

# Effect of biota on soil mineral dissolution in salt marsh sediment

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### **Chapter I: General Introduction**

#### 1. Blue Carbon Ecosystems

Blue carbon ecosystems (BCEs)—including mangrove forests, salt marshes, and seagrass meadows—cover an estimated 36 to 185 million ha globally (Macreadie et al., 2021). Despite their relatively small global extent, BCEs provide numerous co-benefits, such as supporting economic development, enhancing food security, promoting social well-being, and protecting coastal areas (Nellemann, 2009). These ecosystems are increasingly recognized as a nature-based solution to climate change due to their high efficiency as carbon sinks. BCEs capture atmospheric CO<sub>2</sub> and store it in their sediments as both living and non-living biomass over millennial timescales (Duarte et al., 2005), with the potential to store up to 32,650 Tg C (Macreadie et al., 2021) and considerably higher than the terrestrial ecosystem (McLeod et al., 2011).

#### Salt Marshes

Salt marshes are coastal habitats found in the intertidal zones of low-temperate coasts, where they are regularly submerged and exposed to tidal movements, resulting in periodic saltwater inundation (McOwen et al., 2017). Salt marshes rank highest in net primary production among BCEs (Bauer et al., 2013; Duarte et al., 2013) with their trapping efficiency and low rate of organic matter degradation (Spivak et al., 2019) enabling substantial organic carbon burial, estimated at up to 87.2 Tg C yr<sup>-1</sup> (Duarte et al., 2005). As organic carbon in the sediment undergoes various respiration processes, salt marshes become sources of dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC), which are exported to adjacent coastal waters via tidal pore-water exchange (Odum, 1968; Wang & Cai, 2004). DIC, which is a sum of all carbonate species ( $HCO_3^-$ ,  $CO_3^{2-}$ ,  $H_2CO_3^*$  ( $CO_2(aq)$ )), plays a crucial role in regulating coastal ocean carbonate chemistry upon export (Wang et al., 2016). Additionally, salt marshes have been reported to export total alkalinity (TA) (Cai & Wang, 1998; Raymond et al., 2000; Wang & Cai, 2004; Weiss, 2013; Yau et al., 2022), which is equally as important to DIC export

and significantly contributes to the global ocean alkalinity budget (Thomas et al., 2009). The importance of TA is to attenuate ocean acidification caused by rising atmospheric  $CO_2$  concentrations and can enhance the ocean's capacity for binding  $CO_2$ .

#### 2. Ocean Alkalinity

The oceans are the largest global carbon reservoir storing up to  $3.8 \times 10^7$  Tg C and absorbing approximately one-third of anthropogenic CO<sub>2</sub> emissions (McLeod et al., 2011). As a result of the increasing anthropogenic CO<sub>2</sub> emission, the ocean's uptake of CO<sub>2</sub> potentially leads to acidification. Seawater relies on its natural buffering system, known as alkalinity, to counteract the resulting decrease in pH (Zeebe & Wolf-Gladrow, 2001). Alkalinity refers to the capacity of a water body to resist changes in pH, and it is closely tied to the chemical equilibrium between weak acids and their conjugate bases in solution (Raymond & Hamilton, 2018).

#### 2.1. Total alkalinity definition and its ecological functions

In 1981, Dickson defined the modern concept of seawater alkalinity as: "*The total alkalinity of a natural water is thus defined as the number of moles of hydrogen ion equivalent to the excess of proton acceptors (bases formed from weak acids with a dissociation constant*  $K \le 10^{-4.5}$  *and zero ionic strength) over proton donors (acids with*  $K > 10^{-4.5}$ ) *in one kilogram of sample.*" (Dickson, 1981; Middelburg et al., 2020). In seawater, the carbonate system is the dominant buffering system, though other acid-base systems—such as fluoride, sulfate, borate, phosphate, silicate, ammonia, and hydrogen sulfide—also contribute. Based on Dickson's definition, total alkalinity or titration alkalinity can be derived as:

$$TA = [HCO_3^-] + 2[CO_3^{2-}] + [OH^-] + [B(OH)_4^-] + [HPO_4^{2-}] + 2[PO_4^{3-}] + [H_3SiO_4^-] + [NH_3] + [HS^-] + 2[S^{2-}] - [H^+] - [HF] - [HF] - [HSO_4^-] - [H_3PO_4]$$
Equation 1. 1

The dissolution of  $CO_2$  is in equilibrium with  $CO_2(g)$  and dissolved  $CO_2$ , a process that generates H<sup>+</sup> ions due to the dissociation of a weak acid (Equation 1. 2). However, TA, as a buffer, remains unaffected by  $CO_2$  dissolution because proton donors and acceptors are balanced (Bach et al., 2019). An increase in alkalinity (e.g., in the form of  $HCO_3^-$  and  $CO_3^{2-}$ ) disrupts the carbonate chemistry equilibrium, leading to lower dissolved  $CO_2$  concentration than it dissociates. This shift enhances the air-sea  $CO_2$  flux, increasing the ocean's uptake of  $CO_2$  to compensate for the unbalance of dissolved  $CO_2$  and storing it permanently in the ocean (Renforth & Henderson, 2017; National Academies of Sciences & Medicine, 2022).

$$\operatorname{CO}_2(g) \leftrightarrow \operatorname{CO}_2(\operatorname{aq}) + \operatorname{H}_2\operatorname{O} \leftrightarrow \operatorname{H}_2\operatorname{CO}_3 \leftrightarrow \operatorname{HCO}_3^- + \operatorname{H}^+ \leftrightarrow \operatorname{CO}_3^{2-} + 2\operatorname{H}^+$$
 Equation 1. 2

#### 2.2. Biogeochemical processes governing alkalinity generation

The key processes controlling alkalinity generation and consumption in BCEs involve various biogeochemical mechanisms, and they are closely tied to the redox stratification within the sediment (Santos et al., 2021). Organic matter degradation through anaerobic respiration processes—such as sulfate reduction and sulfate reduction coupled with pyrite (FeS<sub>2</sub>) formation—leads to a net gain of alkalinity. Sulfate reduction generates two moles of TA (as  $HCO_3^-$ ) for every mole of S reduced (Krumins et al., 2013; Reithmaier et al., 2021). In some salt marshes along the Australian east coast, sulfate reduction coupled with pyrite formation produces up to four to five moles of TA (as  $HCO_3^-$ ) per mole of pyrite formed (Reithmaier et al., 2021). Mineral (carbonates or silicates) dissolution can increase TA (as  $HCO_3^-$ ) by two moles per mole of  $CO_2$  consumption in the case of carbonates and varying amounts for silicate minerals, depending on the type and quantity of  $CO_2$  involved in the reaction (Hartmann et al., 2013). Conversely, reverse reactions like mineral precipitation tend to consume alkalinity (Middelburg et al., 2020). While aerobic respiration does not directly generate TA (Krumins et al., 2013), the produced  $CO_2$  as a byproduct can induce mineral dissolution, resulting in a gain of TA.

### 3. Inorganic Carbon Stock in Salt Marsh and an Overlook Contribution to Alkalinity Budget

Salt marshes in the Wadden Sea region, including those in the Elbe estuary, are characterized as a minerogenic organic-poor type with high rates of vertical mineral deposition per year (> 5 mm yr<sup>-1</sup>) (Esselink et al., 2017). Unlike other ecosystems, salt marshes accumulate allochthonous inorganic carbon in the form of carbonate shell debris (biogenic carbonates) (Figure 1. 1) rather than producing their carbonates. This is due to the relatively low abundance of calcifying organisms (Saderne et al., 2019; Granse et al., 2024). Most of the shell debris is introduced by storm surges and likely trapped by vegetation (Figure 1. 1B) (Ayyad & El-Ghareeh, 1982; Allen, 1987; Mueller et al., 2023; Granse et al., 2024). These carbonate shells are often fragmented due to the intense predation by birds, crabs, and fish (Figure 1. 1) (Cadée, 1994).



Figure 1. 1: A, B, and C demonstrate the large amount of fragmented carbonate shell debris deposited in the Hamburger Hallig salt marsh, located within the European Wadden Sea. Some of the debris was trapped by vegetation. These pictures were taken in the mid-May 2023.

A previous study on salt marshes in the Wadden Sea reported that inorganic sediment accounted for 7-57% of overall carbon stock and surpassed organic carbon across all the successional gradients (Mueller et al., 2023). Given the significant accumulation and burial of inorganic material within salt marshes (Zwolsman et al., 1993), fragmented shell debris found

in deeper soil cores has been shown to play a role in early marsh development (Mueller et al., 2023).

Research in carbonate-rich sediment (80% CaCO<sub>3</sub> w/w) from Red Sea mangroves emphasized the importance of the inorganic carbon stock and carbonate dissolution in the ocean alkalinity budget and climate mitigation strategies (Saderne et al., 2021). As mentioned in Section 2.2, the dissolution of carbonate can be written as:

$$CaCO_3(s) + CO_2(g) + H_2O(l) \rightarrow Ca^{2+}(aq) + 2HCO_3(aq)$$
 Equation 1.3

Notably, this process consumes one equivalent of atmospheric/soil  $CO_2$  and subsequently produces  $HCO_3^-$  as TA. The subsequent emission of TA in the water column was found to lead to around 0.6 to 0.7 mol of  $CO_2$  per CaCO<sub>3</sub> dissolved (Abril & Frankignoulle, 2001; Saderne et al., 2021). Thus, carbonate dissolution would be considered a  $CO_2$  sink process (Macreadie et al., 2017).

A comprehensive comparison of salt marsh types by Weiss (Weiss, 2013) revealed that most studies have focused on organic-rich sites, where anaerobic respiration is considered the primary driver of alkalinity production (Cai & Wang, 1998; Raymond et al., 2000; Reithmaier et al., 2021; Yau et al., 2022). This highlights a significant gap in understanding the role of inorganic carbon and mineral dissolution in the alkalinity budget, especially in minerogenic salt marshes where inorganic carbon contributes substantially.

In addition to biogenic carbonates, lithogenic and pedogenic carbonates are commonly found in soil and contribute significantly to the soil inorganic carbon pool in salt marshes (Yang & Yang, 2020; Ma et al., 2024). Lithogenic carbonates refer to unweathered minerals that remain relatively stable in the soil (Yang & Yang, 2020). On the other hand, pedogenic carbonates are formed through the re-precipitation of dissolved ions after the dissolution of parent minerals (Zamanian et al., 2016) and can also be induced by root activity (Verrecchia, 2011). The quantitative importance of these carbonates remains largely underexplored in many current salt marsh studies despite their potential contribution to mineral dissolution and the alkalinity budget (Mueller et al., 2023).

#### 4. Plants-Mediated Inorganic Carbon Dynamics

In addition to their role in trapping shell debris, plants, as autotrophic organisms, sequester atmospheric  $CO_2$ . Through photosynthesis, plants transform  $CO_2$  into organic matter and store it within the soil carbon pool. This organic matter is then oxidized by heterotrophic organisms (i.e., bacteria, fungi, and mycorrhiza), releasing  $CO_2$  back into the atmosphere (Kuzyakov, 2006). Rhizosphere is the small volume of soil affected by the vicinity of roots (Hiltner, 1904). Despite its small scale, the rhizosphere is the hotspot for carbon turnover, various respiration processes, and soil  $CO_2$  production.

Plant-microbe interactions in the rhizosphere are particularly strong in environments with low  $O_2$  availability. Salt marsh plants, such as *Spartina spp.*, have developed adaptive traits to supply  $O_2$  to the sediment through their roots, allowing them to survive under anoxic conditions (Koop-Jakobsen & Wenzhöfer, 2015). The increased availability of  $O_2$  and labile organic carbon (root exudates) drives aerobic respiration and leads to  $CO_2$  production (Fakhraee et al., 2023). Additionally, rhizodeposition potentially changes microbial activity and stimulates soil organic matter (SOM) decomposition within the rhizosphere. This process is known as the priming effect and generates  $CO_2$  (Cheng & Kuzyakov, 2005). Roots also respire  $CO_2$  to maintain cell concentration gradient and energy to maintain the metabolism (Kuzyakov & Larionova, 2005). These processes result in concentrated soil *p*CO<sub>2</sub> levels that are hundreds of-fold higher than atmospheric  $CO_2$ . As shown in Figure 1. 2, rhizosphere-derived  $CO_2$  from the roots of *Spartina x. townsendii* can reach concentrations as high as 12%, or 120,000 ppm.



Figure 1. 2: Rhizosphere  $CO_2$  and pH gradients around Spartima x townsendii roots. The color bar of the  $CO_2$  shows a gradient from 0 to 16% (1% = 10,000 ppm) and pH from 6.5 to 8.5. pCO<sub>2</sub> is the most pronounced at the roots with 12% and 2-fold of the bulk soil. pH is lowest at 7.2 in the root with a 0.2 lower than in the bulk sediment. See **Chapter IV** for more details.

Given the high acidity (CO<sub>2</sub>) production and relatively low pH at the rhizosphere compared to the bulk soil (Figure 1. 2), it is suggested that the rhizosphere can be a potential biotic driver of mineral dissolution and may serve as a hotspot for alkalinity generation. However, most carbon or alkalinity export studies focus on broader landscape or ecosystem perspectives. There is still limited data on the small scale, such as the rhizosphere processes (Ren et al., 2022), on driving alkalinity generation and influencing inorganic carbon dynamics. Some previous studies have reported the effect of soil pCO<sub>2</sub> on mineral dissolution. However, most of these studies are based on modeling approaches (Romero-Mujalli et al., 2019a; Romero-Mujalli et al., 2019b) and focus on terrestrial ecosystems (Thaysen et al., 2014; Wen et al., 2021; Lehmann et al., 2023). There clearly remains a lack of experimental evidence directly linking rhizosphere processes to inorganic carbon dynamics, particularly mineral dissolution and alkalinity generation (Mueller et al., 2023).

#### 5. Thesis outline

This thesis aims to improve the understanding of the contribution of the inorganic carbon within the minerogenic salt marsh soil and salt marsh plant-mediated effects on alkalinity generation to the coastal waters (Figure 1. 3). The central research questions are;

- 1. Is the mineral dissolution a dominant source of alkalinity production in the organicpoor minerogenic salt marsh soil?
- 2. Do the biogenic carbonate sources potentially contribute to the alkalinity budget in the coastal waters?
- 3. Do the plants mediate the alkalinity generation via mineral dissolution by rhizosphere process?

Considering the research on estuaries and salt marshes, studies on inorganic carbon dynamics and exports remain limited, particularly on a global scale. Most salt marsh research has been concentrated in organic-rich sediments, leaving a gap in understanding for other regions, especially those with minerogenic sediments. To address this gap, this research was primarily conducted in the organic-poor minerogenic salt marshes of the Wadden Sea, specifically at Kaiser-Wilhelms-Koog of the Elbe estuary (**Chapters II and III**) and Hamburger Hallig (**Chapter IV**).



Figure 1. 3: This diagram illustrates the structure of the thesis. BC refers to biogenic. Soil carbonates are the group of lithogenic and pedogenic carbonates. The dashed lines depict the stimulation of the effect under study. In **Chapter II**, we stimulated the rhizosphere  $CO_2$  to observe its impact on the mineral dissolution. In **Chapter IV**, **Experiment II**, we stimulated the biogenic carbonates (BC) by adding shell-bearing limestone. The solid lines represent the studied effect without any stimulation. Spartina refers to as Spartina x townsendii and Elymus as Elymus arthericus.

All experiments (**Chapters II to IV**) employed a soil-column approach to investigate changes in water chemistry throughout the study. **Chapter II** focused on elucidating the rhizosphere effect by simulating soil *p*CO<sub>2</sub> conditions coupling with the changing of hydrological regimes to examine how alkalinity generation is influenced and to identify the dominant biogeochemical processes responsible for alkalinity production under minerogenic salt marsh soil. **Chapter III** investigated the contribution of shell debris on alkalinity generation, exploring the role of biogenic carbonates. **Chapter IV** consisted of two sub-studies and included a planar optode experiment to illustrate the rhizosphere parameters relevant to the alkalinity generation. Both studies centered on assessing the effects of salt marsh plants on alkalinity generation. **Experiment I** aimed to determine whether different salt marsh plant species (*Spartina x townsendii* and *Elymus athericus*) have distinct effects on alkalinity generation. **Experiment II** explored the impact of adding biogenic carbonates (*Spartina x townsendii*) enhances alkalinity production.

### Chapter II: Soil *p*CO<sub>2</sub> Levels Control Salt Marsh Alkalinity Generation

#### **Chapter Summary**

Recent studies highlighted salt marshes as potential hotspots for alkalinity generation and export to coastal waters. Several studies emphasized the critical role of anaerobic microbial metabolism as a major source of total alkalinity (TA) generation in marsh soils. However, the potential contribution of mineral dissolution to salt marsh TA generation has yet to be extensively studied from a mechanistic viewpoint. Therefore, we conducted a lab-based soil column experiment to investigate the influence of soil  $pCO_2$  levels on TA generation in organic-poor minerogenic salt marsh soils, considering their significance in soil mineral dissolution processes. Results showed a strong positive effect of  $pCO_2$  levels and rainfall intensity on TA generation. Carbonate mineral dissolution was identified as the possible dominant driving force behind TA generation, with an additional contribution from silicate minerals. Our data suggested that elevated  $pCO_2$  levels, a typical rhizosphere environment, might be the hotspot for alkalinity generation via accelerating mineral dissolution processes. Our findings advance the mechanistic understanding of TA generation controls in salt marsh ecosystems and highlight the importance of soil minerals dissolution as a significant source of alkalinity export to coastal waters. Evaluating the contribution of salt marshes or other blue carbon ecosystems to alkalinity production is essential for integrating the roles of both soil organic and inorganic carbon dynamics into climate mitigation assessments.

Keywords: Salt Marsh, Alkalinity, Blue Carbon, Mineral Dissolution

#### 1. Introduction

Salt marshes, mangroves, and seagrasses (so-called blue carbon ecosystems, BCEs) store vast quantities of organic matter in their soils and are recognized as hotspots for carbon sequestration (McLeod et al., 2011; Macreadie et al., 2021). Furthermore, there is an increasing

number of studies that reveal significant rates of inorganic carbon (i.e., dissolved inorganic carbon, DIC) outwelling from salt marshes to coastal waters (Wang & Cai, 2004; Wang et al., 2016; Chu et al., 2018). This is due to the lateral inorganic carbon flux, which is evidenced as the primary biota-mediated carbon sequestration and further controls the seawater carbonate chemistry (Wang et al., 2016).

Recent blue carbon studies started to pay attention to the export of not only DIC but also total alkalinity (TA) from blue carbon ecosystems because exporting of TA enhances the CO<sub>2</sub> removal capacity of the oceans (Cai & Wang, 1998; Middelburg et al., 2020; Saderne et al., 2021). TA usually comprises three primary sources: carbonate, non-carbonate inorganic, and organic alkalinity (Lukawska-Matuszewska et al., 2018). Biogeochemical processes of organic matter degradation (i.e., anaerobic respiration) and mineral dissolution significantly govern TA generation. Additionally, aerobic respiration indirectly controls the change of TA by producing CO<sub>2</sub>, potentially causing mineral dissolution. In contrast, carbonate re-precipitation is known to consume TA (Middelburg et al., 2020). TA export from salt marshes has mainly been discussed as the result of anaerobic respiration processes (Reithmaier et al., 2021; Yau et al., 2022). However, the potential of net mineral dissolution processes, such as carbonate and silicate dissolution, to contribute to salt marsh TA export has not been extensively studied (Saderne et al., 2019; Middelburg et al., 2020; Mueller et al., 2023).

One of the key factors controlling mineral dissolution is soil CO<sub>2</sub>. Microbial and root respiration results in soil pCO<sub>2</sub> being orders of magnitude higher than atmospheric CO<sub>2</sub> levels (Andrews & Schlesinger, 2001; Kuzyakov, 2006; Ikkonen et al., 2018). Soil pCO<sub>2</sub> often increases with root proximity (Blossfeld et al., 2013; Koop-Jakobsen et al., 2018; Lenzewski et al., 2018), implying that the mineral dissolution hotspot might also be found inside the rhizosphere (Vicca et al., 2022). Dissolved CO<sub>2</sub> promotes acidification of the pore water and governs porewater pH by forming carbonic acid (H<sub>2</sub>CO<sub>3</sub>). This effect leads to carbonate or silicate dissolution process releasing in the form of bicarbonate (HCO<sub>3</sub><sup>-</sup>) and carbonate ion (CO<sub>3</sub><sup>2-</sup>) (Bricker et al., 1968; Guo et al., 2012; Hartmann et al., 2013; Saderne et al., 2019), consequently increasing TA (Wolf-Gladrow et al., 2007; Krumins et al., 2013). In addition to soil CO<sub>2</sub>, inorganic solid acids, like H<sub>2</sub>SO<sub>4</sub> and HNO<sub>3</sub>, can stimulate mineral dissolution processes, consequently leading to alterations in TA through increasing bicarbonate ions

(Beaulieu et al., 2011; Martin, 2017). The presence of organic acids (root exudates) also indirectly contributes to TA by inducing mineral dissolution (Drever & Vance, 1994). Moreover, organic acids contribute to TA through organic alkalinity (Yang et al., 2015; Lukawska-Matuszewska et al., 2018) and control carbonate species (Song et al., 2023).

Currently, our quantitative understanding of TA generation and export from coastal ecosystems comes from the ecosystem- and landscape-scale observations (Ren et al., 2022), while smaller-scale investigations, such as rhizosphere CO<sub>2</sub>-induce soil mineral dissolution, that can reveal mechanistic insight into the drivers of soil and sediment TA generation are scarce. Hence, we investigated the importance of mineral dissolution for TA generation in salt marsh soils and assessed the control of soil  $pCO_2$  levels on TA generation. We conducted laboratory experiments using columns filled with soil materials from an organic-poor minerogenic salt marsh ecosystem and exposed the columns to different levels of soil  $pCO_2$ . We hypothesized 1) that TA generation increases with soil  $pCO_2$  due to stimulation of mineral dissolution and 2) that total soil mineral dissolution is a more important TA source than anaerobic respiration in the mineral-rich soils investigated.

#### 2. Materials and methods

To investigate the effect of soil  $pCO_2$  on soil TA generation, we conducted a two-factorial labbased column experiment. Columns were subjected to three levels of  $pCO_2$  stimulating ambient at 440 µatm, bulk soil at 15,000 µatm, and rhizosphere at 150,000 µatm conditions (Mueller, 2017). Site hydrology governs the removal of solutes from the soil system and can affect TA generation. Therefore, we employed three different rainfall regimes for each CO<sub>2</sub> treatment (600, 1200, and 2400 mm yr<sup>-1</sup>). Soils were collected from a minerogenic salt marsh site on the Elbe estuary, Germany. The change in water matrix composition was meticulously monitored over the experimental period using various water chemistry analysis methods.

#### 2.1. Site description, soil characteristics, and soil sampling

The salt marsh site is located at the mouth of the Elbe estuary, Schleswig Holstein, Germany (53.92781°, 8.91424°) (Figure 2. 1). The sampling location was characterized by common salt

marsh grass (*Puccinellia maritima*) and sea aster (*Aster tripolium*), reflecting the typical vegetation composition of low marshes in the Wadden Sea region (Mueller et al., 2020). Annually, the average rainfall totals approximately 800 mm (Saadaoui et al., in prep). Soil chemical composition was characterized by high amounts of Si, Al, Ca, and Fe. It was enriched with SiO<sub>2</sub> (alpha quartz), MgCaCO<sub>3</sub> (carbonate), NaAlSi<sub>3</sub>O<sub>8</sub> (albite), and other clay minerals (see Supporting Information, Table S2. 1). Soil was collected in October 2021 using a spade cutting to a depth of 17 cm below the surface. All chunks were placed in a plastic box for transportation and storage. Samples were stored in a refrigerator at 4°C until the experiment commenced.



Figure 2. 1: A.) Location of the Elbe estuary in the northern part of Germany; B.) Location of the salt marsh site; C.) Photo of the sampling position next to the infrastructure of the RTG2530 long-term research site. Photograph by F. Neiske; maps were generated by ArcGIS 10.7.1.

#### 2.2. Packing column and subsampling

Soil chunks were sectioned into three depths (0-5 cm, 5-10 cm, and 10-17 cm) to keep the sorting of the soil profile, and each layer was homogenized separately. Soil samples were then filled into polycarbonate columns (Figure 2. 2), with each column containing 550 g of soil, corresponding to the original depth intervals (see Supporting Information, Table S2. 2). Large

roots and stems in the topsoil were removed by handpicking to prevent the macropores and preferential flow caused by plants. In total, the series of CO<sub>2</sub>-treated columns consisted of 27 columns. They were randomly assigned to nine columns of ambient-CO<sub>2</sub> (hereafter: Am), nine columns of 15,000  $\mu$ atm CO<sub>2</sub> (hereafter: 1.5%), and another nine columns of 150,000  $\mu$ atm CO<sub>2</sub> (hereafter: 15%). (Table 2. 1)



Figure 2. 2: The simplified sketch of the column experiment performed under unsaturated conditions. The custom-made column was made of polycarbonate (5.5 cm diameter x 25 cm height). The bottom of the column was fitted with a 200  $\mu$ m-phytoplankton mesh allowing only the outflow water to pass through and retain soil. The column was fixed vertically and it was connected with a conical funnel and a polyethylene (PE) bottle to collect the outflow water.

