe^{2+} and Mn^{2+}): 348 samples (Utrecht University)	**3375,6 €**
- pH sensor used in the protocol to measure alkalinity	**265,66 €**
Total	**3641,26**

Extra/additional

- Safety step	**401,08 €**
Total	**5000,65€**

4. Pictures

A set of five pictures (low resolution in this document). The five high resolution pictures should be delivered to VLIZ by email to karen.rappe@vliz.be.

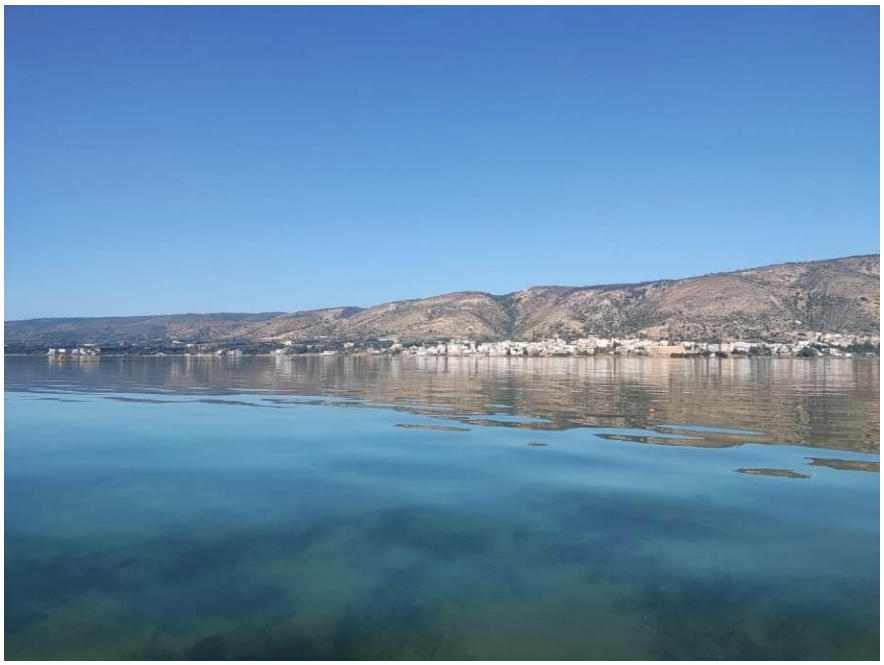

Figure 1: Study site Ghar El Melh Lagoon

Figure 2: Open incubation sampling

Figure 3: Incubation core setup with Rhizons for porewater extraction

Figure 4: Micro-profiling to detect cable bacteria geochemical imprint

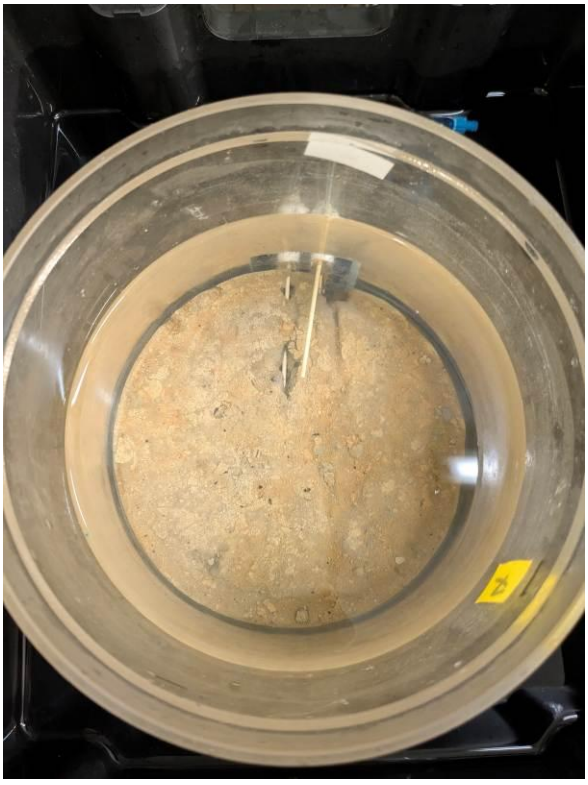

Figure 5: Calcium carbonate crust formation at the sediment-water Interface