#### 2.3. Hydrological regimes and water distribution

Salt marsh hydrology is determined by rainfall and flooding by seawater. Artificial rainwater and seawater (ARW and ASW, respectively) were prepared by dissolving mineral salts in MilliQ water until reaching the natural abundance concentration (see Supporting Information, Table S2. 3 and 2.4). ARW and ASW were drip-fed into the column with a peristaltic pump at a 1 mL min<sup>-1</sup> pumping rate (ISMATEC® MCP-Process IP-65) (Figure 2. 2). Treatment selection was randomly assigned to high, medium, and low rainfall intensity with a fixed flooding regime (Table 2. 1). Each watering cycle was 14 days long, alternating between drying and rewetting periods to stimulate the hydrology at the low marsh. Rainfall events occurred five times per cycle, whereas the simulation of flooding by seawater was two times per cycle, corresponding to the flooding frequency of the Wadden Sea low marshes (Reents et al., 2021). Before feeding to the columns, ASW and ARW were equilibrated with the respective CO<sub>2</sub> treatments: ambient-CO<sub>2</sub> conditions with air, and the 15,000 and 150,000 µatm conditions with

# a CO<sub>2</sub> stream for five minutes. The initial pH and TA of the mixture of inflow water can be found in Table 2. 1.

CO <sub>2</sub> treatment	Rainfall Simplified Sedir regimes name in col		Sediment in column	ent mn Replicates	Experimental period (days)	Amount of water inflow per cycle		Initial condition of the mixture of inflow water (mean ± SD, n=9)		
	(mm yr <sup>*</sup> )					ARW (mL)	ASW (mL)	pН	TA (umol/kg)	
	600	Am_L		3	168	59.5	(1112)		60 ± 16	
Ambient- $CO_2$	1200	Am_M				119		6.4 ± 0.3		
(440 µuiii)	2400	Am_H				237.5				
	600	1.5%_L	Salt marsh soil			59.5		$4.9 \pm$	$74 \pm 6$	
15,000 μatm	1200	1.5%_M				119	71.2			
	2400	1.5%_H		3011			237.5		0.1	
	600	15%_L				59.5				
150,000 μatm	1200	15%_M				119		$4.4 \pm$	$73\pm5$	
	2400	15%_H				237.5		0.0		

Table 2. 1: The series of columns and parameters.

#### 2.4. Experimental setup

For the ambient-CO<sub>2</sub> treatment (Table 2. 1), all nine columns were placed in the windowless laboratory room under the average temperature of  $17.4 \pm 1.8$  °C and  $438.8 \pm 35.4$  ppm (mean  $\pm$  SD) of *p*CO<sub>2</sub> measured by air quality monitor (CO220, EXTECH) (see Supporting Information, Figure S2. 1). The room remained dark for the majority of the time, with lighting activated for one to two hours per day during the watering process. The 15,000 and 150,000 µatm CO<sub>2</sub> experiments were conducted 106 days later in the same room and same experimental duration as ambient-CO<sub>2</sub> treatment (Table 2. 1). However, all columns were placed in a transparent gas-tight box (see Supporting Information, Figure S2. 2). During the CO<sub>2</sub> treatment, each box was flushed with CO<sub>2</sub> gas three times per week for five to ten minutes to maintain the CO<sub>2</sub> inside the box and it was assumed to be stable throughout the experiment. The customized CO<sub>2</sub> gas was produced by Westfalen GmbH, containing either 15,000 µatm or 150,000 µatm CO<sub>2</sub> in the synthetic air.

#### 2.5. Water sampling and chemistry analysis

Water samples were collected in the PE bottle every 14 days (Figure 2. 2). The first watering cycle allowed the soil columns to enter the equilibration period. pH was measured directly after the sampling with a portable pH meter (WTWmulti 3630 IDS, IDS pH/electrode Sentix®940). The pH probe was calibrated daily with three NBS buffer standards at 4.006, 6.865, and 9.180 (WTW standard (DIN/NIST) buffer solution). The remaining sample was filtered with the polyethersulfone (PES) membrane filters 0.45 µm (Sartorius) for TA, major ions (Na<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>), and dissolved silica (DSi). Analysis of TA was conducted after the sampling by using automated titration with 0.1 N HCl to pH 4.3 using Metrohm Titrando and calibrated with certified references by A. Dickson (Amann et al., 2022). Major ion measurement was carried out by ion chromatography (Metrohm 881 Compact IC Pro system) (Amann et al., 2015). Dissolved silica analysis was determined by using the molybdate blue colorimetric method. The analysis procedure was modified after Grasshoff (Grasshoff et al., 2009) and carried out by spectrophotometer HACH DR3900 at a wavelength of 810 nm. It was ensured that all the processes for sampling and preparing the chemicals were handled by avoiding the usage of glassware. For DIC, 12 mL of unfiltered samples were transferred into the sealed glass vial (Labco), and 6  $\mu$ L of HgCl<sub>2</sub> was added to suppress microbial activities. All DIC samples were stored in the dark at 4°C in the fridge until the analysis. DIC was analyzed by cavity ring-down spectroscopy (CRDS) Picarro (G2101-i). The certified reference material (CRM) used for constructing the calibration curve in this experiment was CaCO<sub>3</sub>. The external calibration was measured daily within 0.5 - 2.0 mg of CRM.

#### 2.6. Constituents generating/releasing from soil columns experiment

#### 2.6.1. TA

In this study, measured TA (total alkalinity) refers to titration alkalinity as defined by Dickson (1981), representing the excess of proton acceptors over proton donors relative to the zero level of proton (pK value at 4.5) for each acid-based system as mentioned in Chapter I, Section 2.1 (Dickson, 1981; Middelburg et al., 2020) (see Equation 1. 1).

The absolute amount of TA generation is expressed as moles TA per unit area (mol TA m<sup>-2</sup>). To calculate the absolute amount of TA released from the column after each watering cycle for each column setup, we used the following equation:

$$TA(t) = \frac{(C_{TA\_outflow}(t) \times Wt_{outflow}(t) - C_{TA\_inflow}(t) \times Wt_{inflow}(t))}{A_{column}} \qquad Equation 2.1$$

In Equation 2. 1,  $C_{TA\_outflow}(t)$  and  $C_{TA\_inflow}(t)$  [mol kg<sup>-1</sup>] are the TA concentration at the time t of outflow and inflow water, Wt.<sub>outflow</sub>(t) and Wt.<sub>inflow</sub>(t) [kg] is the weight of water sample at the time t of outflow and inflow water, and  $A_{column}$  [m<sup>2</sup>] is the surface area of the soil column. The calculation is based on assuming that the density of the water sample ( $\rho_{water}$ ) is equal to 1.0 g ml<sup>-1</sup>; therefore, V<sub>water</sub> is equal to the Wt.<sub>water sample</sub>.

#### 2.6.2 DSi

The absolute amount of DSi released from the soil column was reported as moles DSi per unit area (mol DSi m<sup>-2</sup>). Since the inflow water did not contain DSi, it was not considered in Equation 2. 2.

$$DSi(t) = \frac{C_{DSi\_outflow}(t) \times Wt_{outflow}(t)}{A_{column}}$$
 Equation 2.2

In Equation 2. 2, C<sub>DSi</sub> (t) [mol DSi L<sup>-1</sup>] is the concentration of DSi at time t of outflow water.

#### 2.6.3 Ion release

The estimation of cation loss from the soil throughout the experiment period was calculated based on the different amounts of ions entering and leaving the soil column using the following expressions Equation 2. 3.

Cumulative ion release

$$= \sum_{t=1}^{t=max} \left( \frac{C_{ion\_outflow}(t) \times Wt_{outflow}(t) - C_{ion\_inflow}(t) \times Wt_{inflow}(t)}{A_{column}} \right)^{Equation 2.3}$$

Here, we measured the concentration of each ion for both inflow water ( $C_{ion_inflow}(t)$ ) [mol L<sup>-1</sup>] and the outflow water ( $C_{ion_outflow}(t)$ ) [mol L<sup>-1</sup>] at each sampling interval (t). The Wt<sub>outflow</sub> (t) and Wt<sub>inflow</sub> (t) [kg] are the weights of outflow and inflow water at the time step. In this case, the positive and negative values represented the net ions loss and the retaining tendency, respectively. Total ion release from the soil column is reported as moles ion per unit area (mol ion m<sup>-2</sup>).

#### 2.7 Data collection and statistical analysis

To assess the effect of soil  $pCO_2$  on TA generation (hypothesis 1), we used two-way ANOVA testing for the effects of CO<sub>2</sub> level, rainfall, and their interaction on the cumulative outflow of TA. Two-way ANOVA was also used to assess the effects of CO<sub>2</sub> and rainfall on the net loss of Ca<sup>2+</sup> and DSi. Before ANOVA testing, the normal distribution of residuals was determined visually (Tang et al., 2021) and homogeneity of variance was assessed using Levene's test. Tukey's test was used for pairwise comparisons. The significance level at 5% ( $\alpha = 0.05$ ) was used to determine the statistical difference in mean between the experiment series. Statistical analyses were conducted using Python utilizing the statistical packages (scipy.stats, statsmodels.formula.api, statsmodels.stats.anova, and statsmodels.stats.multicomp). To assess the importance of mineral dissolution for TA generations (hypothesis 2), we analyzed the relationships between TA generation and Ca<sup>2+</sup> and DSi outflow using linear regression (sklearn.linear\_model and sklearn.metrics).

#### 3. Results

- 3.1. Cumulative TA, ions release (Ca<sup>2+</sup>), and DSi across CO<sub>2</sub> and rainfall gradients
- 3.1.1 Cumulative absolute amount of TA

The cumulative pattern of TA generation induced by increasing  $pCO_2$  levels and the effect of CO<sub>2</sub> was consistent among rainfall setups (Figure 2. 3 A). The highest TA generation was observed with 150,000 µatm CO<sub>2</sub> and 2400 mm yr<sup>-1</sup> treatment while the lowest value occurred in the ambient-CO<sub>2</sub> and 600 mm yr<sup>-1</sup> setup. In addition, the overall trend of absolute TA at each sampling point across all treatments showed a pattern similar to that of cumulative TA (see Supporting Information, Figure S2. 3). The increase in TA could be double when the CO<sub>2</sub> level changes from 440 µatm to 150,000 µatm CO<sub>2</sub> and up to 1.4 times when the condition changes from 15,000 to 150,000 µatm CO<sub>2</sub> (calculated by high rainfall data). Average cumulative absolute amount TA among CO<sub>2</sub> and rainfall treatments differed significantly and non-significantly (Figure 2. 3 A, see Supporting Information, Table S2. 5 and Table S2. 6). However, there was a significant interaction effect between CO<sub>2</sub> and rainfall (see Supporting Information, Table S2. 5). The CO<sub>2</sub> effect was consistent and significantly pronounced under 2400 mm yr<sup>-1</sup> treatment.

#### 3.1.2 Cumulative ions release

Measuring the release of ions provides insight into soil mineral dissolution processes, particularly concerning cations generated through dissolution. The cumulative release of  $Ca^{2+}$  ions aligns consistently with trends observed in TA and DSi (Figure 2. 3 A, C). The net release ratio of  $Ca^{2+}$  was most pronounced under 150,00 µatm CO<sub>2</sub> and 2400 mm yr<sup>-1</sup> treatment, with an outflow-inflow ratio of 3.0 (see Supporting Information, Table S2. 10). The remaining ions (i.e.,  $Mg^{2+}$ ,  $K^+$ ,  $Na^+$ ,  $Cl^-$ ,  $NO_3^-$ ,  $SO_4^{2-}$ ) did not show a clear pattern. Most ions tended to retain within the soil columns (see Supporting Information, Table S2. 10 and Table S2. 11).  $Mg^{2+}$  leached only under 2400 mm yr<sup>-1</sup> rainfall and 15,000 and 150,000 µatm CO<sub>2</sub> treatments.  $SO_4^{2-}$  showed releasing tendencies without a clear pattern. Since only  $Ca^{2+}$  showed the release across all experimental setups, the later analysis and discussion will only account for  $Ca^{2+}$ .

#### 3.1.3 Cumulative dissolved silica (DSi)

DSi (H<sub>4</sub>SiO<sub>4</sub>) can be used as an implication of the mineral dissolution processes since it is represented as one of the byproducts of silicate dissolution. The overall trend of cumulative DSi indicated an increment of DSi generation corresponding to an increase in soil pCO<sub>2</sub> levels, and the effect of CO<sub>2</sub> was consistent across rainfall setups (Figure 2. 3 C). It was similar to the time series data pattern for DSi generation (see Supporting Information, Figure S2. 4). The statistical results showed a significant difference among CO<sub>2</sub> treatments, especially under the 2400 mm yr<sup>-1</sup> regime (Figure 2. 3 C and see Supporting Information, Table S2. 5 to Table S2. 7).

#### 3.2 TA in relation to DIC, ion (Ca<sup>2+</sup>), and DSi

Samples were collected from three distinct sampling points for DIC analysis. The measured TA and DIC concentrations aligned closely along a 1:1 ratio line, forming distinct clusters within their respective groups (Figure 2. 4 A). Furthermore, a strong and significant positive correlation was observed between the cumulative TA values of all columns and the cumulative release of  $Ca^{2+}$  and DSi (Figure 2. 4 B and C).



Figure 2. 3: Box plot and swarm plot of the cumulative amount of A.) TA B.)  $Ca^{2+}$ , C.) DSi from all twelve sampling points. Each dot represents a replicate for each setup, e.g.  $Am_H_I$ ,  $Am_H_II$ ,  $Am_H_III$ . Tukey's test results are shown in the graphs. ns, \*, \*\*, and \*\*\* referred to non-significant difference, p < 0.05, p < 0.01, and p < 0.001 respectively. Supporting information for statistical analysis can be found in Table S2. 6, Table S2. 7, and Table S2. 9.



Figure 2. 4: A.) The scatter plot of TA vs. DIC of the outflow water at first, sixth, and twelfth sampling and the 1:1-line ratio. The blue circle, orange square, and green triangle represent 440, 15,000, and 150,000  $\mu$ atm CO<sub>2</sub> conditions respectively and it does not differentiate the rainfall regime. B.) and C.) the scatter plot and linear regression analysis between TA and Ca<sup>2+</sup>, and TA and DSi, respectively. The black line indicates a significant correlation.

#### 4. Discussion

#### 4.1. Effect of soil $pCO_2$ on net TA releasing

Increasing soil  $pCO_2$  led to a noticeable increase in net TA releasing (Figure 2. 3 A). The findings imply that a steeper CO<sub>2</sub> gradient in the soil might increase TA production via soil mineral dissolution. This is due to the increased acidity source (CO<sub>2</sub>) for the dissolution agent, which subsequently accelerates the chemical dissolution process. Our results are consistent with reactive transport and thermodynamic models, which show that soil  $pCO_2$  plays a significant role in controlling the dissolution of carbonate minerals and the production of alkalinity (Romero-Mujalli et al., 2019a; Romero-Mujalli et al., 2019b). The reactive model analyses also observed variations in dissolved inorganic carbon in soil water driven by soil  $pCO_2$  (Wen et al., 2021). Similar to studies on spring water, alkalinity displayed a spatiotemporal increased pattern, corresponding with rising soil  $pCO_2$  (Yoshimura et al., 2001;

Calmels et al., 2014). In addition, an increase in  $CO_2$  was shown to relate to the increase in dissolution rate (Maher et al., 2009; Calmels et al., 2014).

The impact of CO<sub>2</sub> on TA and DSi displayed a similar pattern and exhibited significant potential effects, particularly under scenarios of the highest rainfall (Figure 2. 3 A and C). It was suggested that greater water availability could enhance chemical dissolution processes (Maher, 2010; Spatti Júnior et al., 2019; Calabrese & Porporato, 2020; Xie et al., 2022). Additionally, higher amounts of water lead to greater leaching of constituents and products (i.e., dissolved Ca<sup>2+</sup> and HCO<sub>3</sub><sup>-</sup>) from the soil (Vranken et al., 1990). This can drive the departure from thermodynamic equilibrium in the transport-control system and increase the chemical dissolution rate (Maher, 2010). The demonstrated releasing trend of cumulative Ca<sup>2+</sup> from our soil columns experiment aligned with the aforementioned findings (Figure 2. 3 B). Specifically, heightened soil  $pCO_2$  and rainfall intensity increased Ca<sup>2+</sup> leaching, signifying the possibility of elevated carbonate mineral dissolution.

The observed linear increase in cumulative TA with respect to the increasing rainfall gradients highlights the predominant hydrological control on the leaching experiment (Figure 2. 5A); the relationship between cumulative TA and soil  $pCO_2$  exhibits a positive non-linear trend, characterized by a sharp initial increase followed by a plateau (Figure 2. 5 B). This suggests that the dissolution reaction of soil minerals may be reaching saturation to soil  $pCO_2$  levels. The measured TA concentration in the outflow water was more affected by changes in  $pCO_2$  than rainfall intensity (see Supporting Information, Figure S2. 5). Specifically, within the same  $CO_2$  conditions, TA concentrations were consistent across different rainfall intensities. However, the cumulative increase in the absolute amount of TA was due to the larger volume of outflow water, which was strongly influenced by the amount of rainfall. This indicates that the mineral dissolution reaction is not strongly limited by the kinetic factor ( $CO_2$ ), and the absolute amount of TA can still increase with a rise in rainfall intensity.



*Figure 2. 5: Average of cumulative TA as a function of A.) Rainfall intensity and B.) Soil pCO*<sub>2</sub>*. The fitted curves are represented as the solid line.* 

#### 4.2 Soil mineral dissolution as TA generation source

As in Equation 1. 1, TA is a function of various acid-base systems including carbonate alkalinity and non-carbonate inorganic bases (Dickson, 1981; Middelburg et al., 2020). Contrary to those acid-base species, the organic charge group of dissolved organic carbon (DOC) also contributes to TA as organic alkalinity (Lukawska-Matuszewska et al., 2018). However, in this study, we assumed that the contribution of organic alkalinity could be negligible, as indicated by the insensitivity to change and low levels of DOC observed in the outflow water (see Supporting Information, Figure S2. 6). At the observed pH range of 7 - 8 in the water samples (see Supporting Information, Table S2. 5), the predominant contribution to TA is primarily from bicarbonate ions, as this pH range strongly favors the presence of this species (Stumm & Morgan, 1981). Bicarbonate is a shared ion in both TA and DIC, primarily constituting the bulk of both parameters. DIC is expressed as a collective sum of all carbonate species. Therefore, the measured TA in this soil columns experiment mainly contributed to the carbonate system indicated by the TA:DIC ratio.

#### Characteristics of soil mineral dissolution and possible pathways

Different biological mechanisms, such as soil mineral dissolution and anaerobic respiration (specifically sulfate reduction), can significantly change TA (Hu & Cai, 2011; Santos et al., 2021). In this experiment, the expected depletion pattern of  $SO_4^{2-}$  was not observed; instead, the releasing of  $SO_4^{2-}$  from the column was found, suggesting minimal activity of sulfate reduction reactions. Thus, sulfate reduction was assumed to be negligible.

On the other hand, the increasing pH in the outflow water compared to inflow water indicated a dominant role in soil mineral dissolution processes. Observing carbonate alkalinity as a major component in outflow water might result from the net chemical dissolution of available minerals. This observation serves as a possible indication of soil mineral dissolution induced by an increase in soil  $pCO_2$  levels. The net release of Ca<sup>2+</sup> might also indicate that carbonate minerals were undergoing dissolution due to increased  $pCO_2$  and changes in rainfall patterns. The TA-Ca<sup>2+</sup> plot, with a slope of 1.73, implied a correlational change in TA and Ca<sup>2+</sup> following the stoichiometric ratio of dissolution of carbonate found in a salt marsh soil sample with a factor of 2 of bicarbonate (HCO<sub>3</sub><sup>-</sup>) and Ca<sup>2+</sup> (Equation 2. 4). Hence, TA releasing might be primarily driven by carbonate mineral dissolution. This speculation is supported by the study in the Elbe estuary that carbonate dissolution was identified as the main source of TA generation (Weiss, 2013).

Carbonate dissolution:

$$Mg_{0.03}Ca_{0.97}CO_{3}(s) + CO_{2}(g) + H_{2}O(l) \rightarrow$$
  
0.03Mg<sup>2+</sup>(aq) + 0.97Ca<sup>2+</sup>(aq) + 2HCO<sub>3</sub><sup>-</sup>(aq) *Equation 2. 4*

Despite TA and DSi exhibiting a positive correlation, the change in TA concerning DSi appeared disproportionate (Figure 2. 4 C). The soil mineralogy in this study site exhibited a notably higher Si content, mostly in quartz (SiO<sub>2</sub>) and some other silicates (e.g., albite)/clay minerals. DSi was produced from dissolved minerals containing Si and exhibited a mixture signal. The dissolution of quartz does not significantly affect TA. It is more likely that TA generation is associated with the dissolution of albite, characterized by a faster dissolution rate than quartz (Lasaga, 1984). However, we emphasized that in this soil columns experiment, the TA might be mainly driven by carbonate dissolution, with an additional contribution from the

dissolution of silicates. This was attributed to the faster dissolution rate of carbonate minerals compared to silicates (Yadav & Chakrapani, 2006).

#### 4.3 Perspective, research gaps, and implications

#### 4.3.1 Coastal wetland rhizosphere as a hotspot for alkalinity enhancement

The rhizosphere is a microenvironment containing relatively high soil  $pCO_2$  under wetland conditions, roughly 35 to 350-fold higher than the atmosphere (Mueller, 2017). In addition, soil  $pCO_2$  can differ depending on the ecosystem, type of plants, physiochemical soil properties, and microbial activity. By experimentally creating  $pCO_2$  conditions similar to the rhizosphere in salt marshes, we can suggest that the rhizosphere is a potential source of alkalinity generation via enhancing soil mineral dissolution (Figure 2. 6). Regarding future studies, investigating the direct effects of soil  $pCO_2$  in marsh soils will provide us with a better understanding of how TA fluxes from the estuarine marshes to the channels are promoted due to the belowground processes (Mueller et al., 2023). Despite efforts made by researchers in recent decades to understand the mechanisms behind the processes driving TA in coastal wetlands, there is still a surprising lack of data at the rhizosphere scale (Ren et al., 2022), particularly regarding the role of rhizosphere-derived CO<sub>2</sub> in the generation of TA export.

Additionally, in the context of blue carbon, a small number of studies reported that alkalinity export is associated with the degradation of organic carbon via anaerobic microbial respiration (i.e. sulfate reduction, denitrification) (Giblin & Howarth, 1984; Sippo et al., 2016; Wang et al., 2016; Reithmaier et al., 2021; Santos et al., 2021; Yau et al., 2022). Here, our findings suggested that the source of TA production cannot solely be attributed to anaerobic respiration; instead, the role of soil mineral dissolution should also be added into context (Saderne et al., 2021; Fakhraee et al., 2023). Notably, the acidity (CO<sub>2</sub>) required for mineral dissolution may originate from the oxidation of organic matter and microbial activities, which may lead to mineral dissolution and subsequent alkalinity production (Figure 2. 6). Integrating both organic and inorganic systems in future assessments is urgently needed for gaining a comprehensive insight into the function of blue carbon ecosystems in climate change mitigation, such as restoration of coastal habitats or ocean alkalinity enhancement (Fakhraee et al., 2023; Sulpis & Middelburg, 2023).


Figure 2. 6: Conceptual diagram illustrating the role of rhizosphere  $CO_2$  and soil mineral dissolution process on enhancing TA export (through carbonate alkalinity) from minerogenic salt marshes to the adjacent coastal waters. (Generated by Biorender.com)

#### 4.3.2 Alkalinity export responses to climate change in salt marsh

Climate change drivers, such as elevated atmospheric CO<sub>2</sub>, along with climate change consequences like increased rainfall intensity and frequency, can affect wetland biogeochemistry in several ways (Salimi et al., 2021) and potentially affect the carbon pool and DIC export (Marsh et al., 2005). Several studies have provided evidence that elevated atmospheric  $CO_2$  levels can stimulate soil respiration and increase soil  $pCO_2$  due to high root biomass and productivity (van Veen et al., 1991; Andrews & Schlesinger, 2001; Kim et al., 2004; Marsh et al., 2005). Our results proved that high soil  $pCO_2$  yielded relatively high TA and DIC production, consistent with field studies in coastal marshes (Marsh et al., 2005) and terrestrial ecosystems (Andrews & Schlesinger, 2001). Belowground CO<sub>2</sub> likely controls DIC production through soil acidification and mineral dissolution processes, resulting in the export of a high amount of DIC to the adjacent ecosystem. In addition, this present study has also shown a clear link between increased rainfall intensity and TA generation. By 2100, we may experience atmospheric CO<sub>2</sub> levels up to 1000 µatm (Pörtner et al., 2022) and heavier rainfall intensity (Fischer et al., 2014). A projection of the alkalinity export due to climate change is outside the scope of this study but would certainly be a promising next step. Moreover, it is still unknown how the warming climate and sea level rise will facilitate carbon export, and it is crucial to study how resilient the salt marsh and its biota will be against these changes (Figure 2. 7). Carbon sequestration in coastal marshes is a complex process involving various biogeochemical mechanisms. Therefore, future assessments are necessary to improve our understanding of the role of salt marshes on inorganic carbon dynamics in response to climate change.



Figure 2. 7: Conceptual diagram depicting the potential for alkalinity export under present and future climatic conditions. Qualitative insights indicate that increasing in soil  $pCO_2$  levels and rainfall gradients may enhance alkalinity export. However, the impact of climate warming factors on salt marsh alkalinity budgets remains unexplored, representing a promising perspective for future research. (Diagram created using Biorender.com)

#### 4.3.3 Methodological limitations

This soil columns experiment simplified studying the TA export and belowground processes in the marsh-dominated estuaries system. Thus, the results represent carbon generation during the summer, particularly in the high and low marsh areas (low inundation frequency). However, homogenized soil columns disturbed the natural soil structure, potentially exposing more mineral surfaces to dissolution and altering preferential flow paths. The solute residence time within the soil columns, influenced by water flow, hydrology, and tidal dynamics, may also impact the estimated flux to adjacent ecosystems (Maher, 2011). Future research should address and incorporate these factors on the laboratory scale to better understand fluxes generated by rhizosphere processes under varying soil conditions. Additionally, this soil column experiment primarily focuses on the effect of soil  $pCO_2$  gradient on mineral dissolution processes. However, the soil CO<sub>2</sub> feedback was not entirely considered, especially on CO<sub>2</sub>-fixing microbes affecting the inorganic carbon export. The concerted effect of soil  $pCO_2$  on biological processes (microbes and plants) should be considered to provide a realistic picture of the effect of rhizosphere processes on TA export.

## 4.3.4 Implications

Overall, our investigation revealed a significant positive effect of elevated soil  $pCO_2$  on TA enrichment from soil to the outflow water, particularly pronounced under the highest rainfall regime condition. We identified carbonate mineral dissolution as the possible dominant driver of TA generation in organic-poor minerogenic salt marsh soil, with an additional contribution from silicate dissolution. The rhizosphere was highlighted as a possible key factor in alkalinity generation through soil mineral dissolution (Figure 2. 6). Our study quantified the role of soil mineral dissolution in TA generation; however, accounting for individual contributions of all biogeochemical processes remains challenging. This underlines the necessity of soil mineral dissolution and inorganic carbon cycling in future assessments. Since TA is associated with organic and inorganic carbon, integrating both factors is urgently required in future blue carbon research. Moreover, it is essential to investigate how salt marshes and their biota will respond to future climatic conditions, particularly regarding alkalinity production (Figure 2. 7).

# **Supporting Information**

Table

Chemical Composition	mass %	Mineralogical Composition
SiO <sub>2</sub>	72.96	
$\begin{array}{c} Al_2O_3\\ Fe_2O_3\\ MnO\\ MgO\\ CaO\\ Na_2O\\ K_2O\\ TiO_2\\ P_2O_5\\ SO_3\\ LO I^a\end{array}$	$\begin{array}{c} 6.56 \\ 2.45 \\ 0.06 \\ 1.02 \\ 4.33 \\ 1.39 \\ 1.75 \\ 0.40 \\ 0.14 \\ 0.08 \\ 8.84 \end{array}$	SiO <sub>2</sub> (alpha quartz) (Mg <sub>0.03</sub> Ca <sub>0.97</sub> )(CO <sub>3</sub> ) (Mg-bearing calcite), NaAlSi <sub>3</sub> O <sub>8</sub> (Albite), (Mg,Al) <sub>6</sub> (Si,Al) <sub>4</sub> O <sub>10</sub> (OH) <sub>8</sub> (Chlorite-serpentine), Na-Ca-Al- Si <sub>4</sub> O <sub>10</sub> -O (Montmorillonite- chlorite)
Sum	99.97	

Table S2. 1: Chemical and mineralogical composition of salt marsh soil by XRF and XRD

Table S2. 2: The amount of sediment added to the column

CO <sub>2</sub> Treatments	Columns	Total Sediment (g)
	Am_I	550.18
	Am_II	550.88
	Am_III	550.81
	Am_I	550.79
440 µatm	Am_II	550.89
	Am_III	550.44
	Am_I	550.79
	Am_II	551.69
	Am_III	550.16
	1.5%_I	550.1
	1.5%_II	550.4
	1.5%_III	550
	1.5%_I	550.4
15,000 µatm	1.5%_II	550.5
	1.5%_III	550.1
	1.5%_I	550.5
	1.5%_II	550.2
	1.5%_III	550.5
	15%_I	550.5
	15%_II	550
	15%_III	550
	15%_I	550
150,000 µatm	15%_II	550.4
	15%_III	550.8
	15%_I	550
	15%_II	550.2
	15% III	550.7

Species	Natural abundance (mg l <sup>-1</sup> )	ARW (mg l <sup>-1</sup> )
Na <sup>+</sup>	58	58
Mg <sup>2+</sup>	5.6	5.6
K <sup>+</sup>	11	11
Ca <sup>2+</sup>	2.9	2.9
S	13	7.4
Cl-	180	104

Table S2. 3: The final concentration of each ion species in ARW solution in comparison to the natural abundance of rainwater in the North Sea region

*Table S2. 4: The final concentration of each ion species in ASW solution with 35% salinity in comparison to the natural abundance of seawater* 

Natural abundance (g1)	ASW $(g l^{-1})$
11.14	10.34
1.32	1.34
0.42	0.42
0.41	0.39
19.98	19.16
2.79	2.40
	11.14   1.32   0.42   0.41   19.98   2.79

Table S2. 5: Average pH (n=36) and average cumulative TA, DOC, and DSi (n=36) (mean  $\pm$  SD). Two-way ANOVA results are included below (highlighted p-value means there is a significant difference when  $p \leq 0.05$ ). Tukey's test results of cumulative TA, DOC, and DSi can be found in Table S2. 6 to Table S2. 8, respectively.

Rainfall (mm yr <sup>-1</sup> )	CO <sub>2</sub> - treatment (µatm)	Average pH	Average cumulative TA (mol m <sup>-2</sup> )		Average cumulative DOC (mol m <sup>-2</sup> )		Average cumulativ DSi (mol m <sup>-2</sup> )	
600	440	$8.2 \pm 0.2$	2.5 =	± 0.3	7.5	± 0.1	0.24 =	± 0.02
	15,000	$7.1 \pm 0.2$	2.6 =	± 0.3	8.4	$\pm 0.6$	0.30 =	± 0.02
	150,000	$6.8\pm0.2$	4.1 =	± 0.2	7.9	$\pm 0.5$	$0.33 \pm 0.01$	
1200	440	$8.2 \pm 0.3$	$3.4 \pm 0.3$		$7.8 \pm 0.4$		$0.34 \pm 0.01$	
	15,000	$7.1 \pm 0.1$	$4.1 \pm 0.1$		$10.2 \pm 0.3$		$0.45 \pm 0.01$	
	150,000	$6.8 \pm 0.1$	$6.1 \pm 0.5$		$9.2 \pm 0.5$		$0.50\pm0.05$	
2400	440	$8.2 \pm 0.2$	4.9 =	± 0.2	$10.3 \pm 0.6$		$0.52 \pm 0.02$	
	15,000	$7.1 \pm 0.1$	7.1 =	± 1.1	$13.2 \pm 1.1$		$0.75 \pm 0.10$	
	150,000	$6.9 \pm 0.1$	$9.8 \pm 0.9$		12.7	± 1.4	$0.83 \pm 0.03$	
Two-v	vay ANOVA re	sults	F	р	F	р	F	р
	CO <sub>2</sub>		73.22	< 0.001	18.84	< 0.001	50.78	< 0.001
Rainfall		139.54	< 0.001	76.86	< 0.001	244.34	< 0.001	
(	CO <sub>2</sub> x Rainfall		7.15	0.0012	1.90	0.154	6.72	0.0017

Constant variable	Varied variable	p-value
2400 mm/yr	15,000:150,000	< 0.001
	15,000:440	0.0024
	440:150,000	< 0.001
1200 mm/yr	15,000:150,000	0.0058
	15,000:440	0.8366
	440:150,000	< 0.001
600 mm/yr	15,000:150,000	0.0706
	15,000:440	1
	440:150,000	0.0418

Table S2. 6: P-value after Tukey's test for cumulative TA between  $CO_2$  levels and rainfall regimes. The red color represents that there is no significant difference between the means of the group.

*Table S2. 7: P-value after Tukey's test for cumulative DSi between CO*<sub>2</sub> *levels and rainfall regimes.* 

Constant variable	Varied variable	p-value
2400 mm/yr	15,000:150,000	0.2864
	15,000:440	< 0.001
	440:150,000	< 0.001
1200 mm/yr	15,000:150,000	0.8563
	15,000:440	0.063
	440:150,000	0.0032
600 mm/yr	15,000:150,000	0.9969
	15,000:440	0.6679
	440:150,000	0.2604

*Table S2. 8: P-value after Tukey's test for cumulative DOC between CO<sub>2</sub> levels and rainfall regimes.* 

Constant variable	Varied variable	p-value
2400 mm/yr	15,000:150,000	0.9979
	15,000:440	0.0038
	440:150,000	0.0165
1200 mm/yr	15,000:150,000	0.722
	15,000:440	0.0179
	440:150,000	0.3979
600 mm/yr	15,000:150,000	0.9949
	15,000:440	0.788
	440:150,000	0.9955

Constant variable	Varied variable	p-value
2400 mm/yr	15,000:150,000	0.7995
	15,000:440	< 0.001
	440:150,000	< 0.001
1200 mm/yr	15,000:150,000	0.1161
	150,000:440	0.8762
	440:150,000	0.0069
600 mm/yr	15,000:150,000	0.6234
	15,000:440	0.0237
	440:150,000	< 0.001

*Table S2. 9: P-value after Tukey's test for cumulative*  $Ca^{2+}$  *between*  $CO_2$  *levels and rainfall regimes.* 

*Table S2. 10: Mean outflow-inflow water ratio of each ion. The ratio is below and above one referring to retaining tendency and net loss, respectively.* 

Rainfall (mm yr <sup>-1</sup> )	CO <sub>2</sub> -treatment (µatm)	Ca <sup>2+</sup>	$Mg^{2+}$	$K^+$	$Na^+$	Cl-	SO4 <sup>2-</sup>
600	440	1.45	0.88	0.95	0.86	0.85	1.14
	15,000	1.85	0.84	0.96	0.79	0.69	1.11
	150,000	2.17	0.89	1.05	0.85	0.73	1.24
1200	440	1.70	0.86	0.98	0.93	0.90	1.10
	15,000	1.98	0.88	1.01	0.89	1.10	1.16
	150,000	2.31	0.90	1.08	0.85	0.60	0.95
2400	440	1.81	0.91	0.92	0.98	0.98	0.98
	15,000	2.87	1.13	1.35	1.26	1.55	1.37
	150,000	3.15	1.03	1.20	0.88	0.90	0.93

Table S2. 11: Mean total ions release (mol  $m^2$ ) from soil columns experiment (mean  $\pm$  SD). The negative value represents ion retaining within the soil columns and the positive value represents net ion loss. N/D refers to no data.

Rainfall (mm yr <sup>-1</sup> )	CO <sub>2</sub> - treatment (µatm)	Ca <sup>2+</sup>	$Mg^{2+}$	$K^+$	Na <sup>+</sup>	Cl-	NO <sub>3</sub> -*	SO4 <sup>2-</sup>
		$1.57 \pm$	$-1.90 \pm$	-0.15 ±	$-15.88 \pm$	-21.03 ±	$0.03 \pm 0.08$	$0.82 \pm 0.66$
600	440	0.48	0.66	0.23	4.38	5.30	(n=21)	(n=36)
		(n=36)	(n=36)	(n=36)	(n=30)	(n=30)	(11-21)	(II-30)
		$2.77 \pm$	$-2.82 \pm$	$-0.15 \pm$	$-30.24 \pm$	-77.45 ±		$0.75 \pm 0.27$
	15,000	0.03	0.15	0.01	0.46	7.26	N/D	(n=36)
		(n=36)	(n=36)	(n=36)	(n=36)	(n=36)		(11 50)
		$3.36 \pm$	$-1.82 \pm$	$0.15 \pm 0.02$	$-21.21 \pm$	$-46.34 \pm$		$144 \pm 0.05$
	150,000	0.02	0.10	(n=36)	0.07	1.75	N/D	(n=36)
		(n=36)	(n=36)	(11 50)	(n=36)	(n=36)		(1 50)
		$2.63 \pm$	$-2.72 \pm$	$-0.08 \pm$	-9.31 ±	$-16.33 \pm$	$0.17 \pm 0.03$	$0.82 \pm 0.18$
1200	200 440 0.1		0.28	0.05	2.40	4.67	(n=21)	(n=36)
		(n=36)	(n=36)	(n=36)	(n=30) (n=30) (n=30)		(11 21)	(1 50)
		$3.07 \pm$	$-2.06 \pm$	$0.03 \pm 0.23$	$-15.00 \pm$	$19.55 \pm$		$1.02 \pm 0.60$
	15,000	0.36	0.91	(n=36)	7.90	15.28	N/D	(n=36)
		(n=36)	(n=36)	(1 00)	(n=36)	(n=36)		(1 20)
		$4.02 \pm$	$-1.80 \pm$	$0.25 \pm 0.08$	$-23.92 \pm$	$-76.22 \pm$	/	$-0.43\pm$
	150,000	0.05	0.64	(n=36)	4.89	4.48	N/D	0.71
		(n=36)	(n=36)	(1.00)	(n=36)	(n=36)		(n=36)
<b>2 4</b> 0 0	1.10	$3.07 \pm$	$-1.65 \pm$	$-0.38 \pm$	$-2.22 \pm$	$-3.32 \pm$	$0.11 \pm 0.05$	$-0.13 \pm$
2400	440	0.28	0.52	0.07	4.13	5.10	(n=21)	0.24
		(n=36)	(n=36)	(n=36)	(n=30)	(n=30)		(n=36)
	15.000	$5.11 \pm$	$2.15 \pm 0.14$	$1.03 \pm 0.05$	$28.72 \pm$	92.96 ±		$2.31 \pm 0.48$
	15,000	0.28	(n=36)	(n=36)	5.68	25.39	N/D	(n=36)
		(n=36)			(n=36)	(n=30)		
	150,000	$5.8/\pm$	$0.46 \pm 0.06$	$0.66 \pm 0.05$	$-16.05 \pm$	$-6.09 \pm$		$-0.63 \pm$
	150,000	0.11	(n=24)	(n=24)	2.17	0.83	N/D	0.44
		(n=24)			(n=36)	(n=24)		(n=24)

\*Note the inflow water does not contain NO<sub>3</sub><sup>-</sup>. The reported values were calculated from the outflow water.

## Figure



Figure S2. 1: In the ambient conditions experiment, there are two setups, but this paper only discusses the columns on the right side. Each row represents a different rainfall regime, and triplicates of each treatment.



Figure S2. 2: The experimental setup for the  $CO_2$ -treated condition in the gas-tight box. It is designed to control the atmospheric condition within the sealed container and allow the user to perform the work with two-sided gloves. The custom-made Lexan polycarbonate box is composed of two different parts; the vacuum chamber (30 (L) x 26 (W) x 26 (H) cm) and the main compartment (72.2 (L) x 50 (W) x 81.4 (H) cm). The vacuum chamber is mainly used to transport objects in and out of the main channel. The water inlet tubes are connected between columns and peristaltic pumps on one side of the box.



Figure S2. 3: Time series data for absolute TA per unit area of all columns. The green, gray, and brown colors represent 2400, 1200, and 600 mm yr<sup>-1</sup> setups respectively. The circle, square, and triangular markers represent 440, 15,000, and 150,000  $\mu$ atm CO<sub>2</sub> setups respectively. Left: ambient- CO<sub>2</sub> (440  $\mu$ atm) setup. Middle: 15,000  $\mu$ atm CO<sub>2</sub>. Right: 150,000  $\mu$ atm CO<sub>2</sub>. The legend showed the simplified name (Table 2. 1).



Figure S2. 4: Time series data for DSi per unit area of all columns. Left: ambient-  $CO_2$  (440 µatm) setup. Middle: 15,000 µatm  $CO_2$ . Right: 150,000 µatm  $CO_2$ . The legend showed the simplified name (Table 2. 1).



Figure S2. 5: Box plot of TA concentration across CO<sub>2</sub> levels from all the sampling points



Figure S2. 6: Time series data for DOC per unit area of all columns. Left: ambient-  $CO_2$  (440 µatm) setup. Middle: 15,000 µatm  $CO_2$ . Right: 150,000 µatm  $CO_2$ . The legend showed the simplified name (Table 1). For DOC analysis, 15 mL of water samples were filtered through the glass microfibre GF/F with 0.7 µm (Whatman®) and subsequently measured by the Total organic carbon analyzer (TOC-VCSH Shimadzu Corporation, Japan).

# **Chapter III: Fresh Biogenic Carbonates Addition on Salt Marsh Soil and Effect on TA Generation**

## **Chapter Summary**

Salt marshes have recently been highlighted as storage for allochthonous carbonate, presenting the potential for CO<sub>2</sub> removal and enhancing alkalinity production through mineral dissolution processes. However, there is a limited understanding of the quantitative contribution of biogenic carbonates to alkalinity production in salt marsh, unlike in other blue carbon ecosystems. To address this gap, we conducted a two-factorial lab-based soil column experiment, examining the impact of fragmented shell debris under varying rainfall gradients on TA generation in organic-poor minerogenic salt marsh soil. Contrary to our initial hypothesis that sediment enriched with carbonate shells would amplify TA generation with increasing rainfall gradients, our findings revealed a negative effect of adding mussel shells on increasing TA. The primary inorganic carbon sources for mineral dissolution processes and TA generation might be soil carbonates and atmospheric  $CO_2$ . This implies the significance of factors such as shorter water exposure duration and larger grain size of mussel shells, exerting a more pronounced influence on the susceptibility of minerals to chemical dissolution processes in this experimental setting. Despite these observations, we assert that the role of biogenic carbonates should not be overlooked in assessing the efficacy of salt marshes in mitigating anthropogenic CO<sub>2</sub> removal. In the context of blue carbon, future studies should delve into a more comprehensive investigation of the carbonate budget and dynamics and elucidate how these processes impact TA export to coastal ecosystems.

**Keywords**: Salt Marsh, Alkalinity, Blue Carbon, Mineral Dissolution, Soil Carbonate, Biogenic Carbonate

#### 1. Introduction

Salt marshes accumulate inorganic carbon rather than producing carbonate by calcifying microorganisms but mainly from allochthonous carbonate shell debris (biogenic carbonates) through the translocation of storm surges, subsequently trapped by vegetation (Allen, 1987; Saderne et al., 2019). Notably, north-western European salt marshes such as the Scheldt estuary exhibited a high burial rate of shell fragments (Zwolsman et al., 1993), and the Hamburger Hallig salt marsh in Germany showed a dense deposition of fragmented shell debris in deep soil core levels, supporting early marsh development (Mueller et al., 2023). Additionally, in the outer area of the Elbe estuary within the Wadden Sea region, the presence of fragmented carbonate shells results from the intense predation of mollusks by birds, crabs, and fish, exhibiting a diverse range of sizes from <0.1 to 8 mm (Cadée, 1994).

Salt marsh has a significant capacity to sequester atmospheric CO<sub>2</sub> by storing it as organic carbon in sediment (McLeod et al., 2011; Macreadie et al., 2021), showing a high carbon sequestration rate among other blue carbon ecosystems (Ouyang & Lee, 2014). In addition, the role of carbonate minerals through the dissolution process in the salt marsh sediment enhances the overall capacity for CO<sub>2</sub> removal. Salt marshes are likely to be potential sites of net carbonate dissolution (Saderne et al., 2019). The dissolution of carbonate minerals captures CO<sub>2</sub> and converts it into dissolved inorganic carbon (DIC) or bicarbonate (HCO<sub>3</sub><sup>-</sup>) (see Chapter I, Equation 1. 3).

Previous studies highlighted salt marsh as a source of alkalinity production through organic matter degradation via an anaerobic respiration process (Yau et al., 2022; Reithmaier et al., 2023). Research in US marshes has proposed sulfate reduction as the primary biogeochemical pathway for total alkalinity (TA) production (Hines et al., 1989; Cai & Wang, 1998; Koretsky et al., 2003; Reithmaier et al., 2021), while limited reports in the salt marsh have highlighted carbonate mineral dissolution as the primary source of alkalinity generation (Weiss, 2013). Particularly in the Elbe estuary, identified as an organic-poor minerogenic salt marsh (Esselink et al., 2017), TA production is predominantly driven by carbonate sediment (Weiss, 2013). The dissolution of minerals in sediment is stimulated by the production of CO<sub>2</sub> around the rhizosphere (Koop-Jakobsen et al., 2018) or through the degradation of organic matter, resulting in porewater acidification and subsequent alkalinity generation. Alkalinity export,

mainly present as HCO<sub>3</sub><sup>-</sup>, contributes significantly to enhancing the ocean's long-term capacity to sequester atmospheric CO<sub>2</sub> and serves as a buffer against ocean acidification (Hartmann et al., 2013; Middelburg et al., 2020; Saderne et al., 2021; Reithmaier et al., 2023; Sulpis & Middelburg, 2023). This underlines the crucial role of carbonate in directly and indirectly driving atmospheric CO<sub>2</sub> removal through mineral dissolution and alkalinity production (Sulpis & Middelburg, 2023); however, this has been widely ignored in BCE research on CO<sub>2</sub> removal.

Even though the carbonate mineral dissolution process was thoroughly investigated in many aspects, quantitative analyses of the feedback from biogenic carbonates on alkalinity generation, especially in the salt marsh, remain limited compared to other blue carbon ecosystems (Van Dam et al., 2019; Saderne et al., 2021; Mueller et al., 2023). Therefore, this study investigated the effect of adding fresh biogenic carbonates (mussel shell debris) on the alkalinity generation of organic-poor minerogenic salt marsh soil. We conducted a lab-based soil column experiment on different levels of rainfall intensity. We hypothesized that the presence of mussel shells will significantly enhance TA generation in salt marsh soil; however, the mineral source responsible for TA generation primarily originates from soil carbonates.

#### 2. Materials and methods

To examine the influence of carbonate shell deposition on salt marsh soil with regard to the production of TA, a laboratory-based column experiment was conducted employing a two-factorial design. The experimental setup was composed of adding non-mussel shells and mussel shells, with each treatment subjected to three distinct rainfall regimes representative of rainfall levels at the site of interest (600, 1200, and 2400 mm yr<sup>-1</sup>). The soil samples were obtained from a minerogenic salt marsh site in the Elbe estuary in Germany. Throughout the experiment, the composition of the outflow water was monitored using various water chemistry analytical methods.

## 2.1 Site description and soil sampling

The study site is identical to Chapter II, and soil sampling was conducted on the same day as the experiment outlined in Chapter II, Section 2.1. Soil samples were stored at 4°C before the experiment.

## 2.2 Mineralogical composition of mussel shells

The mineralogical composition and quantity of significant elements data of the mussel shells were drawn from the literature (Cubillas et al., 2005). Mussel shells are solely composed of carbonate minerals and contain a high content of Ca (Table 3. 1). Salt marsh mineralogical composition and major constituents can be found in Chapter II, Section 2.1 and Supporting Information Figure S2. 1.

Table 3. 1: Mussel shells mineralogical and chemical composition

Mineralogical	Major constituents (mass %)												
composition	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	MnO	MgO	CaO	Na <sub>2</sub> O	K <sub>2</sub> O	TiO <sub>2</sub>	P <sub>2</sub> O <sub>5</sub>	SO <sub>3</sub>	L.O.I <sup>a</sup>	Sum
Calcite (90%),													
Aragonite	0.00	0.10	0.10	0.00	0.20	53.70	0.20	0.00	0.00	0.00	-	46.00	100.30
(10%)													

<sup>a</sup>Loss on ignition. It is accounted for the mass lost from such volatile substances during the heating process (Lechler & Desilets, 1987).

## 2.3 Packing column and subsampling

The soil columns followed the design from Chapter II, including packing and subsampling methods (see Section 2.2, Chapter II). The experiment consisted of 18 columns, divided into two sets: one without mussel shells (B) and one with mussel shells (M). Two kilograms of fresh mussels from the North Sea were purchased from the supermarket in Oct 2021, shells air-dried after rinsing, ground, and sieved into five size ranges (<500  $\mu$ m, 500 – 800  $\mu$ m, 800  $\mu$ m – 1 mm, 1 – 1.25 mm, and 1.25 -2 mm), and 4 g (3.5% w/w) of each range mixed in a 1:1:1:1:1 ratio for amendment on the top layer of the soil columns (see Supporting Information, Table S3. 1).

### 2.4 Hydrological regimes and water distribution

Mirroring the methodology detailed in Chapter II, the present experiment incorporated identical types of inflow water (ARW and ASW), hydrological regimes, watering cycles, and watering techniques (see Section 2.3, Chapter II). All soil columns were randomly assigned to different hydrological regimes (varied ARW and fixed ASW) as shown in Table 3. 2.

Mussel shells	ells Rainfall Simplified Solid in columns Replicate		Replicates	Experimental	Amount of water inflow per cycle		
treatments	$(mm yr^{-1})$	name		1	period(days)	ARW (mL)	ASW (mL)
	600	BL	unpretreated soil			59.5	
Non-mussel	ssel 1200 BM unpretreated soil			119			
shells addition	2400	BH	unpretreated soil			237.5	
	600	ML	unpretreated soil + ground mussel shells	3	168	59.5	71.2
Mussel shells addition	1200	MM	unpretreated soil + ground mussel shells	ls		119	
	2400	MH	unpretreated soil + ground mussel shells			237.5	

Table 3. 2: The series of soil columns and parameters

2.5 Experimental setup, water sampling, and chemistry analysis

During the experiment, all 18 columns were positioned within a windowless laboratory room similar condition to the experiment in Chapter II (see Section 2.4 and see Supporting Information Figure S2. 1). The stock solutions of ARW and ASW were introduced directly into the columns using a peristaltic pump (ISMATEC® MCP-Process IP-65), following the designated hydrological regimes. Initially, the pH of the solution and TA were in equilibrium with the laboratory room's ambient conditions, with average values of  $6.4 \pm 0.3$  and  $60 \pm 16 \mu mol/kg$ , respectively (mean  $\pm$  SD). Water sampling and chemical analysis followed the same procedures outlined in Chapter II (see Section 2.5). The carbon isotopic composition of DIC ( $\delta^{13}$ C-DIC) referred to a molar ratio of heavier isotope ( $^{13}$ C) to lighter isotope ( $^{12}$ C) and was analyzed by cavity ring-down spectroscopy (CRDS) Picarro. Three types of certified reference material (CRM) used for constructing the calibration curve in this experiment were NBS18, NBS19, and CaCO<sub>3</sub> with certified  $\delta^{13}$ C-VPDB values of -5.014 ‰, 1.95 ‰, and -9.650 ‰. The external calibration was measured daily within 0.5 – 2.0 mg of CRM.

## 2.6 TA leaching and total ion release calculation

TA definition for this chapter followed the acid-based systems of carbonate alkalinity and noncarbonate alkalinity (Dickson, 1981) (see Equation 1. 1, Chapter I). The quantification of the absolute amount of TA leaching and cumulative total ion release resulting from the soil column experiment was performed using Equation 2. 1 and Equation 2. 3 as detailed in Section 2.6, Chapter II, respectively.

#### 2.7 Saturation Indices (SI)

The geochemical program PhreeqC (version 3) (Parkhurst & Appelo, 2013) was used to calculate the saturation indices (SI) of the relevant minerals such as calcite and aragonite in the water inflow and outflow water samples, and ionic strength of the solution. The computation used the "phreeqc.dat" database and used the alkalinity, major ions, pH, and temperature at 23 °C as input variables. SI refers to the thermodynamic tendency of a particular mineral concerning an aqueous solution and as a function of the based-10 logarithm of saturation state ( $\Omega$ ) (Equation 3. 1).

$$SI = log\left(\frac{a_{Ca^{2+}}a_{CO_{3}^{2-}}}{K_{sp}}\right)$$
 Equation 3. 1

 $a_{Ca2+}$  and  $a_{CO32-}$  are the activity of  $Ca^{2+}$  and  $CO_3^{2-}$  in the aqueous solution and it can be defined by the multiplication between  $\gamma$  (activity coefficient after ionic strength) and concentration of respective ions.  $K_{sp}$  denotes the equilibrium constant of the respective carbonate minerals. In other words,  $K_{sp}$  is expressed as the stoichiometric concentration of constituents at equilibrium conditions. Thus, SI > 0 (or  $\Omega > 1$ ) indicates a supersaturated condition, SI < 0 (or  $\Omega < 1$ ) undersaturated condition, and SI = 0 (or  $\Omega = 1$ ) equilibrium state.

#### 2.8 Data collection and statistical analysis

Two-way ANOVA was used to analyze data from a two-factorial design experiment (two mussel treatments and three rainfall regimes). Two-way ANOVA was selected to investigate the existence of significant differences and the effect and interaction of adding mussel and

rainfall regimes on cumulative TA and Ca<sup>2+</sup>. Normal distribution was determined visually (Tang et al., 2021) and assessed by the Shapiro-Wilk test. The homogeneity of variance was assessed by using Levene's test. After the assumptions test, two-way ANOVA is subsequently conducted followed by Tukey's test. The statistical analysis was conducted in Python utilizing the statistical package (scipy.stats, statsmodels.formula.api, statsmodels.stats.anova, and statsmodels.stats.multicomp). The significance level at 5% ( $\alpha = 0.05$ ) was used to determine the statistical difference in mean between the experiment series.

## 3. Results

3.1 pH, TA, cumulative Ca<sup>2+</sup> release, and saturation indices of non-mussel and mussel shells addition columns across rainfall regimes

3.1.1 pH

The pH values of all treatment groups exhibited a level of stability at approximately pH 8 throughout the experimental period (Table 3. 3). However, the decline on the 56<sup>th</sup> day was attributed to a modification in the pH measurement methodology, whereby pH readings were directly measured after 30 s. The pH values obtained for the mussel-addition columns closely paralleled those of the non-mussel treatments, as visually depicted in Figure 3. 1A and B. The difference in its initial pH value of the water inflow was represented at two pH units higher.



Figure 3. 1: A.) pH of the outflow water of all the columns over 168 days. The blue and orange colors represent without and with mussel shells addition respectively. The circle, square, and triangle markers represent 2400, 1200, and 600 mm yr<sup>-1</sup> respectively. B.) Box plot and swarm plot of average pH over the experimental period.

#### 3.1.2 TA

The time series data of TA generation as mol TA per area exhibited a gradual decline until the 56<sup>th</sup> day (4<sup>th</sup> sampling), after which it maintained relatively stable for the remaining duration (Figure 3. 2A). Furthermore, the time series data for TA generation and cumulative absolute amount TA generation within the columns containing non-mussel shells closely resembled those observed in the columns with mussel shells across all rainfall setups (Figure 3. 2B and Table 3. 3). Variance analysis after Turkey's test proved that there is no significant difference in the mean cumulative TA levels between the mussel and non-mussel treatment within the same rainfall setup (Figure 3. 2B). There was no significant interaction effect between mussel shells and rainfall.



Figure 3. 2: A.) The absolute amount of TA in the outflow water of all the columns over 168 days. B.) Box plot and swarm plot of the accumulative absolute amount of TA over the experimental period. Tukey's test results are shown in the graphs and ns refers to non-significant differences (p > 0.05).

## 3.1.3 Cumulative Ca<sup>2+</sup> release

 $Ca^{2+}$  ions serve as key indicators for monitoring carbonate dissolution. The analysis revealed no noticeable upward trend in total  $Ca^{2+}$  between columns containing non-mussel and mussel shells (Figure 3. 3). The behavior of total  $Ca^{2+}$  did not exhibit consistency across various rainfall scenarios. Only the non-mussel shell columns demonstrated an ascending trend in  $Ca^{2+}$ release with increasing rainfall regimes. In contrast, other ions (Mg<sup>2+</sup>, K<sup>+</sup>, Na<sup>+</sup>, and Cl<sup>-</sup>) tended to be retained within the soil columns. At the same time,  $SO_4^{2-}$  exhibited a leaching pattern, with an observed ratio of around one (see Supporting Information, Table S3. 2).



Figure 3. 3: Box plot and swarm plot of the cumulative amount of  $Ca^{2+}$  over the experimental period. ns and \*\*\* referred to non-significant difference (p > 0.5), and p < 0.00, respectively.

Table 3. 3: Average pH (n=36) and average cumulative TA and  $Ca^{2+}$  (n=36) (mean ± SD). Two-way ANOVA results are included below (highlighted p-value means there is a significant difference when  $p \le 0.05$ ). Detailed results of Tukey's test of average cumulative TA and  $Ca^{2+}$  can be found in Table S3. 3 and Table S3. 4 in Supporting Information, respectively.

Rainfall regimes (mm yr <sup>-1</sup> )	Mussel shells treatment	Average pH	Ave cumu TA (m	rage lative ol m <sup>-2</sup> )	Ave cumula (mo	erage tive Ca <sup>2+</sup> l m <sup>-2</sup> )	
600	Non-Mussel shells	$8.20\pm0.06$	$2.47 \pm 0.26$		$1.57\pm0.48$		
000	Mussel shells	$8.13\pm0.03$	1.84 =	$1.84 \pm 0.32$		$2.85 \pm 0.25$	
1200	Non-Mussel shells	$8.18\pm0.01$	$3.38\pm0.26$		$2.63 \pm 0.12$		
1200	Mussel shells	$8.18\pm0.02$	$2.98\pm0.34$		$2.92 \pm 0.13$		
2400	Non-Mussel shells	$8.20\pm0.05$	4.91 =	± 0.17	$3.07 \pm 0.28$		
2400	Mussel shells	$8.19\pm0.04$	$4.91\pm0.34$		$2.53 \pm 0.17$		
Tv	vo-way ANOVA results		F	р	F	р	
	Mussel shells		6.40	0.026	7.35	0.012	
	Rainfall		139.73	< 0.001	9.24	0.004	
	1.80	0.207	17.49	< 0.001			

#### 3.1.4 Carbonate Minerals Saturation Indices

The time series SIs for calcite and aragonite are presented in Figure 3. 4. The calculation by PhreeqC shows that the outflow water was supersaturated regarding calcite and aragonite over the whole experiment. In addition, the SIs of all columns were in the same range, and there was no difference between non-mussel shells or mussel shells-amended columns.



Figure 3. 4: Saturation indices of calcite (upper) and aragonite (lower). The calculation is based on the database of Parkhurst and Appelo (Parkhurst & Appelo, 2013) in PhreeqC and the composition of the outflow water. From day 14 to 140, the calculation included the Na<sup>+</sup> and Cl<sup>-</sup>. From 154 to 168, Na<sup>+</sup> and Cl<sup>-</sup> were excluded.

# 3.2 TA: DIC ratio and $\delta^{13}$ C-DIC in the outflow water

The relationship between measured TA and DIC ratio from the samples on the 14<sup>th</sup>, 83<sup>rd</sup>, and 168<sup>th</sup> days falls into the area between the theoretical TA: DIC of calcium carbonate dissolution (slope = 2) and sulfate reduction (slope =1) (Figure 3. 5A). On average, measured TA values were higher than measured DIC values, with differences ranging from -66% to 10%. The carbon isotopic composition of all treatments did not show a distinct isotopic signal. The results showed that the  $\delta^{13}$ C-DIC of all the columns was around -8 to -11 ‰ with the cluster at around -10 ‰ (Figure 3. 5B).



Figure 3. 5: A.) The scatter plot of TA vs. DIC of the outflow water and theoretical line for the biogeochemical processes (Chen & Wang, 1999). The plot does not differentiate the sampling points. B.) The inverted box plot showing the range of  $\delta^{13}$ C-DIC from all columns (BH, MH, BM, MM, BL, BM) on day  $83^{rd}$  (6<sup>th</sup> sampling). \* $\delta^{13}$ C of soil inorganic carbon is between -10 to 0‰ (Salomons & Mook, 1976; Cerling, 1984; Ramnarine et al., 2012). \*\* $\delta^{13}$ C of atmospheric CO<sub>2</sub> is between -9 to -8‰ (Cerling et al., 1991). \*\* $\delta^{13}$ C of soil organic carbon from the salt marsh of the Elbe estuary ranges between -25 to -21‰ (Mueller et al., 2023). \*\*\* $\delta^{13}$ C of mussel shells in the North Sea region is between -7 to -2‰ (Gillikin et al., 2006).

#### 4. Discussion

4.1 Soil carbonates control TA signal over fresh mussel shells (biogenic carbonates)

The deposition of mussel shells on the top layer of salt marsh soil did not significantly increase TA leaching in this experimental setup (Figure 3. 2A and B). The release of Ca<sup>2+</sup> ions also remained unchanged when columns were amended with mussel shells, even across varying rainfall gradients. These findings suggest that the deposition of mussel shells on the soil surface may not substantially contribute to TA generation in this soil column experiment. This finding contradicts previous studies that have reported increased alkalinity generation through the dissolution of biogenic shells (Uster et al., 2015).

The observed increase in the pH of outflow water compared to the inflow solution may indicate soil carbonate dissolution occurring within the soil column. The presence of supersaturated conditions for calcite and aragonite in the outflow water, irrespective of the experimental setups, indicated that further dissolution of carbonate minerals was halted (Figure 3. 4). Soil carbonates, which are likely more susceptible to dissolution (Briese et al., 2017), may have dissolved first, driving the system to a supersaturated state. Meanwhile, other mineral sources, such as biogenic carbonates, likely remained unweathered. The lack of significant TA

generation from mussel shell dissolution in these soil columns may be due to the duration of water exposure and the grain size of the shells. Theoretically, residence time is critical in controlling dissolution processes (Maher, 2010). In this experiment, the mussel shells placed on the top layer of salt marsh soil (1–2 cm) were in contact with water for a shorter time, reducing their susceptibility to chemical dissolution. Conversely, the longer residence time of water passing through the soil particles likely enhances the interaction between water and soil minerals, promoting greater dissolution within the soil matrix. The chemical dissolution rate is inversely related to grain size (Senna, 1989; Israeli & Emmanuel, 2018). Given that the crushed shells have a larger grain size than the fine minerals in the soil, mineral dissolution is likely more pronounced in the salt marsh soil layer than in the mussel shells.

A previous study estimated a high carbonate content dissolution rate in the Scheldt estuary within the Wadden Sea (Vranken et al., 1990). Field observations in mangrove ecosystems have further demonstrated a substantial contribution of carbonate sediments to TA production through carbonate dissolution (Saderne et al., 2021). It is suggested that the carbonate contributing to TA might originate from old shells deposited long ago, which must first undergo breakdown into smaller particles to become more susceptible to chemical dissolution and responsible for TA production in the marshland. Notably, the erosion rate positively correlates with chemical dissolution, as highlighted in several studies (Maher, 2010; Hack, 2020).

The breakdown of shells encompasses various processes, including physical breakdown through waves and currents, bioerosion, and chemical and biological weathering (Lescinsky et al., 2002; Zuschin et al., 2003). Moreover, the processes can significantly depend on various factors and specific conditions in the marshland, spanning several years (Říhová et al., 2018). This complexity implies that carbonate shells may not undergo an abrupt chemical dissolution process; instead, it may require considerable time before the shells become susceptible to dissolution and contribute to TA or DIC through the estuarine channel.

In this soil columns experiment, only the change of hydrological gradients (rainfall) led to an increase in the TA leaching, regardless of mussel addition (Figure 3. 2). The greater amount of water tends to remove a greater amount of mineral dissolution products and other constituents

(i.e.,  $Ca^{2+}$  (Figure 3. 3)), causing a shift of thermodynamic equilibrium and accelerating chemical dissolution rates (Maher, 2010).

#### 4.2 Inorganic carbon source responsible for TA generation

The relationship between measured TA and DIC (Figure 3. 5A) suggests that TA production results from a combination of net mineral dissolution and redox processes (Saderne et al., 2021). However, we were unable to quantify the proportion of TA generated specifically from mineral dissolution in this study. Based on findings from Chapter II, mineral dissolution appears to be the dominant process driving TA production in the minerogenic salt marsh soils of the Elbe estuary. Therefore, we considered stable isotopic data to further identify the mineral sources contributing to TA.

The isotopic signature of  $\delta^{13}$ C-DIC reflects a mixture of CO<sub>2</sub> and carbonate species resulting from mineral dissolution processes. In the case of carbonate dissolution involving dissolved CO<sub>2</sub>, approximately half of the DIC originates from minerals, with the remaining portion derived from CO<sub>2</sub> sources. In contrast, DIC produced from silicate dissolution is entirely derived from CO<sub>2</sub>. In this experiment, the primary sources of inorganic carbon include atmospheric CO<sub>2</sub> (introduced through inflow water) and respired CO<sub>2</sub> from organic matter decomposition, as well as inorganic carbon from soil carbonates (found in salt marsh soil) and mussel shells. The  $\delta^{13}$ C of atmospheric CO<sub>2</sub> is typically between -9‰ and -8‰ (Cerling et al., 1991), while the  $\delta^{13}$ C of respired CO<sub>2</sub> mirrors that of soil organic carbon (SOC), with values in the Elbe estuary salt marsh soil ranging from -25 to -21 ‰ (Mueller et al., 2023). Soil carbonates  $\delta^{13}$ C have been reported to range from -10‰ to 0‰, heavily influenced by parent material and soil processes (Salomons & Mook, 1976; Cerling, 1984; Ramnarine et al., 2012). Additionally, the  $\delta^{13}$ C signature of mussel shells in the Scheldt estuary within the North Sea region varies from -7‰ to -2‰, depending significantly on the carbon sources in the mussels' growing environment (Gillikin et al., 2006).

We assumed the  $\delta^{13}$ C-DIC endmember for CO<sub>2</sub>-induced soil carbonate dissolution to range from approximately -9‰ to -4.5‰ when atmospheric CO<sub>2</sub> is the primary dissolution agent, and from -15.5‰ to -12‰ when respired CO<sub>2</sub> is the primary agent. Similarly, we assumed the  $\delta^{13}$ C-DIC endmember for CO<sub>2</sub>-induced dissolution of mussel shells (excluding contributions from soil carbonate) to range from approximately -8% to -5% when atmospheric CO<sub>2</sub> is the main agent, and from -16% to -12.5% when respired CO<sub>2</sub> is the primary agent.

The carbon isotopic composition showed enrichment in <sup>13</sup>C (Figure 3. 5B), making it difficult to precisely identify the mineral source of DIC based on this data alone. The contribution of mussel shells to DIC cannot be conclusively determined through stable isotopic analysis, as actual isotopic signatures for soil carbonate and mussel shells were unavailable in this study, and existing literature values for these sources overlap considerably. However, our findings suggest that respired CO<sub>2</sub> from microbial activity was likely not the main source of CO<sub>2</sub> for mineral dissolution in this column experiment. Instead, atmospheric CO<sub>2</sub> introduced through inflow water is proposed as the primary dissolution agent, as the observed  $\delta^{13}$ C-DIC values align with the expected  $\delta^{13}$ C-DIC endmember ranges for atmospheric CO<sub>2</sub>-induced mineral dissolution.

Considering TA leaching and Ca<sup>2+</sup> release patterns (Figure 3. 2 and Figure 3. 3), we speculated that mussel shells do not significantly contribute to TA generation. In this soil columns experiment, we assumed that soil carbonates emerged as the possible primary source responsible for TA generation. This assumption aligns well with previous research findings, as Chapter II discussed (Weiss, 2013), which suggest that carbonates are likely the primary driver of TA in the Elbe estuary.

#### 4.3 Implications

Overall, this present study provided evidence that the fresh biogenic carbonates on the topsoil might not be relevant to an increase in TA production, contrary to our initial hypothesis. Our findings do not clearly indicate the actual inorganic carbon source. However, in this experiment, we postulated that soil carbonate and atmospheric CO<sub>2</sub> might represent the primary sources driving mineral dissolution and TA generation. The absence of carbonate shell contribution to TA may be related to less water exposure time and larger grain size, causing the shells less susceptibility to dissolution. This might not imply the negligible role of fragmented shell debris on TA generation, we assumed that the breakdown of substantial shell fragments is a prerequisite before undergoing the dissolution process, a transformation that may extend over an extended period, ranging from days to years in the natural system. Overall,

future studies should focus on understanding the role of carbonate minerals in salt marsh ecosystems, investigating its budget, dissolution rate, and reprecipitation in soil, and examining how these dynamics contribute to TA export to coastal ecosystems and the ocean's capacity for  $CO_2$  removal.

## **Supporting Information**

Table

Table S3.	1:	The	amount of	of.	solid	in	the	columns
				./				

		g Sedi	ment			20 g Mus	ssel shells (1	:1:1:1:1)	
Simplified name	0.5 am	5 10 am	10.17 am	Total	< 500 um	500 -	800 µm -	1 - 1.25	1.25 - 2
	0-3 cm	3-10 CIII	10-17 cm	Total	< 300 µm	800 µm	1mm	mm	mm
BH_I	171.47	145.93	232.78	550.18	-	-	-	-	-
BH_II	169.86	155.04	225.98	550.88	-	-	-	-	-
BH_III	165.92	156.28	228.61	550.81	-	-	-	-	-
BM_I	168.97	150.24	231.58	550.79	-	-	-	-	-
BM_II	158.83	158.94	233.12	550.89	-	-	-	-	-
BM_III	149.38	162.94	238.12	550.44	-	-	-	-	-
BL_I	188.31	149.91	212.57	550.79	-	-	-	-	-
BL_II	162.54	150.33	238.82	551.69	-	-	-	-	-
BL_III	163.74	161.74	224.68	550.16	-	-	-	-	-
MH_I	172.76	147.65	229.91	550.32	4	4	4.01	3.99	4.008
MH_II	146.94	157.04	246.15	550.09	4	4	4	4	4.001
MH_III	168.79	152.27	229.57	550.63	4	4.004	4	4	4
MM_I	172.41	157.62	220.39	550.42	3.999	3.999	4	4.002	4.003
MM_II	189.66	148.49	212.73	550.88	4.003	4.007	4.002	4.008	3.996
MM_III	172.39	147.54	230.71	550.64	4.003	3.997	4.001	4.002	4.004
ML_I	169.36	154.75	226.3	550.41	3.999	4.001	4.001	4	4.006
ML_II	160.45	169.04	221.13	550.62	4.002	4.007	4.002	4.007	4.007
ML_III	167.96	157.59	224.7	550.25	4.002	4.007	3.999	4.001	4.002

Rainfall regimes (mm yr <sup>-1</sup> )	Mussel shells treatment	Ca <sup>2+</sup> (n=36)	Mg <sup>2+</sup> (n=36)	K <sup>+</sup> (n=36)	Na <sup>+</sup> (n=30)	Cl <sup>-</sup> (n=30)	SO4 <sup>2-</sup> (n=36)
600	Non Mussel shells	$1.45 \pm$	$0.88 \pm$	$0.95 \pm$	$0.86 \pm$	$0.85 \pm$	$1.14 \pm$
000	Non-wusser shens	0.11	0.03	0.06	0.03	0.03	0.09
	Mussel shalls	$1.90 \pm$	$0.99 \pm$	$1.05 \pm$	$0.96 \pm$	$0.95 \pm$	$1.38 \pm$
	Mussel silens	0.10	0.02	0.02	0.04	0.04	0.03
1200	Non Mussel shalls	$1.70 \pm$	$0.86 \pm$	$0.98 \pm$	$0.93 \pm$	$0.90 \pm$	$1.10 \pm$
1200	Non-wusser snens	0.02	0.01	0.01	0.02	0.02	0.02
	Mussal shalls	$1.80 \pm$	$0.82 \pm$	$0.93 \pm$	$0.88 \pm$	$0.85 \pm$	$1.02 \pm$
	Mussel shens	0.04	0.01	0.02	0.02	0.03	0.02
2400	Non Mussel shalls	$1.81 \pm$	0.91 ±	$0.92 \pm$	$0.98 \pm$	$0.98 \pm$	$0.98 \pm$
2400	Non-wusser shens	0.06	0.02	0.01	0.03	0.03	0.02
	M	$1.66 \pm$	$0.89 \pm$	$0.85 \pm$	$0.93 \pm$	$0.92 \pm$	$0.95 \pm$
	Mussel shells	0.04	0.03	0.05	0.05	0.05	0.03

*Table S3. 2: Mean total outflow-inflow ratio of each ion (mean*  $\pm$  *SD). The ratio is below and above one referring to retaining tendency and net loss, respectively.* 

Table S3. 3: P-value after two-way ANOVA approach and Tukey's test for accumulative TA between rainfall regimes and mussel treatment. Red color represents that there is no significant difference between the means of the group.

Category	Group	P-value
High	Non-mussel: Mussel	1
Med	Non-mussel: Mussel	0.5635
Low	Non-mussel: Mussel	0.1517

Table S3. 4: P-value after two-way ANOVA approach and Tukey's test for accumulative  $Ca^{2+}$  release between rainfall regimes and mussel treatment. Red color represents that there is no significant difference between the means of the group.

Category	Group	P-value
High	Non-mussel: Mussel	0.2018
Med	Non-mussel: Mussel	0.77
Low	Non-mussel: Mussel	0.0008

# **Chapter IV: Plants Mediated Alkalinity Enhancement in Salt Marsh Ecosystem**

## **Chapter Summary**

Plants play a significant role in carbon sequestration and influence key processes in inorganic carbon dynamics within salt marshes. However, the plant-mediated effects on alkalinity generation via mineral dissolution remain poorly understood. This study evaluated alkalinity generation mediated by salt marsh plants (Spartina x townsendii and Elymus athericus) in organic-poor minerogenic salt marsh soil. Additionally, the effect of adding shell-bearing limestone as stimulation of biogenic carbonates to salt marsh soil in the presence of S. xtownsendii was examined. A combination of methodologies was employed to track changes in total alkalinity (TA) concentration, identify biogeochemical pathways, and monitor rhizosphere CO<sub>2</sub> and pH using planar optode technology. Our results demonstrated a 10-fold increase in TA generation in planted columns compared to non-planted columns, which correlated with elevated CO<sub>2</sub> production around the roots. The  $\delta^{13}$ C-DIC signature of the planted columns suggested that plants potentially induced mineral dissolution and increased alkalinity production. Even with the presence of plants, adding powdered carbonate minerals in the minerogenic salt marsh soil did not significantly enhance TA generation compared to the control condition. However, the  $\delta^{13}$ C-DIC signature implied that the added minerals contributed to the TA. In the context of blue carbon, understanding the rhizosphere traits that facilitate alkalinity generation is essential for comprehending the role of plants in carbon cycling. Furthermore, evaluating the buffering capacity provided by different plant species is crucial for developing strategies to enhance TA production, supporting the restoration of blue carbon ecosystems, and improving coastal ecosystem management and carbon sequestration efforts.

**Keywords:** Salt Marsh, Alkalinity, Blue Carbon, Mineral Dissolution, Spartina x townsendii, Elymus athericus

### 1. Introduction

Recognition of salt marsh or other vegetated coastal ecosystems as a major carbon sequestration site has been acknowledged as a potential strategy for climate change regulation (Nellemann, 2009; Duarte et al., 2013; Macreadie et al., 2021). Their natural function is to sequester a great amount of atmospheric carbon dioxide ( $CO_2$ ) and store a large pool of organic matter within their ecosystems (McLeod et al., 2011). In addition, the focus on carbon outwelling to the coastal waters in terms of alkalinity export has increasingly gained scientific interest due to the potential to be a long-term carbon sink (Middelburg et al., 2020) and this may represent an overlooked mechanism for carbon sequestration (Saderne et al., 2020; Reithmaier et al., 2023). It can enhance the oceanic capacity to remove anthropogenic  $CO_2$  emissions and act as a buffering capacity to prevent ocean acidification (Thomas et al., 2009).

Alkalinity production within salt marsh ecosystems is a function of different biogeochemical processes (Middelburg et al., 2020). The organic carbon pool within the ecosystem is decomposed by anaerobic microbial activities (denitrification or sulfate reduction) releasing carbonate alkalinity ( $HCO_3^-$  or  $CO_3^{2-}$ ). On the other hand, inorganic carbon such as soil carbonates or fragmented calcareous shells within the salt marsh sediment undergoes a dissolution process triggered by microbially respired  $CO_2$  and produces carbonate alkalinity as a by-product.

Plants play a significant role in sequestering carbon and directly and indirectly governing biogeochemical processes relevant to alkalinity generation. Current knowledge indicates that plants exert strong control over organic acids (root exudates) and oxygen release in sediment (Lynch & Whipps, 1990; Colmer, 2003; Hartmann et al., 2009), significantly affecting microbial organic matter decomposition (Wolf et al., 2007; Kuzyakov, 2010). Root-respired CO<sub>2</sub>, a by-product of plant-microbe interactions (rhizosphere processes) (Kuzyakov, 2006), serves as an acidity source, accelerating mineral dissolution within the sediment (Calmels et al., 2014) by facilitating the vertical transport of CO<sub>2</sub> through roots (Wen et al., 2021). While

the effect of vascular plants on mineral dissolution has been widely studied in terrestrial ecosystems, studies are limited in coastal ecosystems. Former reports indicated that seasonal plants highly control bicarbonate production in salt marshes (Wang & Cai, 2004) and governed pore water chemistry (Koretsky et al., 2008). However, experimental evidence is still lacking to illustrate the important link between rhizosphere processes and inorganic carbon dynamics (Mueller et al., 2023).

Dynamics of rhizosphere CO<sub>2</sub> and pH are promising parameters to advance mechanistic and quantitative understanding of the effect of plant-mediated alkalinity generation within the salt marsh ecosystem. The quantitative imaging of roots analysis, the so-called planar optode, can overcome the limitations of the invasive conventional method (Blossfeld & Gansert, 2012). It presents a state-of-the-art technology for non-invasive rhizosphere assessment allowing us to observe the distribution of CO<sub>2</sub> and pH around the roots over time (Blossfeld & Gansert, 2012). However, a few applications of planar optode have already been applied on salt marsh plants (Santner et al., 2015) and it is limited to only *Spartina anglica* (O<sub>2</sub>, CO<sub>2</sub>, and pH) (Koop-Jakobsen & Wenzhöfer, 2015; Koop-Jakobsen et al., 2018) and *Elymus athericus* (only O<sub>2</sub>) (Koop-Jakobsen et al., 2021). The study of their derivative species and some rhizosphere parameters is still limited.

From an ecosystem perspective, the understanding of alkalinity export has largely reported on specific biogeochemical processes driving its production. However, to the best of our knowledge, the role of biota and small-scale biotic interactions, such as those within the marsh rhizosphere, in enhancing alkalinity within salt marsh ecosystems has not been extensively explored. Thus, this study focused on illustrating the effect of salt marsh plants' rhizosphere specifically on alkalinity generation.

This chapter was divided into two sub-experiments. The first experiment aimed to investigate the effects of *Spartina x townsendii* and *Elymus athericus*, on alkalinity enhancement in organic-poor minerogenic salt marsh soil. We addressed three key questions: 1) How much greater is alkalinity generation under planted versus non-planted conditions? 2) Are there species-specific differences in driving alkalinity production? and 3) What is the potential biogeochemical mechanism by which plants control alkalinity generation?

The second experiment expanded on the findings of Chapter III by investigating *S. x townsendii*'s effects on alkalinity production in limestone-added minerogenic salt marsh soil. This research focused on two additional questions: 1) How much higher is alkalinity generation in carbonate-rich soil compared to normal salt marsh soil? and 2) Does *S. x townsendii* enhance alkalinity production when the soil is rich in carbonate? To achieve this, we used a soil-column approach to monitor changes in water chemistry (alkalinity) and identify biogeochemical pathways (using  $\delta^{13}$ C-DIC signatures) over time in planted and non-planted conditions. Additionally, we utilized a novel planar optode technology to observe CO<sub>2</sub> and pH distribution around the roots, providing insight into the influence of plant roots on alkalinity generation processes.

#### 2. Materials and Methods

## 2.1 Sampling site description, soil characteristics, soil sampling

The sampling site was at Hamburger Hallig (54.6020983, 8.8181099), a tidal flat salt marsh within the European Wadden Sea. This salt marsh is characterized as minerogenic due to its high rate of mineral sediment deposition (Esselink et al., 2017). Additionally, this site has been reported that the soil inorganic carbon stocks at this site exceeded the soil organic carbon stocks across all successional gradients. The Hamburger Hallig marsh contained a significant number of calcareous shell fragments within the sediment supporting marsh development (Mueller et al., 2023). The vegetation composition of this location was characterized by *Spartina anglica* dominating in the pioneer zone, *Puccinellia maritima* dominating at the low marsh, and *E. athericus* dominating the high marsh (Mueller et al., 2020). Soil was sampled on Sep 2023 at the low marsh station during low tide by scooping the wet soil and transferring it into a bucket. The soil samples were stored at room temperature until the experiment was conducted.

## 2.2 Vegetation sampling and culturing

*S. x townsendii* was sourced from leftover plants from a study on the distribution of *Spartina* cytotypes along the European Wadden Sea region (Granse et al., 2022). These plants had been re-cultivated in the greenhouse at the University of Hamburg for at least three years before our

columns experiment commenced. *E. athericus* was collected on Sep 2023 in late autumn from the high marsh of Hamburger Hallig (54.6017619, 8.8185737). Four squares of *E. athericus* vegetation measuring 25 x 25 x 25 cm were dug out with a spade and brought back to the greenhouse, where the plants were prepared and cultured. Soils were gently washed away from the roots and rhizomes. Both *S. x townsendii* and *E. athericus* were divided into smaller shoots and replanted directly in pots with the soil that served as the substrate for the respective experiments (Experiment I: salt marsh soil; Experiment II: limestone added salt marsh soil). The plants were left in these conditions for a month to adapt to the experimental environment before the soil columns experiment.

## 2.3 Packing soil columns

These experiments used an identical soil column design as explained in Chapter II. Before the soil packing step, the soil needed to be homogenized. A small amount of water was added to the buckets of sediment, and a power drill with a long stirrer extension was used to mix the soils into a consistent texture. Subsequently, soil was wet sieved through a sifter with 2.50 mm-sized holes to remove rocks, plant detritus, and larger shells. Soil was left in the bucket for a few days, and the excess water on top of the soil layer was removed before packing the soil columns.

#### Experiment I

Approximately 550 g of homogenized salt marsh soil was filled into each column (see Supporting Information, Table S4. 1). For the planted columns, the soil was added until half the column height, the plants were placed into the columns, and then the columns were refilled with the remaining soil, ensuring that all plant roots were covered. The series of soil column experiments were randomly assigned to five columns of non-planted (control, hereafter: S), five columns of *S. x townsendii* treatment (hereafter: SP4), and five columns of *E. athericus* treatment (hereafter: SP3) (Figure 4. 1).

#### Experiment II

The solid in the columns was a mixture of salt marsh soil and 20% w/w of shell-bearing limestone to recreate the condition of soil with relatively high carbonate mineral content.

Limestone was purchased from Mühldorfer Nutrition AG and it was pulverized into the fine powder (<1  $\mu$ m) with the planetary ball mill (PM100, Retsch GmbH). Approximately 440 g of homogenized salt marsh soil mixed with 110 g of powdered limestone were filled into the columns (see Supporting Information, Table S4. 1) and repeated the steps as in Experiment I. The series of soil columns were randomly assigned to five columns of non-planted carbonaterich salt marsh soil (hereafter: SC), and five columns of *S. x townsendii* with limestone-added salt marsh soil (hereafter: SCP4) (Figure 4. 2). For the later analysis, the data of S and SP4 from Experiment I were used to compare the plant effect on alkalinity generation under relatively high carbonate content salt marsh soil.

#### 2.4 Soil columns experiment: Experimental Setup and Water Distribution

Both experiments ran simultaneously and all 25 columns were placed on racks in a greenhouse, maintained under controlled temperatures of 16°C during the day and 14°C at night, from the end of Sep 2023 to mid-Feb 2024. The position of each column was assigned randomly to avoid bias. The greenhouse lights were programmed to turn on daily from 8 am to 4 pm. Throughout the experiment, all columns were watered daily with an identical amount of MilliQ water, which was distributed using a syringe (Figure 4. 1 and Figure 4. 2).



Figure 4. 1: Experimental I setup for soil columns experiment including TA, DIC, and  $\delta^{13}$ C-DIC measurement of the outflow water, and planar optode investigations for rhizosphere CO<sub>2</sub> and pH with VisiSens system. Generated by Biorender.com



Figure 4. 2: Experimental II setup for soil columns experiment including TA, DIC, and  $\delta^{13}$ C-DIC measurement of the outflow water, and planar optode investigations for rhizosphere CO<sub>2</sub> and pH with VisiSens system. Generated by Biorender.com

2.5 Soil columns experiment: Sample Collecting and Water Chemistry Analysis (TA, DIC,  $\delta^{13}$ C-DIC)

Water samples were collected 13, 35, 69, and 145 days after the start of the columns experiment. The first sampling at 13 days was conducted to ensure the soil columns reached initial equilibrium. Untreated water samples were measured for pH using a lab bench pH meter (WTW multi 9310 IDS, pH electrode SenTix®81). Only for the last sampling point, 12 mL of the remaining samples were carefully transferred into sealed glass vials (Labco) for measuring dissolved inorganic carbon (DIC) and stable isotope analysis ( $\delta^{13}$ C-DIC) without HgCl<sub>2</sub> treatment, as the samples were measured directly a few weeks after sampling. Samples were kept in a refrigerator at 4°C prior to the water chemistry measurements.

Measured TA referred to the titration alkalinity composed of carbonate alkalinity and noncarbonate alkalinity as mentioned in Chapter I (see Equation 1. 1). All TA samples were analyzed by potentiometric titration using the VINDTA 3C (Versatile Instrument for Determination of Titration Alkalinity by MARIANDA). The quality of all measurements was ensured through daily calibration with certified reference materials (CRM batch #162, #157, #198, and #196) provided by Andrew G. Dickson, Scripps Institution of Oceanography, USA, for oceanic CO<sub>2</sub> measurement. DIC was analyzed using the AIRICA (Automated InfraRed Inorganic Carbon Analyzer by Marianda) equipped with a LICOR detector (LI-7000 CO2/H2O Analyzer). The quality of the DIC measurements was ensured using certified reference material (CRM batch #196) provided by Andrew G. Dickson, Scripps Institution of Oceanography, USA. The  $\delta^{13}$ C-DIC was measured by coupling the AIRICA with a cavity ring-down spectrometer (CRDS) from Picarro (Call et al., 2017). Sodium carbonate ( $\delta^{13}$ C = -10.6 ± 0.3 ‰) and potassium carbonate ( $\delta^{13}$ C = -41.4 ± 0.7 ‰) were used as a standard for two-point calibration.

#### 2.5.1 Corrected TA concentration

We observed that there were differences between inflow and outflow water throughout the experiment (see Supporting Information, Figure S4. 1), and we assumed that the water loss was due to evaporation and transpiration without accounting for the changes in soil water storage capacity and water storage in biomass (Amann et al., 2020). Therefore, we calculated the corrected TA concentration by accounting for the loss through evaporation and transpiration and normalized it with the volume of inflow water (Equation 4. 1).

Corrected TA concentration

Equation 4. 1

$$= TA_{measured} \frac{V_{out}}{V_{in}} - TA_{loss} \frac{(V_{out} - V_{in})}{V_{in}}$$

Here,  $TA_{measured}$  was the measured TA concentration (µmol kg<sup>-1</sup>). V<sub>out</sub> was the outflow water volume (mL) and we assumed that the density of water was 1 g mL<sup>-1</sup>. V<sub>in</sub> was the inflow water volume (mL). V<sub>out</sub> – V<sub>in</sub> indicated the loss of volume of water (mL). TA concentration loss (TA<sub>loss</sub>) due to evaporation and transpiration was assumed to be 0 µmol kg<sup>-1</sup>.

2.6 Planar Optode: Rhizobox preparation, foils calibration, optode measurement, and imaging analysis

After the columns experiment, three out of five replicates from each SP4, SP3, and SCP4 treatment were randomly selected for planar optode measurement. The plants and their salt marsh soil from the columns were transferred into 14.8 x 3 x 21 cm Rhizoboxes (containers designed to study rhizosphere processes, Vienna Scientific Instruments GmbH). The Rhizoboxes consist of a black back plate and a transparent front plate, allowing for clear visual inspection during the optode measurement (Figure 4. 1 and Figure 4. 2).

Three to four roots were selected for measuring rhizosphere  $CO_2$  and pH. A 5 x 5 cm piece of  $CO_2$  foil and a 3 x 3 or 4 x 3 cm piece of pH foil were placed directly on the sediment over the roots, with the sensitive side facing down against the soil and roots, and covered with a transparent plate (Figure 4. 1 and Figure 4. 2). The front plate was tightened to ensure that the roots, foil, and front plate maintained strong contact, preventing the foil from sliding and soil water from gliding onto the foil surface during the measurement.

The optode experiment was conducted using CO<sub>2</sub> foil (PreSens GmbH; SF-CD1R; size 10 x 10 cm; range  $1 - 25\% pCO_2$ ) and pH foil (PreSens GmbH; SF-HP5R; size 15 x 20 cm; range pH 5 - 8). Before preparing the Rhizoboxes, the optode sensor foils were calibrated according to the procedures outlined in the VisiSens Scientifical software. CO<sub>2</sub> calibration was achieved using a series of N<sub>2</sub>-CO<sub>2</sub> gas mixtures (Air Products GmbH) with CO<sub>2</sub> concentrations of 0%, 3%, 10%, and 20%. pH calibration was conducted using a series of PBS buffers with six points of calibration within the pH range of 5 to 8. Since the original size of the foil was larger than needed for the experiment, the foils were cut to the desired size for measurement.

The Rhizobox was incubated in an aquarium with MilliQ water, submerging it halfway. Specifically, the water level was raised a few centimeters above the optode foils. Measurements began after a 24-hour incubation, using a perpendicular setup between the camera and a light source positioned against the roots and sediment (Figure 4. 1 and Figure 4. 2) at 23 cm from the camera and the internal LED light source. To capture CO<sub>2</sub> and pH dynamics around the rhizosphere and the designated area, the optode measurement was conducted over 24 hours, alternating between 12 hours of light on and 12 hours of light off.
During the light-on period, plants were illuminated with LEDs at room temperature (25°C) and followed the protocol described in Mittmann-Goetsch's study (Mittmann-Goetsch et al., 2024).

The optode recorded the dynamics of  $CO_2$  and pH simultaneously, capturing images every 6 minutes over 24 hours, resulting in 240 images. Image analysis was conducted using VisiSens Scientifical software, allowing observation of changes in  $CO_2$  and pH in selected areas of the roots and bulk soil within the optode foils. The cross-sectional profile of  $CO_2$  and pH was measured using the live profile function (a plug-in within the software) by selecting the point where  $CO_2$  and pH were stable, typically the last phase of each measurement session.

#### 2.7 Statistical Analysis

#### Experiment I

Since some of the observations in the dataset were missing due to insufficient water sample volume for chemical analysis, we employed a linear mixed-effect model (LMM) (Gałecki & Burzykowski, 2013) to assess the general effect of plants and differences between salt marsh plant species on alkalinity generation. LMM is an alternative method to handle the missing dataset without imputation of the missing data, ensuring the analysis could still be performed without bias (Gabrio et al., 2022). The significance level at 5% ( $\alpha = 0.05$ ) was used to determine the statistical difference in mean values between experimental conditions. The fixed effects were the relationship between the dependent variable (TA concentration) and independent variables (days of sampling and column setups). The statistical analyses were conducted in Python using the statsmodels library, particularly the statsmodels.formula.api for fitting the mixed-effects model.

#### Experiment II

We used two-way repeated measurement ANOVA to assess the effect of adding carbonate minerals (limestone) into the minerogenic salt marsh soil and plants on alkalinity generation. The significance level at 5% ( $\alpha = 0.05$ ) was used to determine the statistical difference in mean between the experiment series. The normality was determined by the Shapiro-Wilk test before

the ANOVA analysis. Two-way repeated measurement ANOVA was used to assess the effect of times and all column conditions on TA concentration. Tukey's test was used for pairwise comparisons if significant effects were found. Statistical analyses were conducted using Python utilizing the statistical packages (scipy.stats, statsmodels.api, and statsmodels.stats.anova).

#### 3. Results

#### Experiment I

3.1 Alkalinity production in the absence of plants and across different plant species

At day 69<sup>th</sup>, TA concentration changes were most pronounced in the planted treatments resulting in approximately a 10-fold increase compared to the non-planted treatment (Figure 4.3). After day 69<sup>th</sup>, both SP4 and SP3 exhibited a sharp decline in TA concentration, reaching lower levels by day 145<sup>th</sup> due to the plants were dying off. While the sampling days did not significantly impact TA levels, both planted columns demonstrated a significant effect on TA production (see Supporting Information, Table S4. 2). Additionally, a significant difference was observed between the SP4 and SP3 setups (see Supporting Information, Table S4. 2). Average pH range of S, SP4, and SP3 was around 8.0 – 9.0 across the whole course of the experiment (see Supporting Information, Table S4. 5).



Figure 4. 3: Time series data for average corrected TA concentration of S (unplanted, solid blue line and blue filled circle), SP4 (planted with S. x townsendii, dash orange line, and unfilled orange circle), and SP3 (planted with E. athericus, dash green line, and unfilled green circle). n = 5 for all the sampling points except for SP3 on days 69 and 145 n = 3 due to insufficient sample volume for TA measurement

#### 3.2 CO<sub>2</sub> and pH distribution within its rhizosphere

For all examined single roots and root aggregates from *S. x townsendii* and *E. athericus*, CO<sub>2</sub> levels significantly increased over time, stabilizing at a higher concentration in the later stages of measurement (see Supporting Information; Figure S4. 3, Figure S4. 5, Figure S4. 9, Figure S4. 11, and Figure S4. 12). In some setups, the CO<sub>2</sub>-enhanced zone remained constant throughout the observation period (see Supporting Information; Figure S4. 7). Conversely, the pH around the roots consistently decreased over time (see Supporting Information; Figure S4. 4, Figure S4. 6, Figure S4. 8, Figure S4. 10, and Figure S4. 13). The points where each measurement showed the highest CO<sub>2</sub> concentration and the lowest pH were selected for future comparisons and analysis.

#### S. x townsendii – $CO_2$ and pH

Each root exhibited a distinct maximum amount of respired CO<sub>2</sub>, ranging from 5.5% to 12.8%, with an average of  $8.9 \pm 2.7$  % (Figure 4. 4A, B, and C). CO<sub>2</sub> levels at the root were twice as high as in the bulk sediment. The affected zone, measured from the root to the bulk sediment (white double-headed arrow), averaged 20.7 ± 4.6 mm in width. The pH gradient was consistently lower around the roots, with an average minimum pH of 7.4 ± 0.1, 0.2 units lower than in the bulk sediment (Figure 4. 4D, E, and F). The affected pH zone was markedly smaller than the CO<sub>2</sub> zone, averaging 5.7 ± 0.6 mm.

#### *E.* athericus $-CO_2$ and pH

The single roots effect of *E. athericus* cannot be determined due to the resolution from the 2D image (Figure 4. 5A and D). Therefore, the root aggregates effect was measured for CO<sub>2</sub> and pH distribution in some setups. CO<sub>2</sub> accumulated within the root aggregates, averaging  $6.0 \pm 0.5$  %, while rhizomes in SP35 showed relatively higher CO<sub>2</sub> concentrations around 9.9% (Figure 4. 5B). CO<sub>2</sub> levels in the roots and rhizome areas were 1.5- to 2-fold higher than in the bulk sediment. The affected zone from root aggregates appeared larger than that of a single root; however, the definite area couldn't be determined (Figure 4. 5C). The rhizome-affected zone in SP35 was 20.8 mm, similar to the single roots of *S. townsendii*. The pH around the roots was about 0.6 units lower than in the bulk sediment, with an affected zone of around 3.2 mm, smaller than that for CO<sub>2</sub> (Figure 4. 5E and F).



Figure 4. 4: A.) First row: images of the selected roots of S. x townsendii chosen randomly from three out of five columns of SP4; second row: planar optode images of rhizosphere  $CO_2$  with the color bar gradients shown as %  $CO_2$  (1% = 10,000 ppm); third row:  $CO_2$  cross-sectional profile from last measurement shown as a function of the distant from root (0 mm) to bulk soil (± 30 mm). B.) Table showing the range and average  $CO_2$  around the selected roots and bulk soil. C.) Table showing the radius of  $CO_2$  distribution around the roots. The white two-sided arrows in A. indicate the position chosen to measure the single root affected zone from VisiSens software. D.) First row: images of the selected roots of S. x townsendii chosen randomly from three out of five columns of SP4; second row: planar optode images of rhizosphere pH with the color bar gradients; third row: pH cross-sectional profile from the last measurement shown as a function of the distant from root (0 mm) to bulk soil (± 30 mm). SP42 setup could not observe the clear rhizosphere pH; therefore, the cross-sectional profile showed the pH of the area where the roots should be. E.) Table showing the range and average pH around the selected roots and bulk soil. F.) Table showing the radius of pH distribution around the roots. The white two-sided arrows in D. indicated the position chosen to measure the root affected zone.



Figure 4. 5: A.) First row: images of the selected roots of Elymus chosen randomly from three out of five columns of SP3; second row: planar optode images of rhizosphere CO<sub>2</sub> with the color bar gradients shown as % CO<sub>2</sub> (1% = 10,000 ppm); third row: CO<sub>2</sub> cross-sectional profile from last measurement shown as a function of the distant from root (0 mm) to bulk soil ( $\pm$  30 mm). SP33 and SP34 show an additive effect of aggregation of fine roots on the distribution of CO<sub>2</sub>. B.) Table showing the range and average CO<sub>2</sub> around the aggregated roots, rhizome, and bulk soil. C.) Table showing the radius of CO<sub>2</sub> distribution around the roots. The white two-sided arrow in A. indicate the position chosen to measure the single root affected zone from VisiSens software. D.) First row: images of the selected roots of Elymus chosen randomly from three out of five columns of SP3; second row: planar optode images of rhizosphere pH with the color bar gradients; third row: pH cross-sectional profile from the last measurement shown as a function of the distant from root (0 mm) to bulk soil ( $\pm$  30 mm). SP35 setup could not observe the clear rhizosphere pH; therefore, the cross-sectional profile showed the pH of the area where the roots should be. SP34 could not analyze data due to the resolution of the image. E.) Table showing pH around the selected roots and bulk soil. F.) Table showing the radius of pH distribution around the root.

#### 3.3 Stable carbon isotopes signature of the water outflow

Understanding carbon isotope imprints is useful for obtaining information for interpreting biogeochemical pathways. Figure 4. 6 illustrates the range of isotopic compositions in the water outflow from the last sampling point of different soil columns, alongside reference values from the literature for soil inorganic carbon, soil organic carbon, C<sub>4</sub> plant (as a reference for *S. x townsendii*), and C<sub>3</sub> plant (as a reference for *E. athericus*). The  $\delta^{13}$ C-DIC values for non-planted soil columns (S) ranged from -11.95 to -8.45 ‰, with an average of -9.85 ± 1.30

‰ (n=5, ±SD). In contrast, the δ<sup>13</sup>C-DIC values for planted columns of *S. x townsendii* (SP4) varied from -7.35 to -0.36 ‰, with an average of -3.68 ± 2.80 ‰ (n=5, ±SD). Due to insufficient sample volume, some replicates from *E. athericus* (SP3) columns could not be measured. The δ<sup>13</sup>C-DIC values for the measured replicates of *E. athericus* exhibited minimal variation, averaging -7.17 ± 0.09 ‰ (n=2, ±SD).



Figure 4. 6: The inverted box plot showing the range of  $\delta^{13}$ C-DIC from all columns (S, SP4, and SP3). \*The range of  $\delta^{13}$ C soil inorganic carbon was taken from literature with the range of -10 to 0‰ (Cerling, 1984; Cerling et al., 1991; Ramnarine et al., 2012). \*\* $\delta^{13}$ C of soil organic carbon at the Hamburger Hallig site with the range of -25 to -21 ‰ (Mueller et al., 2023). \*\*\* $\delta^{13}$ C data for Spartina spp. (C<sub>4</sub> plant) from different locations was taken from literature with the range of -19 to -6 ‰ (Gratton & Denno, 2006; Ju et al., 2016). \*\*\*\*There is no data available for E. athericus (C<sub>3</sub> plant). Therefore,  $\delta^{13}$ C data for organic matter of C<sub>3</sub> plant (some studies use Phragmites australis) was also taken from literature with the range of -30 to -22 ‰ (Cerling et al., 1997; Van Geldern et al., 2015).

#### Experiment II

3.4 Alkalinity Production under limestone added salt marsh soil

TA production was more pronounced under planted conditions in both soil types with significant differences between planted and non-planted columns (see Supporting Information, Table S4. 3 and Table S4. 4), with an increase of up to 8-fold for SP4 compared to S and 6-fold for SC compared to SCP4 by day 69 (Figure 4. 7). After day 69, TA concentrations in the planted columns tended to decline due to die-off of plants. At the same time, TA levels remained relatively constant in the non-planted columns. Although no significant differences were observed among the SC vs S and SCP4 vs SP4 (see Supporting Information, Table S4. 3), the normal salt marsh soil conditions generally tended to release more TA

compared to the limestone-amended salt marsh soil. Average pH range of SC, and SCP4 was around 8.0 - 9.0 across the whole course of the experiment (see Supporting Information, Table S4. 5)



Figure 4. 7: Time series data for average corrected TA concentration of S (unplanted, solid blue line and blue filled circle), SP4 (planted with S. x townsendii, dash blue line, and unfilled blue circle), SC (unplanted, solid orange line and orange filled circle) and SCP4 (planted with S. x townsendii, dash orange line, and unfilled orange circle). n = 5 for all the sampling points.

#### 3.5 CO<sub>2</sub> and pH distribution within its rhizosphere

For all examined single roots from *S. x townsendii*, CO<sub>2</sub> levels increased and remained constant at elevated concentrations throughout the experiment (see Supporting Information, Figure S4. 14, Figure S4. 16, and Figure S4. 18). In contrast, pH levels showed a decreasing trend and stabilized over time (see Supporting Information, Figure S4. 15, Figure S4. 17, and Figure S4. 19). For subsequent analysis of the cross-section profile, we selected the measurements exhibiting the highest CO<sub>2</sub> concentration and the lowest pH

#### S. x townsendii – $CO_2$ and pH

Each root of *S. x townsendii* in limestone-added soil exhibited distinct CO<sub>2</sub> concentrations in the rhizosphere, ranging from 5.7% to 11.9%, with an average of  $7.6 \pm 3.4\%$ , approximately twice as high as in the bulk soil (Figure 4. 8A, B). The CO<sub>2</sub>-affected zone in the rhizosphere was  $21.2 \pm 5.6$  mm in width (Figure 4. 8C). Rhizosphere pH averaged  $7.3 \pm 0.1$ , which was 0.3 units lower than in the bulk soil (Figure 4. 8D, E). The pH-affected zone was smaller than the CO<sub>2</sub>-affected zone, averaging  $8.9 \pm 1.5$  mm (Figure 4. 8F).



Figure 4. 8: A.) First row: images of the selected roots of S. x townsendii chosen randomly from three out of five columns of SCP4; second row: planar optode images of rhizosphere CO<sub>2</sub> with the color bar gradients shown as % CO<sub>2</sub> (1% = 10,000 ppm); third row: CO<sub>2</sub> cross-sectional profile from last measurement shown as a function of the distant from root (0 mm) to bulk soil ( $\pm$  30 mm). SCP44 could not identify the root area due to the resolution; therefore, we selected the tip of the root\_1 for further analysis. B.) Table showing the range and average CO<sub>2</sub> around the selected roots and bulk soil. C.) Table showing the radius of CO<sub>2</sub> distribution around the roots. The white two-sided arrows in A. indicate the position chosen to measure the single root affected zone from VisiSens software. D.) First row: images of the selected roots of S. x townsendii chosen randomly from three out of five columns of SCP4; second row: planar optode images of rhizosphere pH with the color bar gradients; third row: pH cross-sectional profile from the last measurement shown as a function of the distant from root (0 mm) to bulk soil ( $\pm$  30 mm). SCP42 setup could not observe the clear rhizosphere pH with the root bar gradients; third row: pH cross-sectional profile from the last measurement shown as a function of the distant from root (0 mm) to bulk soil ( $\pm$  30 mm). SCP42 setup could not observe the clear rhizosphere pH; therefore, the cross-sectional profile showed the pH of the area where the roots should be. E.) Table showing the range and average pH around the selected roots and bulk soil. F.) Table showing the radius of pH distribution around the roots. The white two-sided arrows in D. indicate the position chosen to measure the root affected zone from VisiSens software.

3.6 Stable carbon isotopes signature of the water outflow

Figure 4. 9 illustrates  $\delta^{13}$ C data from the last sampling point of SC, and SCP4 (carbonate-rich salt marsh soil), along with the data from Experiment I (S and SP4) and reference values for potential inorganic carbon sources. The  $\delta^{13}$ C-DIC values for the non-planted carbonate-rich

soil columns (SC) ranged from -8.06 to -5.94 ‰, with an average of -7.11 ± 0.81 ‰ (n = 5, ±SD). In contrast, the  $\delta^{13}$ C-DIC values for the planted carbonate-rich soil columns tended to shift to more positive values, ranging from -4.78 to -1.37 ‰, with an average of -3.52 ± 1.23 ‰ (n = 5, ±SD).



Figure 4. 9: The inverted box plot showing the range of  $\delta^{13}$ C-DIC from all columns (S, SP4, SC, and SCP4). The reference values of soil inorganic carbon, soil organic carbon, and C4 plant were similar to Figure 4. 6. \*\*  $\delta^{13}$ C data for limestone was taken from the literature with the range of -2 to +2 ‰ (Jeffery et al., 1955).

#### 4 Discussion

#### 4.1 Plants as a TA driver within the salt marsh ecosystem

The substantial TA release observed under *S. x townsendii* and *E. athericus* conditions suggested that these plants significantly controlled TA production in salt marsh soils. This finding aligned with studies comparing *Spartina alterniflora*-vegetated and unvegetated sites, demonstrating that *S. alterniflora* enhanced alkalinity production, which was less pronounced in unvegetated areas (Koretsky et al., 2008). Previous high-resolution research in salt marsh demonstrated that vegetation and root zonation are closely correlated to water geochemistry (Moffett & Gorelick, 2016).

Two potential mechanisms may explain how plants enhance alkalinity production. Firstly, the root respired-CO<sub>2</sub> profile of both *S. x townsendii* and *E. athericus* showed markedly higher magnitudes than the bulk soil, where the influence of plant roots is absent. Intense CO<sub>2</sub> production around the vicinity of roots occurs due to roots releasing CO<sub>2</sub> (Kuzyakov & Larionova, 2005) and modulating microbial activity (Koop-Jakobsen et al., 2018). This

potentially can drive mineral dissolution (Mueller et al., 2023), increasing alkalinity production (Fakhraee et al., 2023). Consistent with this, the experimental observations in Chapter II showed increases in soil  $pCO_2$  were related to enhanced TA production with a possibility linked to mineral dissolution processes in minerogenic salt marsh soil. Alternatively, the roots of wetland plants, such as *Spartina spp.*, are capable of oxidizing sulfide and iron(II) through the release of  $O_2$  (Lee et al., 1999). The by-products of this oxidation process can serve as electron acceptors for specific anaerobes, producing the net gain of HCO<sub>3</sub><sup>-</sup> and thereby leading to a net gain of alkalinity (Middelburg et al., 2020). In contrast, non-planted columns lacked the influence of plant roots, resulting in relatively low TA production via both mechanisms.

pH profile was evidence of root oxygenation and directly indicative of microbial respiration producing proton (Koretsky & Miller, 2008). pH of both *S. x townsendii* and *E. athericus* in the vicinity of the roots demonstrated pH gradients within their rhizospheres, aligning with observations from another planar optode study (Koop-Jakobsen et al., 2018) and a field study (Ca çador et al., 1996). However, we found that high CO<sub>2</sub> accumulation was not likely to cause a drop in pH around the roots. This finding contrasts with previous studies on *Spartina anglica* in the Danish Wadden Sea salt marsh (Koop-Jakobsen et al., 2018) and the Minjiang River estuary (Zheng et al., 2019), which reported pH values around 6 or lower in the rhizosphere. It was speculated that this discrepancy might be directly linked to the soil inorganic carbon content at the study site. In minerogenic salt marshes, inorganic carbon can buffer against pH drops. In contrast, organic-rich sediments, such as those in the Minjiang Delta (Chen et al., 2020), are highly affected by acidification.

#### 4.2 Species-specific TA production

*S. x townsendii* and *E. athericus* exhibited statistically significant differences in TA production. However, the underlying reasons for these species-specific contributions are beyond the scope of this study. We observed intra- and interspecific variations in CO<sub>2</sub> and pH within small sections of single roots, fine root aggregates, and rhizomes of both species. We speculated that rhizosphere traits may play a role in driving TA generation. From a rhizosphere perspective, *Spartina* grasses facilitate oxygen transport to the belowground sediment through their roots and rhizomes, particularly at the root tip (Koop-Jakobsen et al., 2018). The O<sub>2</sub>-leaking barrier developed by *Spartina* enhances oxygen transport and increases root length (Ejiri et al., 2021), potentially influencing root morphology and controlling rhizosphere parameters (Bezbaruah & Zhang, 2005). In contrast, *E. athericus* oxygenates sediment more uniformly across its root and rhizome system (Koop-Jakobsen et al., 2021). As shown in Figure 4. 4A, D, and Figure 4. 5A, D, *S. x townsendii* and *E. athericus* differ in root density, diameter, and biomass; however, we were unable to collect sufficient quantifiable data to fully explore these effects. Future studies should investigate rhizosphere traits, respiration rate per root area, and other factors influencing alkalinity generation, and include cross-species comparisons among salt marsh plants to better understand plant responses to inorganic carbon dynamics in salt marsh ecosystems.

#### 4.3 Possible Responsible Biogeochemical Pathway

The outflow water from distinct column setups of Experiment I differ markedly in  $\delta^{13}$ C-DIC composition. The control experiment (S) displays low  $\delta^{13}$ C-DIC and low TA concentration, whereas under the presence of plants (SP4 and SP3) are isotopically heavier and richer in TA concentration (see Supporting Information, Figure S4. 20A). Two major inorganic carbon sources can be considered within the belowground system: soil carbonates ( $\delta^{13}$ C  $\approx$  -10 to 0‰ (Salomons & Mook, 1976; Cerling, 1984; Ramnarine et al., 2012)) and respired-CO<sub>2</sub> from root and microbial respiration. The isotopic composition of respired-CO<sub>2</sub> is identical to soil organic carbon (SOC) (ŠantRůČková et al., 2000), which is derived from both autochthonous and allochthonous inputs (Middelburg et al., 1997). In this experiment, SOC is represented by the C4 plant as *Spartina* ( $\delta^{13}$ C  $\approx$  -19 to -6 ‰ (Gratton & Denno, 2006; Ju et al., 2016)), C3 plant as *Elymus* ( $\delta^{13}$ C  $\approx$  -30 to -22 ‰ (Cerling et al., 1997; Van Geldern et al., 2015)) and SOC from salt marsh soil at Hamburger Hallig site ( $\delta^{13}$ C  $\approx$  -25 to -21 ‰ (Mueller et al., 2023)).

A precise answer to the last question is challenging due to the limits of the acquired data. Determination of the relative distribution between all sources, which could clarify the roles of mineral dissolution or anaerobic/aerobic respiration, would require the isotope mixing model calculations and the initial signature and exact amount of all possible sources (Phillips et al., 2005). However, some perspectives to explain the possible biogeochemical pathway can be drawn from the obtained data. Theoretically, when carbonate mineral dissolution is a

predominant process, the  $\delta^{13}$ C-DIC signature will derive equally from soil carbonates and respired-CO<sub>2</sub>. In contrast, during the dissolution of silicate minerals, the  $\delta^{13}$ C-DIC signature will reflect  $\delta^{13}$ C of respired-CO<sub>2</sub>. Similar to anaerobic respiration, if this process dominates the system, the  $\delta^{13}$ C-DIC signature will likely derive from SOC.

The enrichment of  $\delta^{13}$ C-DIC value and TA enhancement in *S. x townsendii* and *E. athericus* could represent mixing between soil carbonates and C<sub>4</sub>- or C<sub>3</sub>-induced respired-CO<sub>2</sub> and indicate the occurrence of carbonate mineral dissolution occurring in the system. In the absence of plants, low  $\delta^{13}$ C-DIC, and low TA concentrations may indicate an absence of root respiration, resulting in fewer sources of acidity to induce mineral dissolution. It can be speculated that carbonate mineral dissolution also occurred, as indicated by the shift in the  $\delta^{13}$ C signature to a heavier isotopic value. However, the extent of mineral dissolution within the system is likely less than in conditions where plants are present.

#### Experiment II

4.4 High addition of carbonate content in minerogenic salt marsh soil does not necessarily increase TA generation

The significant release of TA in *S. x townsendii*-planted columns across both soil types underscores the plant effect, consistent with the findings from Experiment I. In terms of  $\delta^{13}$ C-DIC composition, an enrichment in isotopic values and higher TA concentrations were observed in SCP4 and SP4 compared to SC and S (see Supporting Information, Figure S4. 20B), suggesting that plants may enhance TA generation through mineral dissolution, as discussed earlier in Experiment I.

However, adding carbonate minerals (limestone) to minerogenic salt marsh soil did not result in a significant increase in TA generation compared to the control treatments (S and SP4), even in the presence of *S. x townsendii*. Rhizosphere CO<sub>2</sub> concentrations and pH levels in *S. x townsendii* under high carbonate content were similar to those in normal salt marsh soil, suggesting that other factors may influence TA production. The distinct pattern in  $\delta^{13}$ C-DIC composition, with a shift toward a heavier  $\delta^{13}$ C signature in SC and SCP4 compared to S and SP4, indicates a likely potential contribution of limestone ( $\delta^{13}$ C  $\approx$  -2 to +2 ‰ (Jeffery et al., 1955)) to the mineral dissolution process. Although limestone appeared to contribute to mineral dissolution, we were unable to quantify the relative contributions of limestone and soil minerals.

Despite the presence of limestone, TA concentrations were similar to, or slightly lower than, those in the normal salt marsh soil. From a thermodynamic perspective, carbonate minerals (limestone and soil carbonate) should dissolve, releasing alkalinity and raising the respective carbonate minerals saturation state ( $\Omega$ ) (Sulpis et al., 2022). Perhaps the one possible explanation may be related to the degree of saturation of the outflow water. The saturation state ( $\Omega$ ) is the key factor driving either mineral precipitation ( $\Omega > 1$ ) or dissolution ( $\Omega < 1$ ) (see Chapter III, Section 2.7) (Millero, 1979; Mucci, 1983). It is possible that the outflow water in these columns was supersaturated ( $\Omega > 1$ ) with respect to carbonate minerals such as calcite, aragonite, or Mg-calcite, preventing further mineral dissolution and leading to mineral precipitation to maintain equilibrium. Unfortunately, we were unable to obtain ion concentration data to calculate the saturation states and support this hypothesis.

#### Experiment I and II

- 4.5 Perspective, knowledge gaps, and implications
- 4.5.1 Can plant-mediated salt marsh alkalinity export enhance coastal waters' buffering capacity?

Ocean-based climate solutions have considered restoration of the coastal vegetation (blue carbon ecosystems) as a potential strategy to enhance anthropogenic CO<sub>2</sub> uptake (Gattuso et al., 2021). The importance of these ecosystems lies in their ability to export alkalinity and shape the carbonate chemistry within the coastal waters (Wang & Cai, 2004; Sippo et al., 2016; Yau et al., 2022; Reithmaier et al., 2023). A recent study has offered new insights into the benefits of restoration, particularly in enhancing local alkalinity (Fakhraee et al., 2023). Our results similarly demonstrate increased alkalinity production in the presence of plants within minerogenic salt marsh sediment, likely driven by mineral dissolution coupled with microbial respiration.

On the one hand, examining alkalinity can reflect the acid-neutralizing capacity of the seawater. On the other hand, the changes in relative proportion between TA and DIC can provide insight into the buffering capacity or acidification potential of coastal waters (Wang et al., 2016). This proportional change reflects the Revelle factor and other buffer factors (Egleston et al., 2010; Middelburg et al., 2020; Reithmaier et al., 2023), and has been described as a proxy of the buffering effect or driver of coastal acidification in the literature across BCEs and ocean acidification research (Sippo et al., 2016; Yau et al., 2022; Reithmaier et al., 2023). The TA:DIC ratio can be used to indicate seawater pH. A greater amount of DIC outwelling than TA (ratio <1) can potentially drop the water pH and likely cause coastal water acidification (Robinson et al., 2018). On the contrary, the ratio above one indicates the potential to increase the pH of water and thus increase buffering capacity (Sippo et al., 2016).

The observed TA:DIC ratio within this study falls into the similar ranges reported in US salt marshes (Reithmaier et al., 2023). The ratio of more than one in some of the plant setups suggested that TA export under plants like S. x townsendii might be able to increase buffering capacity (Figure 4. 10). Conversely, the greater amount of DIC than TA under control of E. athericus and high carbonate content salt marsh soil setups are likely to cause acidification (Figure 4. 10). The general pattern showed some inconsistencies in variations; therefore, it is important to note that these observations are limited to two plant species, laboratory conditions, limited replications per setup, and short experimental period. As reported in the former study, TA:DIC ratio could change seasonally thus affecting the season change of buffering capacity (Wang et al., 2016). Therefore, it is insufficient to draw a conclusive answer. Nevertheless, our observations indicated that while plants contribute to alkalinity enhancement, buffering capacity largely depends on both TA and DIC. As reported in the salt marshes and other BCEs research (Reithmaier et al., 2023), some sites indicated buffering capacity and others tended to acidify the coastal waters. For future research, it is crucial to understand the mechanism behind the conditions under which TA outwelling is higher than DIC and to develop strategies to enhance TA production relative to DIC for the potential restoration project of BCEs.



Figure 4. 10: TA:DIC ratios distribution within the group in water outflow and the reference line at 1 is a proxy for acidification (TA:DIC <1) (Reithmaier et al., 2020) or buffering effect (TA:DIC >1) (Sippo et al., 2016)

#### 4.5.2 Implications

Overall, this study demonstrated that salt marsh plants are potential drivers of alkalinity within salt marsh ecosystems. In Experiment I, *S. x townsendii* and *E. athericus* can significantly enhance alkalinity export to adjacent coastal waters by modulating feedback mechanisms, including microbial respiration (redox processes) and mineral dissolution. We provided evidence that high acidity production from respired  $CO_2$  in the rhizosphere can induce mineral dissolution, particularly in minerogenic salt marshes. However, there are intra- and interspecific variations in the rhizosphere parameters of *S. x townsendii* and *E. athericus*, suggesting the need for further research to fully understand how rhizosphere traits and root morphology influence alkalinity production across different plant species.

In Experiment II, the addition of shell-bearing limestone as a stimulation of biogenic carbonates to the minerogenic salt marsh soil appeared to contribute to mineral dissolution; however, did not significantly increase TA generation. This was likely due to the control exerted by the water's saturation state, which may have prevented further mineral dissolution. Unfortunately, the available data did not allow us to fully validate this hypothesis, indicating a gap in our understanding of processes controlling inorganic carbon dynamics.

Collectively, understanding these mechanisms is crucial for developing effective restoration strategies for blue carbon ecosystems. Future research should focus on increasing TA outwelling and identifying plant species and conditions that favor TA production over DIC, thereby enhancing the buffering capacity of coastal waters and contributing to climate change mitigation.

## **Supporting Information**

Table

Simplified name	g sediment	g limestone	
	Salt marsh soil (S)		
<b>S</b> 1	550.4	-	
S2	550.1	-	
S3	550.2	-	
S4	550.2	-	
S5	550.8	-	
Salt marsh soil + S. x townsendii (SP4)			
SP41	550.5	-	
SP42	550.3	-	
SP43	550.5	-	
SP44	549.9	-	
SP45	550.1	-	
Sa	lt marsh soil + E. athericus (S	SP3)	
SP31	550.7	-	
SP32	550.5	-	
SP33	550.6	-	
SP34	550.3	-	
SP35	550.7	-	
Salt ma	rsh soil + shell-bearing limes	tone (SC)	
SC1	440.4	110.3	
SC2	440.7	110	
SC3	440.5	110.3	
SC4	440.2	110	
SC5	440.7	110	
Salt marsh soil + s	Salt marsh soil + shell-bearing limestone + S. x townsendii (SCP4)		
SCP41	440.8	110.1	
SCP42	440.3	110.5	
SCP43	440.2	110.1	
SCP44	440.3	110.3	
SCP45	440.2	110.4	

Table S4. 1: The amount of solid added in the columns for Experiment I and Experiment II

Table S4. 2: Linear-mixed model effect (LMM) measurement results are included below. These results were run without imputation of the missing data (highlighted p-value means there is a significant difference when  $p \le 0.05$  and red color represents that there is no significant difference). Estimate indicates how much the dependent variable (TA concentration) is expected to change when there is a change of independent variable (days or columns). Std. Error indicates the preciseness. z (z-value) represents how many standard deviations the coefficient estimate is from zero, indicating the strength of evidence for the effect of a variable in the model. 95% confidence interval (95% CI) indicates uncertainty around the estimate.

	Estimate	Std. Error	Z	р	95%CI
Days	15.79	17.31	0.912	0.362	[-18.96, 50.54]
SP3 vs S	5213.47	2148.95	2.426	0.015	[901.28, 9525.65]
SP4 vs S	8104.73	2018.95	4.014	0.001	[4053.41, 12205.66]
SP4 vs SP3	2891.26	1214.57	2.380	0.017	[653.72, 5128.81]

Table S4. 3: Two-way repeated measures ANOVA results are included below (highlighted p-value means there is a significant difference when  $p \le 0.05$ ).

	F	р
Days	8.287	0.003
Column setups	30.323	< 0.001
Days x Column setups	3.029	0.008

Table S4. 4: Tukey's test results of TA concentration after two-way repeated measures ANOVA. The red color represents that there is no significant difference between the means of the group.

group1	group2	р
S	SC	0.9737
S	SCP4	0.0053
S	SP4	< 0.001
SC	SCP4	0.0013
SC	SP4	< 0.001
SCP4	SP4	0.471

Columns	Day of sampling (days)	pH (mean $\pm$ SD, n=5)
S	13	$8.0 \pm 0.2$
SP4	13	$8.2 \pm 0.2$
SP3	13	$8.2 \pm 0.2$
SC	13	$8.0 \pm 0.4$
SCP4	13	$8.4 \pm 0.3$
S	35	$8.1 \pm 0.1$
SP4	35	$8.6 \pm 0.1$
SP3	35	$8.3 \pm 0.2$
SC	35	$8.0 \pm 0.1$
SCP4	35	$8.5 \pm 0.2$
S	69	$8.6 \pm 0.1$
SP4	69	$9.0 \pm 0.2$
SP3	69	$8.7 \pm 0.3$
SC	69	$8.4 \pm 0.1$
SCP4	69	$9.0 \pm 0.2$
S	145	$8.9 \pm 0.1$
SP4	145	$9.1 \pm 0.4$
SP3	145	$8.9 \pm 0.2$
SC	145	$9.0 \pm 0.2$
SCP4	145	$9.0 \pm 0.2$

Table S4. 5: Average pH of all the columns (n=5 at each sampling point)

### Figure



Figure S4. 1: The amount of water inflow (mL) and outflow (g) vs day of sampling. Assumed that the water density is 1 g mL<sup>-1</sup>. The solid and dashed lines represent the water inflow and outflow, respectively. The shadow area represented a 95% confidence interval.



Figure S4. 2: TA and DIC relation from the last samples on day 145th. S (unplanted, solid blue line and blue filled circle), SP4 (planted with S. x townsendii, dash orange line, and unfilled orange circle), and SP3 (planted with E. athericus, dash green line, and unfilled green circle).



Figure S4. 3: Time series data of  $CO_2$  around the roots of S. x townsendii from SP42 setup and bulk soil over 24 h alternating between 0-12 h light on and 12-24 h light off



Figure S4. 4: Time series data of pH around the root of S. x townsendii from SP42 setup and bulk soil over 24 h alternating between 0-12 h light on and 12-24 h light off



Figure S4. 5: Time series data of  $CO_2$  around the roots of S. x townsendii from SP43 setup and bulk soil over 24 h alternating between 0-12 h light on and 12-24 h light off



*Figure S4. 6: Time series data of pH around the root of S. x townsendii from SP43 setup and bulk soil over 24 h alternating between 0-12 h light on and 12-24 h light off* 



Figure S4. 7: Time series data of  $CO_2$  around the root of S. x townsendii from SP44 setup and bulk soil over 24 h alternating between 0-12 h light on and 12-24 h light off



Figure S4. 8: Time series data of pH around the roots of S. x townsendii from SP44 setup and bulk soil over 24 h alternating between 0-12 h light on and 12-24 h light off



Figure S4. 9: Time series data of  $CO_2$  around the roots of *E*. athericus from SP33 setup and bulk soil over 24 h alternating between 0-12 h light on and 12-24 h light off



Figure S4. 10: Time series data of pH around the roots of E. athericus from SP33 setup and bulk soil over 24 h alternating between 0-12 h light on and 12-24 h light off



Figure S4. 11: Time series data of  $CO_2$  around the roots of E. athericus from SP34 setup and bulk soil over 24 h alternating between 0-12 h light on and 12-24 h light off



Figure S4. 12: Time series data of  $CO_2$  around the roots of E. athericus from SP35 setup and bulk soil over 24 h alternating between 0-12 h light on and 12-24 h light off



*Figure S4. 13: Time series data of pH around the roots of E. athericus from SP35 setup and bulk soil over 24 h alternating between 0-12 h light on and 12-24 h light off* 



Figure S4. 14: Time series data of  $CO_2$  around the roots of S. x townsendii from SCP41 setup and bulk soil over 24 h alternating between 0-12 h light on and 12-24 h light off



*Figure S4. 15: Time series data of pH around the roots of S. x townsendii from SCP41 setup and bulk soil over 24 h alternating between 0-12 h light on and 12-24 h light off* 



Figure S4. 16: Time series data of  $CO_2$  around the roots of S. x townsendii from SCP43 setup and bulk soil over 24 h alternating between 0-12 h light on and 12-24 h light off



*Figure S4. 17: Time series data of pH around the roots of S. x townsendii from SCP43 setup and bulk soil over 24 h alternating between 0-12 h light on and 12-24 h light off* 



Figure S4. 18: Time series data of  $CO_2$  around the roots of S. x townsendii from SCP44 setup and bulk soil over 24 h alternating between 0-12 h light on and 12-24 h light off



*Figure S4. 19: Time series data of pH around the roots of S. x townsendii from SCP44 setup and bulk soil over 24 h alternating between 0-12 h light on and 12-24 h light off* 



Figure S4. 20: A.) and B.) represent  $\delta^{13}$ C-DIC against 1/TA of Experiment I and II, respectively.

## **Chapter V: General Discussion**

## 1. Is the mineral dissolution a dominant source of alkalinity production in the organicpoor minerogenic salt marsh soil?

Carbonate mineral dissolution may likely be the dominant source of net alkalinity production in organic-poor minerogenic salt marsh soils, especially when soil  $pCO_2$  is elevated, as observed in rhizosphere conditions. This process may be supplemented by silicate dissolution (see Chapter II). Only a few studies on salt marshes showed the extent of carbonate mineral dissolution within sediments (Reaves, 1986; Vranken et al., 1990; Lin et al., 2020). Notably, the study in the Elbe estuary is one of the first to report carbonate mineral dissolution accounted for approximately 42.6% of net TA generation in minerogenic salt marshes (Weiss, 2013). Similarly, another study suggested that 90% of the TA exported from the Elbe estuary to coastal waters was derived from carbonate dissolution, with 10% attributed to anaerobic respiration (Norbisrath et al., 2022).

These findings challenged the prevailing notion that sulfate reduction, either independently or coupled with Fe(III) reduction (see Chapter I, Section 2.2) is the dominant metabolic pathway contributing to alkalinity sources in salt marshes (Cai & Wang, 1998; Raymond et al., 2000; Kostka et al., 2002a; Cai et al., 2003; Tobias & Neubauer, 2019; Reithmaier et al., 2021; Yau et al., 2022), given that these electron acceptors are typically the most abundant in salt marsh sediments. These differences in dominant processes between other studies and salt marshes in this study are likely reflected in salt marsh characteristics, particularly between organic-rich and organic-poor soils (Weiss, 2013). Especially in the Elbe estuary, high particulate inorganic carbon import is the source of carbonate dissolution (Norbisrath et al., 2022). This proves that the biogeochemical pathway responsible for alkalinity production is highly site-specific (Liu et al., 2021).

While several studies have explicitly measured metabolic activity to determine the prevailing biogeochemical pathways within salt marsh sediment (Howarth & Teal, 1979; Hines et al., 1989; Kostka et al., 2002b; Wang et al., 2018), others have relied on the theoretical TA:DIC ratio (e.g., carbonate dissolution (+2), sulfate reduction (+1), Fe(III) reduction (+8) (Sippo et

al., 2016)) as an indicator of dominant biogeochemical process within the sediment (Liu et al., 2021; Yau et al., 2022). However, using this ratio can oversimplify the complex interactions within the system and lead to misinterpretations. The TA:DIC ratio response may align with theoretical values when the system contains only a single process (Hartmann et al., 2023). In natural systems, however, the net TA:DIC ratio is likely the result of multiple interacting reactions (He et al., 2022). Observing the ratio alone is insufficient to provide detailed information on biogeochemical pathways, the relative contributions of each process, or the involvement of inorganic carbon, which is often not discussed in studies.

# 2. Do the biogenic carbonate sources potentially contribute to the alkalinity budget in the coastal waters?

Coarse biogenic carbonates on the soil surface, such as mussel shell debris, do not significantly contribute to the soil-water matrix's alkalinity. Instead, the primary source of mineral dissolution for alkalinity generation may likely come from soil carbonates (see Chapter III). It is speculated that shell debris is less susceptible to dissolution than soil carbonates due to its lower specific surface area and limited water contact, similar to findings regarding competition between pedogenic carbonates and bedrock fragments in karst systems (Domínguez-Villar et al., 2022). The pedogenic carbonates as part of soil carbonates are easily dissolved due to their smaller size and higher specific surface area (Briese et al., 2017). For shell debris to become more susceptible to dissolution, it should undergo a breakdown transformation and become more enclosed within the soil (Domínguez-Villar et al., 2022).

Experiment II of Chapter IV was designed to test the assumption from Chapter III's findings by adding plants to stimulate respiration and CO<sub>2</sub> production, making the system more corrosive to biogenic carbonates (Waldbusser et al., 2011). A contribution of biogenic carbonates to mineral dissolution and alkalinity generation was observed when powdered shellbearing limestone was homogeneously mixed with minerogenic salt marsh soil (see Chapter IV, Section 4.4). However, increasing the availability of biogenic carbonate minerals (from 3.5% in Chapter III to 20% w/w in Chapter IV) did not produce a relatively significant increase in alkalinity generation compared to control setups, despite literature suggesting that shell dissolution can enhance alkalinity (Schwichtenberg et al., 2020; Ericson & Ragg, 2022). One possible explanation is that during periods when the water is undersaturated with carbonate minerals (SI < 0 or  $\Omega$  <1, see Chapter III, Section 2.7), carbonates will dissolve until the solution becomes supersaturated (SI > 0 or  $\Omega$  >1), thereby preventing further mineral dissolution. This thermodynamic constraint or saturation indices is sensitive to soil *p*CO<sub>2</sub>, ions constituent, salinity, pressure, and temperature (Adkins et al., 2021). Assuming these factors are identical within the replicates of Chapter III and Chapter IV, each column is likely to have a dissolution threshold, beyond which no further dissolution occurs unless one of these controlling factors—such as soil *p*CO<sub>2</sub>—is altered, as in Experiment II of Chapter IV with plants.

As a result, the outflow water from Chapter III (see Section 3.1.4, Figure 3. 4) was found to be supersaturated, and the same is expected for the outflow water of Chapter IV. However, the lack of available data prevents conclusive proof of this assumption. Furthermore, the rate at which biogenic particles contribute to alkalinity generation remains uncertain, as this process could take years depending on the rate of shell decomposition (Říhová et al., 2018).

# 3. Do the plants mediate the alkalinity generation via mineral dissolution by rhizosphere process?

Salt marsh plants appear to mediate alkalinity generation in minerogenic salt marsh soils, likely through rhizosphere processes that induce mineral dissolution (see Chapter IV). This mediation is thought to occur because root respiration and the oxidation of organic matter (Kuzyakov & Larionova, 2005; Kuzyakov, 2006) elevate CO<sub>2</sub> levels within the rhizosphere (see Chapter IV, Section 3.2) consequently enhancing alkalinity production (see Chapter II). Figure 4. 4 and Figure 4. 5 illustrate that individual roots produced significantly higher  $pCO_2$  than the bulk sediment, and the root zone affected by CO<sub>2</sub> appeared to be relatively large. In densely rooted zones of *S. x townsendii* and *E. athericus*, the cumulative CO<sub>2</sub> effect may be more pronounced, leading to more corrosive conditions that potentially promote mineral dissolution (Berner, 1992). Similar rhizosphere effects on mineral dissolution have been observed in laboratory and field experiments with terrestrial plants (Hinsinger et al., 2001; Meheruna & Akagi, 2006).

Salt marshes are known for their exceptional ability to dissolve carbonate minerals (Vranken et al., 1990). This process may be linked to plants such as *Spartina spp.*, which can potentially reduce inorganic carbon stock density (Yang & Yang, 2020). Additionally, plants can increase microbial activity and carbon turnover, creating acidic conditions that affect the saturation state of carbonate (Reaves, 1986). However, as discussed in Section 4.1 of Chapter IV, plants also indirectly increase the possibility of promoting alkalinity generation through anaerobic respiration, such as sulfate reduction or Fe(III) reduction.

While this thesis primarily investigated mineral dissolution induced by rhizosphere processes, the experimental design of this thesis does not allow for a conclusive determination of the proportional contributions of rhizosphere-inducing alkalinity generation via mineral dissolution versus anaerobic respiration. Future research is needed to explore these contributions in detail, to clarify whether plants primarily induce alkalinity through mineral dissolution or anaerobic respiration.

#### 4. Methodological suggestion

All column studies were conducted with the non-isotopic approach using planted or unplanted soil columns to assess the salt marsh plant's effect on enhancing alkalinity generation by measuring water chemistry dynamics, with the addition of planar optode technology. However, this experimental design does not provide conclusive insight into the quantitative contribution of anaerobic respiration and mineral dissolution-inducing alkalinity mediated by plants. Consideration of inclusively incorporating the isotopic approach, such as quantifying the carbon isotopic imprint of rhizosphere-derived CO<sub>2</sub> and soil carbonate coupling with high-precision stable isotope analysis, or labeling of plants in atmospheric <sup>14</sup>CO<sub>2</sub> or <sup>13</sup>CO<sub>2</sub> to track the partitioning of rhizosphere-derived CO<sub>2</sub> (Nguyen et al., 1999; Kuzyakov & Larionova, 2005; Kuzyakov, 2006; Zhu & Cheng, 2011), or <sup>13</sup>C-carbonate label method to capture the dissolution rate (Subhas et al., 2015), could also further provide solid evidence of the biogeochemical pathway and carbonate minerals dissolution. In addition, measuring total carbonate minerals dissolution, while analyzing the SO<sub>4</sub><sup>2-</sup> depletion rate could detect sulfate reduction, which is the dominant anerobic respiration in the salt marsh (Wolf et al., 2007).

#### 5. Perspective for future study and knowledge gaps

5.1. Climate change impact on salt marshes and their biota: implications for alkalinity generation

Salt marshes and their biota are susceptible to climate change-driven stressors, for example sea level rise, elevated atmospheric CO<sub>2</sub>, warming, storms, and increased rainfall, which have the potential to alter carbon sequestration efficiency, vegetation composition, pore water chemistry, plant productivity, and soil stability (Morris et al., 2002; Macreadie et al., 2019; Spivak et al., 2019; Lovelock & Reef, 2020; Koop-Jakobsen & Dolch, 2023). Understanding how salt marshes and their associated biota will respond to future climate conditions is crucial for predicting the role of blue carbon ecosystems in mitigating climate change (Macreadie et al., 2019).

#### Sea level rise (SLR)

Salt marshes possess natural mechanisms to adapt to vertical elevation changes through feedback loops between below- and aboveground parts of plants (Kirwan & Megonigal, 2013). By influencing plant responses, SLR can indirectly affect SOM decomposition (SOM-derived CO<sub>2</sub> production). This process is closely linked to increases in aboveground biomass, which helps regulate microbial activity and compensate for changes in O<sub>2</sub> availability and substrate inputs resulting from flooding (Mueller et al., 2016). Intraspecific variations among salt marsh plants lead to different responses to hydrological changes driven by SLR, as demonstrated by differences in the production of rhizomes, shoots, and leaves (Reents et al., 2021). These variations also shape the response of soil microbial communities and carbon turnover within the sediment (Tang et al., 2021).

#### Elevated atmospheric CO<sub>2</sub>

Elevated atmospheric CO<sub>2</sub> is a well-studied stressor regarding climate impact on plant response. C<sub>3</sub> plants, such as *Elymus athericus*, increase growth and photosynthesis to a larger extent than C<sub>4</sub> plants, such as *Spartina spp.*, under elevated atmospheric CO<sub>2</sub> conditions (Bowes, 1993; Lenssen et al., 1993; Reddy et al., 2010). Multiple studies have shown that atmospheric CO<sub>2</sub> enrichment promotes soil respiration, leading to increased soil  $pCO_2$ production due to higher root biomass and productivity (van Veen et al., 1991; Andrews & Schlesinger, 2001; Kim et al., 2004; Marsh et al., 2005). As more carbon is allocated to the soil through plant roots providing available substrates, increasing plant biomass can lead to increased  $O_2$  release via roots. Consequently, as a hotspot for microbial activity, the rhizosphere should also experience accelerated soil organic matter (SOM) decomposition under elevated  $CO_2$  conditions (Wolf et al., 2007; Kuzyakov, 2010).

#### Warming

Rising temperatures are frequently studied in corporations with elevated  $CO_2$  conditions to assess relative plant responses. C<sub>3</sub> and C<sub>4</sub> species are generally expected to exhibit enhanced biomass production under these conditions (Gray & Mogg, 2001). A recent study reported a counteractive notion that biomass production in *S. anglica* was sensitive to the combined effects of elevated  $CO_2$  and temperature, whereas *E. athericus* showed an insensitivity to these changes (Koop-Jakobsen & Dolch, 2023). Additionally, some studies have shown that C<sub>4</sub> plants are more tolerant to warming-induced drought (Ward et al., 1999); however, severe drought also leads to vegetation dieback (McKee et al., 2004). On another hand, increased soil temperatures also stimulate enzyme activity, accelerating soil organic matter (SOM) decomposition (Kuzyakov, 2010; Spivak et al., 2019). Theoretical predictions suggest that warming will increase ecosystem respiration faster rate than primary production (Yvon-Durocher et al., 2010).

#### Altered rainfall intensity and extreme storm events

Increasing rainfall intensity enhances soil moisture, supporting plant growth and altering elemental cycling (Sun et al., 2021). Wetter conditions are likely to accelerate soil organic matter (SOM) decomposition (Kuzyakov, 2010), as microbes tend to decompose organic matter more efficiently in wet substrates (Foote & Reynolds, 1997). This decomposition acceleration may offset potential productivity gains (Charles & Dukes, 2009). When combined with increased temperatures, this can further amplify the decomposition rate (Foote & Reynolds, 1997). Conversely, decomposition tends to slow down under drought conditions (Charles & Dukes, 2009). However, in salt marshes, decomposition may depend on oxygen levels influenced by flooding. This proliferates anaerobic respiration and slows down decomposition(Davidson & Janssens, 2006). Extreme rainfall events associated with storm

surges can lead to mass mortality of salt marsh vegetation if they exceed the threshold for plant survival (Stagg et al., 2021).

This doctoral thesis provided a glimpse into the impact of certain climate change stressors (e.g., altered rainfall intensity) and their consequences (e.g., increased soil  $pCO_2$ ) which resulted in an increasing TA production (see Chapter II, Section 4.1). However, assessing the effects of climate change on alkalinity generation influenced by salt marsh biota is beyond the focus of this study. As noted in previous examples, those stressors can directly or indirectly, and positively or negatively, influence primary productivity in salt marsh ecosystems. The biota's response to these stressors can, in turn, potentially drive processes related to alkalinity generation, such as CO<sub>2</sub> production, mineral dissolution, and microbial respiration (see Chapter I, Section 2.2). However, this response has been largely overlooked across BCEs (see Chapter II, Section 4.3.2 and Figure 2. 7). Hence, this underlies the clear need to assess environmental stressors integrating with biota's feedback and to properly evaluate the resilience of salt marsh vegetation in offsetting the effects of climate stressors. This should include understanding how they maintain their carbon sequestration efficiency through organic carbon burial or alkalinity export to adjacent coastal waters.

#### 5.2. Ocean-based CO<sub>2</sub> removal by salt marsh restoration

The degradation and human conversion of the coastal environment for agriculture and aquaculture (Howard et al., 2017) have caused 25-50% of the global loss of salt marshes (McOwen et al., 2017), reducing the CO<sub>2</sub> sink efficiency and potentially increasing the greenhouse gas emission (Le Quéré et al., 2009; Duarte et al., 2013). Restoration and conservation of salt marshes and other BCEs have been identified as eco-engineering solutions to maintain sequestration potential and mitigate the climate impacts on coastal ecosystems (Duarte et al., 2013). Beyond those perspectives, recent scientific findings suggest that restoring BCEs could enhance oceanic CO<sub>2</sub> removal by increasing local alkalinity export (Fakhraee et al., 2023). This doctoral thesis provided the first empirical evidence supporting the role of plants and their rhizospheres in promoting alkalinity generation within salt marsh ecosystems, which may contribute to increased alkalinity export to adjacent coastal waters. To date, restoration efforts have primarily focused on the fate of organic carbon, often overlooking

the critical role of inorganic carbon and alkalinity generation (Sulpis & Middelburg, 2023). This underscores the need to incorporate the role of plants controlling the fate of inorganic carbon into restoration strategies, particularly given the limited global data on salt marshes relative to other BCEs (Fakhraee et al., 2023). Additionally, intra- and interspecific variations among plants and their rhizospheres may influence the enhancement of alkalinity and the buffering capacity of exported water chemistry differently (see Chapter IV, Section 4.5). Continued investigation into plant response to these processes and their ecosystem function is essential for developing more effective restoration management and climate change mitigation strategies.

## References

- Abril, G., & Frankignoulle, M. (2001). Nitrogen–alkalinity interactions in the highly polluted scheldt basin (belgium). *Water Research*, 35(3), 844-850. https://doi.org/https://doi.org/10.1016/S0043-1354(00)00310-9
- Adkins, J. F., Naviaux, J. D., Subhas, A. V., Dong, S., & Berelson, W. M. (2021). The Dissolution Rate of CaCO<sub>3</sub> in the Ocean. *Annual Review of Marine Science*, *13*(Volume 13, 2021), 57-80. https://doi.org/https://doi.org/10.1146/annurev-marine-041720-092514
- Allen, J. R. L. (1987). Late Flandrian shoreline oscillations in the Severn Estuary: the Rumney formation at its typesite (Cardiff area). *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*, 315(1171), 157-184. https://doi.org/doi:10.1098/rstb.1987.0006
- Amann, T., Hartmann, J., Hellmann, R., Pedrosa, E. T., & Malik, A. (2022). Enhanced weathering potentials—the role of in situ CO<sub>2</sub> and grain size distribution. *Frontiers in Climate*, 4. <u>https://doi.org/10.3389/fclim.2022.929268</u>
- Amann, T., Hartmann, J., Struyf, E., De Oliveira Garcia, W., Fischer, E. K., Janssens, I., Meire, P., & Schoelynck, J. (2020). Enhanced Weathering and related element fluxes – a cropland mesocosm approach. *Biogeosciences*, 17(1), 103-119. <u>https://doi.org/10.5194/bg-17-103-2020</u>
- Amann, T., Weiss, A., & Hartmann, J. (2015). Inorganic Carbon Fluxes in the Inner Elbe Estuary, Germany. *Estuaries and Coasts*, 38(1), 192-210. <u>https://doi.org/10.1007/s12237-014-9785-6</u>
- Andrews, J. A., & Schlesinger, W. H. (2001). Soil CO<sub>2</sub> dynamics, acidification, and chemical weathering in a temperate forest with experimental CO<sub>2</sub> enrichment. *Global Biogeochemical Cycles*, 15(1), 149-162. <u>https://doi.org/10.1029/2000gb001278</u>
- Ayyad, M. A., & El-Ghareeh, R. E. M. (1982). Salt marsh vegetation of the Western Mediterranean desert of Egypt. *Vegetatio*, 49(1), 3-19. <u>https://doi.org/10.1007/bf00051557</u>
- Bach, L. T., Gill, S. J., Rickaby, R. E. M., Gore, S., & Renforth, P. (2019). CO<sub>2</sub> Removal With Enhanced Weathering and Ocean Alkalinity Enhancement: Potential Risks and Co-benefits for Marine Pelagic Ecosystems [Original Research]. *Frontiers in Climate*, 1. <u>https://doi.org/10.3389/fclim.2019.00007</u>
- Bauer, J. E., Cai, W.-J., Raymond, P. A., Bianchi, T. S., Hopkinson, C. S., & Regnier, P. A. G. (2013). The changing carbon cycle of the coastal ocean. *Nature*, 504(7478), 61-70. <u>https://doi.org/10.1038/nature12857</u>

- Beaulieu, E., Goddéris, Y., Labat, D., Roelandt, C., Calmels, D., & Gaillardet, J. (2011). Modeling of water-rock interaction in the Mackenzie basin: Competition between sulfuric and carbonic acids. *Chemical Geology*, 289(1), 114-123. https://doi.org/https://doi.org/10.1016/j.chemgeo.2011.07.020
- Berner, R. A. (1992). Weathering, plants, and the long-term carbon cycle. *Geochimica et Cosmochimica Acta*, 56(8), 3225-3231. <u>https://doi.org/10.1016/0016-7037(92)90300-8</u>
- Bezbaruah, A. N., & Zhang, T. C. (2005). Quantification of oxygen release by bulrush (Scirpus validus) roots in a constructed treatment wetland. *Biotechnology and Bioengineering*, 89(3), 308-318. <u>https://doi.org/10.1002/bit.20332</u>
- Blossfeld, S., & Gansert, D. (2012). The Use of Planar Optodes in Root Studies for Quantitative Imaging. In (pp. 83-92). Springer Berlin Heidelberg. <u>https://doi.org/10.1007/978-3-642-22067-8\_5</u>
- Blossfeld, S., Schreiber, C. M., Liebsch, G., Kuhn, A. J., & Hinsinger, P. (2013). Quantitative imaging of rhizosphere pH and CO<sub>2</sub> dynamics with planar optodes. *Annals of Botany*, 112(2), 267-276. <u>https://doi.org/10.1093/aob/mct047</u>
- Bowes, G. (1993). Facing the Inevitable: Plants and Increasing Atmospheric CO<sub>2</sub>. *Annual Review of Plant Biology*, *44*(Volume 44, 1993), 309-332. https://doi.org/https://doi.org/10.1146/annurev.pp.44.060193.001521
- Bricker, O. P., Godfrey, A. E., & Cleaves, E. T. (1968). Mineral-Water Interaction During the Chemical Weathering of Silicates. In *Trace Inorganics In Water* (Vol. 73, pp. 128-142). AMERICAN CHEMICAL SOCIETY. <u>https://doi.org/doi:10.1021/ba-1968-0073.ch006</u>
- Briese, L., Arvidson, R. S., & Luttge, A. (2017). The effect of crystal size variation on the rate of dissolution A kinetic Monte Carlo study. *Geochimica et Cosmochimica Acta*, 212, 167-175. https://doi.org/https://doi.org/10.1016/j.gca.2017.06.010
- Ca çador, I., Vale, C., & Catarino, F. (1996). Accumulation of Zn, Pb, Cu, Cr and Ni in Sediments Between Roots of the Tagus Estuary Salt Marshes, Portugal. *Estuarine, Coastal and Shelf Science*, 42(3), 393-403. <u>https://doi.org/https://doi.org/10.1006/ecss.1996.0026</u>
- Cadée, G. C. (1994). Eider, shelduck, and other predators, the main producers of shell fragments in the Wadden Sea: palaeoecological implications. *Palaeontology*, *37*, 181-202.
- Cai, W.-J., & Wang, Y. (1998). The chemistry, fluxes, and sources of carbon dioxide in the estuarine waters of the Satilla and Altamaha Rivers, Georgia. *Limnology and Oceanography*, 43(4), 657-668. <u>https://doi.org/10.4319/lo.1998.43.4.0657</u>
- Cai, W.-J., Wang, Y., Krest, J., & Moore, W. S. (2003). The geochemistry of dissolved inorganic carbon in a surficial groundwater aquifer in North Inlet, South Carolina, and the carbon fluxes to the coastal ocean. *Geochimica et Cosmochimica Acta*, 67(4), 631-639. <u>https://doi.org/https://doi.org/10.1016/S0016-7037(02)01167-5</u>
- Calabrese, S., & Porporato, A. (2020). Wetness controls on global chemical weathering. *Environmental Research Communications*, 2(8), 085005. <u>https://doi.org/10.1088/2515-7620/abad7b</u>
- Call, M., Schulz, K. G., Carvalho, M. C., Santos, I. R., & Maher, D. T. (2017). Technical note: Coupling infrared gas analysis and cavity ring down spectroscopy for autonomous, hightemporal-resolution measurements of DIC and & δ<sup>13</sup>C–DIC. *Biogeosciences*, 14(5), 1305-1313. <u>https://doi.org/10.5194/bg-14-1305-2017</u>
- Calmels, D., Gaillardet, J., & François, L. (2014). Sensitivity of carbonate weathering to soil CO<sub>2</sub> production by biological activity along a temperate climate transect. *Chemical Geology*, 390, 74-86. <u>https://doi.org/https://doi.org/10.1016/j.chemgeo.2014.10.010</u>
- Cerling, T. E. (1984). The stable isotopic composition of modern soil carbonate and its relationship to climate. *Earth and Planetary Science Letters*, 71(2), 229-240. https://doi.org/https://doi.org/10.1016/0012-821X(84)90089-X
- Cerling, T. E., Harris, J. M., Macfadden, B. J., Leakey, M. G., Quade, J., Eisenmann, V., & Ehleringer, J. R. (1997). Global vegetation change through the Miocene/Pliocene boundary. *Nature*, 389(6647), 153-158. <u>https://doi.org/10.1038/38229</u>
- Cerling, T. E., Solomon, D. K., Quade, J., & Bowman, J. R. (1991). On the isotopic composition of carbon in soil carbon dioxide. *Geochimica et Cosmochimica Acta*, 55(11), 3403-3405. https://doi.org/10.1016/0016-7037(91)90498-t
- Charles, H., & Dukes, J. S. (2009). Effects of warming and altered precipitation on plant and nutrient dynamics of a New England salt marsh. *Ecological Applications*, 19(7), 1758-1773. <u>https://doi.org/10.1890/08-0172.1</u>
- Chen, C. T. A., & Wang, S. L. (1999). Carbon, alkalinity and nutrient budgets on the East China Sea continental shelf. *Journal of Geophysical Research: Oceans*, 104(C9), 20675-20686. <u>https://doi.org/10.1029/1999jc900055</u>
- Chen, J., Wang, D., Li, Y., Yu, Z., Chen, S., Hou, X., White, J. R., & Chen, Z. (2020). The Carbon Stock and Sequestration Rate in Tidal Flats From Coastal China. *Global Biogeochemical Cycles*, 34(11). <u>https://doi.org/10.1029/2020gb006772</u>

- Cheng, W., & Kuzyakov, Y. (2005). Root Effects on Soil Organic Matter Decomposition. In *Roots and Soil Management: Interactions between Roots and the Soil* (pp. 119-143). https://doi.org/https://doi.org/10.2134/agronmonogr48.c7
- Chu, S. N., Wang, Z. A., Gonneea, M. E., Kroeger, K. D., & Ganju, N. K. (2018). Deciphering the dynamics of inorganic carbon export from intertidal salt marshes using high-frequency measurements. *Marine Chemistry*, 206, 7-18. <u>https://doi.org/https://doi.org/10.1016/j.marchem.2018.08.005</u>
- Colmer, T. D. (2003). Long-distance transport of gases in plants: a perspective on internal aeration and radial oxygen loss from roots. *Plant, Cell & Coll & Coll*
- Cubillas, P., Köhler, S., Prieto, M., Chaïrat, C., & Oelkers, E. H. (2005). Experimental determination of the dissolution rates of calcite, aragonite, and bivalves. *Chemical Geology*, *216*(1), 59-77. https://doi.org/https://doi.org/10.1016/j.chemgeo.2004.11.009
- Davidson, E. A., & Janssens, I. A. (2006). Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature*, 440(7081), 165-173. <u>https://doi.org/10.1038/nature04514</u>
- Dickson, A. G. (1981). An exact definition of total alkalinity and a procedure for the estimation of alkalinity and total inorganic carbon from titration data. *Deep Sea Research Part A. Oceanographic Research Papers*, *28*(6), 609-623. <u>https://doi.org/10.1016/0198-0149(81)90121-7</u>
- Domínguez-Villar, D., Bensa, A., Švob, M., & Krklec, K. (2022). Causes and implications of the seasonal dissolution and precipitation of pedogenic carbonates in soils of karst regions – A thermodynamic model approach. *Geoderma*, 423, 115962. <u>https://doi.org/https://doi.org/10.1016/j.geoderma.2022.115962</u>
- Drever, J. I., & Vance, G. F. (1994). Role of Soil Organic Acids in Mineral Weathering Processes. In E. D. Pittman & M. D. Lewan (Eds.), *Organic Acids in Geological Processes* (pp. 138-161). Springer Berlin Heidelberg. <u>https://doi.org/10.1007/978-3-642-78356-2\_6</u>
- Duarte, C. M., Losada, I. J., Hendriks, I. E., Mazarrasa, I., & Marbà, N. (2013). The role of coastal plant communities for climate change mitigation and adaptation. *Nature Climate Change*, *3*(11), 961-968. <u>https://doi.org/10.1038/nclimate1970</u>
- Duarte, C. M., Middelburg, J. J., & Caraco, N. (2005). Major role of marine vegetation on the oceanic carbon cycle. *Biogeosciences*, 2(1), 1-8. <u>https://doi.org/10.5194/bg-2-1-2005</u>

- Egleston, E. S., Sabine, C. L., & Morel, F. M. M. (2010). Revelle revisited: Buffer factors that quantify the response of ocean chemistry to changes in DIC and alkalinity. *Global Biogeochemical Cycles*, *24*(1). <u>https://doi.org/https://doi.org/10.1029/2008GB003407</u>
- Ejiri, M., Fukao, T., Miyashita, T., & Shiono, K. (2021). A barrier to radial oxygen loss helps the root system cope with waterlogging-induced hypoxia. *Breed Sci*, 71(1), 40-50. <u>https://doi.org/10.1270/jsbbs.20110</u>
- Ericson, J. A., & Ragg, N. L. C. (2022). Effects of crushed mussel, *Perna canaliculus*, shell enrichment on seawater carbonate buffering and development of conspecific larvae exposed to near-future ocean acidification. *Journal of the World Aquaculture Society*, *53*(1), 271-289. https://doi.org/10.1111/jwas.12779
- Esselink, P., van Duin, W., Bunje, J., Cremer, J., Folmer, E. O., Frikke, J., Glahn, M., de Groot, A., Hecker, N., Hellwig, U., Jensen, K., Körber, P., Petersen, J., & Stock, M. (2017). Salt marshes. In: S. Klöpper et al. (eds). Wadden Sea Quality Status Report 2017. Common Wadden Sea Secretariat, Wilhelmshaven.
- Fakhraee, M., Planavsky, N. J., & Reinhard, C. T. (2023). Ocean alkalinity enhancement through restoration of blue carbon ecosystems. *Nature Sustainability*. <u>https://doi.org/10.1038/s41893-023-01128-2</u>
- Fischer, E. M., Sedláček, J., Hawkins, E., & Knutti, R. (2014). Models agree on forced response pattern of precipitation and temperature extremes. *Geophysical Research Letters*, 41(23), 8554-8562. <u>https://doi.org/10.1002/2014gl062018</u>
- Foote, A. L., & Reynolds, K. A. (1997). Decomposition of Saltmeadow Cordgrass (Spartina patens) in Louisiana Coastal Marshes. *Estuaries*, 20(3), 579. <u>https://doi.org/10.2307/1352616</u>
- Gabrio, A., Plumpton, C., Banerjee, S., & Leurent, B. (2022). Linear mixed models to handle missing at random data in trial-based economic evaluations. *Health Economics*, *31*(6), 1276-1287. https://doi.org/10.1002/hec.4510
- Gałecki, A., & Burzykowski, T. (2013). Linear Mixed-Effects Model. In (pp. 245-273). Springer New York. https://doi.org/10.1007/978-1-4614-3900-4\_13
- Gattuso, J.-P., Williamson, P., Duarte, C. M., & Magnan, A. K. (2021). The Potential for Ocean-Based Climate Action: Negative Emissions Technologies and Beyond. *Frontiers in Climate*, 2. <u>https://doi.org/10.3389/fclim.2020.575716</u>
- Giblin, A. E., & Howarth, R. W. (1984). Porewater evidence for a dynamic sedimentary iron cycle in salt marshes1. *Limnology and Oceanography*, 29(1), 47-63. <u>https://doi.org/10.4319/lo.1984.29.1.0047</u>

- Gillikin, D. P., Lorrain, A., Bouillon, S., Willenz, P., & Dehairs, F. (2006). Stable carbon isotopic composition of Mytilus edulis shells: relation to metabolism, salinity, δ<sup>13</sup>C-DIC and phytoplankton. Organic Geochemistry, 37(10), 1371-1382. <u>https://doi.org/10.1016/j.orggeochem.2006.03.008</u>
- Granse, D., Romeiro Motta, M., Suchrow, S., von Schwartzenberg, K., Schnittger, A., & Jensen, K. (2022). The Overlooked Hybrid: Geographic Distribution and Niche Differentiation Between Spartina Cytotypes (Poaceae) in Wadden Sea Salt Marshes. *Estuaries and Coasts*, 45(5), 1409-1421. <u>https://doi.org/10.1007/s12237-021-00985-4</u>
- Granse, D., Wanner, A., Stock, M., Jensen, K., & Mueller, P. (2024). Plant-sediment interactions decouple inorganic from organic carbon stock development in salt marsh soils. *Limnology and Oceanography Letters*, 9(4), 469-477. <u>https://doi.org/10.1002/lol2.10382</u>
- Grasshoff, K., Kremling, K., & Ehrhardt, M. (2009). *Methods of seawater analysis*. John Wiley & Sons.
- Gratton, C., & Denno, R. F. (2006). Arthropod Food Web Restoration Following Removal Of An Invasive Wetland Plant. *Ecological Applications*, *16*(2), 622-631. <u>https://doi.org/https://doi.org/10.1890/1051-0761(2006)016[0622:AFWRFR]2.0.CO;2</u>
- Gray, A. J., & Mogg, R. J. (2001). Climate impacts on pioneer saltmarsh plants. *Climate Research*, *18*(1/2), 105-112. <u>http://www.jstor.org/stable/24861564</u>
- Guo, J., Zhang, W., Zhang, M., Zhang, L., & Bian, X. (2012). Will elevated CO<sub>2</sub> enhance mineral bioavailability in wetland ecosystems? Evidence from a rice ecosystem. *Plant and Soil*, 355(1-2), 251-263. <u>https://doi.org/10.1007/s11104-011-1096-0</u>
- Hack, H. R. G. K. (2020). Weathering, Erosion, and Susceptibility to Weathering. In M. Kanji, M. He, & L. Ribeiro e Sousa (Eds.), *Soft Rock Mechanics and Engineering* (pp. 291-333). Springer International Publishing. <u>https://doi.org/10.1007/978-3-030-29477-9\_11</u>
- Hartmann, A., Schmid, M., Tuinen, D. V., & Berg, G. (2009). Plant-driven selection of microbes. *Plant and Soil*, 321(1-2), 235-257. <u>https://doi.org/10.1007/s11104-008-9814-y</u>
- Hartmann, J., Suitner, N., Lim, C., Schneider, J., Marín-Samper, L., Arístegui, J., Renforth, P., Taucher, J., & Riebesell, U. (2023). Stability of alkalinity in ocean alkalinity enhancement (OAE) approaches – consequences for durability of CO<sub>2</sub> storage. *Biogeosciences*, 20(4), 781-802. <u>https://doi.org/10.5194/bg-20-781-2023</u>
- Hartmann, J., West, A. J., Renforth, P., Köhler, P., De La Rocha, C. L., Wolf-Gladrow, D. A., Dürr, H. H., & Scheffran, J. (2013). Enhanced chemical weathering as a geoengineering strategy to reduce atmospheric carbon dioxide, supply nutrients, and mitigate ocean acidification. *Reviews of Geophysics*, *51*(2), 113-149. <u>https://doi.org/https://doi.org/10.1002/rog.20004</u>

- He, S., Maiti, K., Swarzenski, C. M., Elsey-Quirk, T., Groseclose, G. N., & Justic, D. (2022). Porewater chemistry of Louisiana marshes with contrasting salinities and its implications for coastal acidification. *Estuarine, Coastal and Shelf Science*, 268, 107801. <u>https://doi.org/10.1016/j.ecss.2022.107801</u>
- Hiltner, L. (1904). Über neuere Erfahrungen und Probleme auf dem Gebiet der Bodenbakteriologie und unter besonderer Berücksichtigung der Gründüngung und Brache. *Arbeiten der deutschen landwirtschaftlichen gesellschaft*, 98, 59.
- Hines, M. E., Knollmeyer, S. L., & Tugel, J. B. (1989). Sulfate reduction and other sedimentary biogeochemistry in a northern New England salt marsh. *Limnology and Oceanography*, 34(3), 578-590. <u>https://doi.org/10.4319/lo.1989.34.3.0578</u>
- Hinsinger, P., Fernandes Barros, O. N., Benedetti, M. F., Noack, Y., & Callot, G. (2001). Plantinduced weathering of a basaltic rock: experimental evidence. *Geochimica et Cosmochimica Acta*, 65(1), 137-152. <u>https://doi.org/https://doi.org/10.1016/S0016-7037(00)00524-X</u>
- Howard, J., Sutton-Grier, A., Herr, D., Kleypas, J., Landis, E., Mcleod, E., Pidgeon, E., & Simpson, S. (2017). Clarifying the role of coastal and marine systems in climate mitigation. *Frontiers in Ecology and the Environment*, 15(1), 42-50. https://doi.org/https://doi.org/10.1002/fee.1451
- Howarth, R. W., & Teal, J. M. (1979). Sulfate reduction in a New England salt marsh1. *Limnology* and Oceanography, 24(6), 999-1013. <u>https://doi.org/10.4319/lo.1979.24.6.0999</u>
- Hu, X., & Cai, W.-J. (2011). An assessment of ocean margin anaerobic processes on oceanic alkalinity budget. *Global Biogeochemical Cycles*, 25(3). <u>https://doi.org/10.1029/2010gb003859</u>
- Ikkonen, E. N., García-Calderón, N. E., Ibáñez-Huerta, A., Etchevers-Barra, J. D., & Krasilnikov, P. V. (2018). Seasonal Dynamics of Soil CO<sub>2</sub> Concentration and CO<sub>2</sub> Fluxes from the Soil of the Former Lake Texcoco, Mexico. *Eurasian Soil Science*, 51(6), 674-681. https://doi.org/10.1134/s106422931806008x
- Israeli, Y., & Emmanuel, S. (2018). Impact of grain size and rock composition on simulated rock weathering. *Earth Surface Dynamics*, 6(2), 319-327. <u>https://doi.org/10.5194/esurf-6-319-2018</u>
- Jeffery, P. M., Compston, W., Greenhalgh, D., & de Laeter, J. (1955). On the carbon-13 abundance of limestones and coals. *Geochimica et Cosmochimica Acta*, 7(5), 255-286. <u>https://doi.org/https://doi.org/10.1016/0016-7037(55)90037-5</u>

- Ju, R.-T., Chen, Y.-Y., Gao, L., & Li, B. (2016). The extended phenology of Spartina invasion alters a native herbivorous insect's abundance and diet in a Chinese salt marsh. *Biological Invasions*, 18(8), 2229-2236. <u>https://doi.org/10.1007/s10530-015-0981-5</u>
- Kim, S.-Y., Kang, H., & Freeman, C. (2004). Effects Of Elevated Atmospheric CO<sub>2</sub> On Wetland Soils. In S.-K. Hong, J. A. Lee, B.-S. Ihm, A. Farina, Y. Son, E.-S. Kim, & J. C. Choe, *Ecological Issues in a Changing World* Dordrecht.
- Kirwan, M. L., & Megonigal, J. P. (2013). Tidal wetland stability in the face of human impacts and sea-level rise. *Nature*, *504*(7478), 53-60. <u>https://doi.org/10.1038/nature12856</u>
- Koop-Jakobsen, K., & Dolch, T. (2023). Future climate conditions alter biomass of salt marsh plants in the Wadden Sea. *Marine Biodiversity*, 53(3). <u>https://doi.org/10.1007/s12526-023-01347-y</u>
- Koop-Jakobsen, K., Meier, R. J., & Mueller, P. (2021). Plant-Mediated Rhizosphere Oxygenation in the Native Invasive Salt Marsh Grass Elymus athericus [Original Research]. Frontiers in Plant Science, 12. <u>https://doi.org/10.3389/fpls.2021.669751</u>
- Koop-Jakobsen, K., Mueller, P., Meier, R. J., Liebsch, G., & Jensen, K. (2018). Plant-Sediment Interactions in Salt Marshes – An Optode Imaging Study of O<sub>2</sub>, pH, and CO<sub>2</sub> Gradients in the Rhizosphere. *Frontiers in Plant Science*, 9. <u>https://doi.org/10.3389/fpls.2018.00541</u>
- Koop-Jakobsen, K., & Wenzhöfer, F. (2015). The Dynamics of Plant-Mediated Sediment Oxygenation in Spartina anglica Rhizospheres—a Planar Optode Study. *Estuaries and Coasts*, 38(3), 951-963. <u>https://doi.org/10.1007/s12237-014-9861-y</u>
- Koretsky, C. M., Haveman, M., Cuellar, A., Beuving, L., Shattuck, T., & Wagner, M. (2008). Influence of Spartina and Juncus on Saltmarsh Sediments. I. Pore Water Geochemistry. *Chemical Geology*, 255(1), 87-99. https://doi.org/https://doi.org/10.1016/j.chemgeo.2008.06.013
- Koretsky, C. M., & Miller, D. (2008). Seasonal Influence of the Needle Rush Juncus roemarianus on Saltmarsh Pore Water Geochemistry. *Estuaries and Coasts*, 31(1), 70-84. <u>https://doi.org/10.1007/s12237-007-9000-0</u>
- Koretsky, C. M., Moore, C. M., Lowe, K. L., Meile, C., Dichristina, T. J., & Van Cappellen, P. (2003). Seasonal Oscillation of Microbial Iron and Sulfate Reduction in Saltmarsh Sediments (Sapelo Island, GA, USA). *Biogeochemistry*, 64(2), 179-203. <u>http://www.jstor.org/stable/1469675</u>
- Kostka, J. E., Gribsholt, B., Petrie, E., Dalton, D., Skelton, H., & Kristensen, E. (2002a). The rates and pathways of carbon oxidation in bioturbated saltmarsh sediments. *Limnology and Oceanography*, 47(1), 230-240. <u>https://doi.org/https://doi.org/10.4319/lo.2002.47.1.0230</u>

- Kostka, J. E., Roychoudhury, A., & Van Cappellen, P. (2002b). Rates and controls of anaerobic microbial respiration across spatial and temporal gradients in saltmarsh sediments. *Biogeochemistry*, 60(1), 49-76. <u>https://doi.org/10.1023/a:1016525216426</u>
- Krumins, V., Gehlen, M., Arndt, S., Van Cappellen, P., & Regnier, P. (2013). Dissolved inorganic carbon and alkalinity fluxes from coastal marine sediments: model estimates for different shelf environments and sensitivity to global change. *Biogeosciences*, 10(1), 371-398. <u>https://doi.org/10.5194/bg-10-371-2013</u>
- Kuzyakov, Y. (2006). Sources of CO<sub>2</sub> efflux from soil and review of partitioning methods. Soil Biology and Biochemistry, 38(3), 425-448. <u>https://doi.org/https://doi.org/10.1016/j.soilbio.2005.08.020</u>
- Kuzyakov, Y. (2010). Priming effects: Interactions between living and dead organic matter. Soil Biology and Biochemistry, 42(9), 1363-1371. <u>https://doi.org/https://doi.org/10.1016/j.soilbio.2010.04.003</u>
- Kuzyakov, Y., & Larionova, A. A. (2005). Root and rhizomicrobial respiration: A review of approaches to estimate respiration by autotrophic and heterotrophic organisms in soil. *Journal of Plant Nutrition and Soil Science*, 168(4), 503-520. <u>https://doi.org/10.1002/jpln.200421703</u>
- Lasaga, A. C. (1984). Chemical kinetics of water-rock interactions. *Journal of Geophysical Research:* Solid Earth, 89(B6), 4009-4025. <u>https://doi.org/https://doi.org/10.1029/JB089iB06p04009</u>
- Le Quéré, C., Raupach, M. R., Canadell, J. G., Marland, G., Bopp, L., Ciais, P., Conway, T. J., Doney, S. C., Feely, R. A., Foster, P., Friedlingstein, P., Gurney, K., Houghton, R. A., House, J. I., Huntingford, C., Levy, P. E., Lomas, M. R., Majkut, J., Metzl, N., . . . Woodward, F. I. (2009). Trends in the sources and sinks of carbon dioxide. *Nature Geoscience*, 2(12), 831-836. <u>https://doi.org/10.1038/ngeo689</u>
- Lechler, P. J., & Desilets, M. O. (1987). A review of the use of loss on ignition as a measurement of total volatiles in whole-rock analysis. *Chemical Geology*, 63(3), 341-344. <u>https://doi.org/https://doi.org/10.1016/0009-2541(87)90171-9</u>
- Lee, R. W., Kraus, D. W., & Doeller, J. E. (1999). Oxidation of sulfide by Spartina alterniflora roots. *Limnology and Oceanography*, 44(4), 1155-1159. <u>https://doi.org/10.4319/lo.1999.44.4.1155</u>
- Lehmann, N., Lantuit, H., Böttcher, M. E., Hartmann, J., Eulenburg, A., & Thomas, H. (2023). Alkalinity generation from carbonate weathering in a silicate-dominated headwater catchment at Iskorasfjellet, northern Norway. *Biogeosciences*, 20(16), 3459-3479. <u>https://doi.org/10.5194/bg-20-3459-2023</u>

- Lenssen, G. M., Lamers, J., Stroetenga, M., & Rozema, J. (1993). Interactive effects of atmospheric CO<sub>2</sub> enrichment, salinity and flooding on growth of C3 (Elymus athericus) and C4 (Spartina anglica) salt marsh species. *Vegetatio*, 104-105(1), 379-388. <u>https://doi.org/10.1007/bf00048167</u>
- Lenzewski, N., Mueller, P., Meier, R. J., Liebsch, G., Jensen, K., & Koop-Jakobsen, K. (2018). Dynamics of oxygen and carbon dioxide in rhizospheres of Lobelia dortmanna - a planar optode study of belowground gas exchange between plants and sediment. *New Phytologist*, 218(1), 131-141. <u>https://doi.org/10.1111/nph.14973</u>
- Lescinsky, H. L., Edinger, E., & Risk, M. J. (2002). Mollusc Shell Encrustation and Bioerosion Rates in a Modern Epeiric Sea: Taphonomy Experiments in the Java Sea, Indonesia. *PALAIOS*, 17(2), 171-191. <u>http://www.jstor.org/stable/3515682</u>
- Lin, C. Y., Turchyn, A. V., Krylov, A., & Antler, G. (2020). The microbially driven formation of siderite in salt marsh sediments. *Geobiology*, 18(2), 207-224. <u>https://doi.org/10.1111/gbi.12371</u>
- Liu, Y., Jiao, J. J., Liang, W., Santos, I. R., Kuang, X., & Robinson, C. E. (2021). Inorganic carbon and alkalinity biogeochemistry and fluxes in an intertidal beach aquifer: Implications for ocean acidification. *Journal of Hydrology*, 595, 126036. <u>https://doi.org/https://doi.org/10.1016/j.jhydrol.2021.126036</u>
- Lovelock, C. E., & Reef, R. (2020). Variable Impacts of Climate Change on Blue Carbon. *One Earth*, 3(2), 195-211. <u>https://doi.org/10.1016/j.oneear.2020.07.010</u>
- Lukawska-Matuszewska, K., Grzybowski, W., Szewczun, A., & Tarasiewicz, P. (2018). Constituents of organic alkalinity in pore water of marine sediments. *Marine Chemistry*, 200, 22-32. https://doi.org/https://doi.org/10.1016/j.marchem.2018.01.012
- Lynch, J. M., & Whipps, J. M. (1990). Substrate flow in the rhizosphere. *Plant and Soil*, *129*(1), 1-10. <u>https://doi.org/10.1007/bf00011685</u>
- Ma, Z., Wu, Y., Cui, Y., Pan, Y., Zhao, S., Liu, J., Zhang, Z., & Zhang, M. (2024). Coastal distribution and driving factors for blue carbon fractions in the surface soil of a warmtemperate salt marsh in China. *Chemosphere*, 350, 141044. <u>https://doi.org/https://doi.org/10.1016/j.chemosphere.2023.141044</u>
- Macreadie, P. I., Anton, A., Raven, J. A., Beaumont, N., Connolly, R. M., Friess, D. A., Kelleway, J. J., Kennedy, H., Kuwae, T., Lavery, P. S., Lovelock, C. E., Smale, D. A., Apostolaki, E. T., Atwood, T. B., Baldock, J., Bianchi, T. S., Chmura, G. L., Eyre, B. D., Fourqurean, J. W., . . . Duarte, C. M. (2019). The future of Blue Carbon science. *Nature Communications*, 10(1). https://doi.org/10.1038/s41467-019-11693-w

- Macreadie, P. I., Costa, M. D. P., Atwood, T. B., Friess, D. A., Kelleway, J. J., Kennedy, H., Lovelock, C. E., Serrano, O., & Duarte, C. M. (2021). Blue carbon as a natural climate solution. *Nature Reviews Earth & amp; Environment*, 2(12), 826-839. <u>https://doi.org/10.1038/s43017-021-00224-1</u>
- Macreadie, P. I., Serrano, O., Maher, D. T., Duarte, C. M., & Beardall, J. (2017). Addressing calcium carbonate cycling in blue carbon accounting. *Limnology and Oceanography Letters*, 2(6), 195-201. <u>https://doi.org/10.1002/lol2.10052</u>
- Maher, K. (2010). The dependence of chemical weathering rates on fluid residence time. *Earth and Planetary Science Letters*, 294(1), 101-110. https://doi.org/https://doi.org/10.1016/j.epsl.2010.03.010
- Maher, K. (2011). The role of fluid residence time and topographic scales in determining chemical fluxes from landscapes. *Earth and Planetary Science Letters*, *312*(1), 48-58. <u>https://doi.org/https://doi.org/10.1016/j.epsl.2011.09.040</u>
- Maher, K., Steefel, C. I., White, A. F., & Stonestrom, D. A. (2009). The role of reaction affinity and secondary minerals in regulating chemical weathering rates at the Santa Cruz Soil Chronosequence, California. *Geochimica et Cosmochimica Acta*, 73(10), 2804-2831. <u>https://doi.org/10.1016/j.gca.2009.01.030</u>
- Marsh, A. S., Rasse, D. P., Drake, B. G., & Patrick Megonigal, J. (2005). Effect of elevated CO<sub>2</sub> on carbon pools and fluxes in a brackish marsh. *Estuaries*, 28(5), 694-704. https://doi.org/10.1007/bf02732908
- Martin, J. B. (2017). Carbonate minerals in the global carbon cycle. *Chemical Geology*, 449, 58-72. https://doi.org/https://doi.org/10.1016/j.chemgeo.2016.11.029
- McKee, K. L., Mendelssohn, I. A., & D. Materne, M. (2004). Acute salt marsh dieback in the Mississippi River deltaic plain: a drought-induced phenomenon? *Global Ecology and Biogeography*, 13(1), 65-73. <u>https://doi.org/https://doi.org/10.1111/j.1466-882X.2004.00075.x</u>
- McLeod, E., Chmura, G. L., Bouillon, S., Salm, R., Björk, M., Duarte, C. M., Lovelock, C. E., Schlesinger, W. H., & Silliman, B. R. (2011). A blueprint for blue carbon: toward an improved understanding of the role of vegetated coastal habitats in sequestering CO<sub>2</sub>. *Frontiers in Ecology and the Environment*, 9(10), 552-560. <u>https://doi.org/10.1890/110004</u>
- McOwen, C., Weatherdon, L., Bochove, J.-W., Sullivan, E., Blyth, S., Zockler, C., Stanwell-Smith, D., Kingston, N., Martin, C., Spalding, M., & Fletcher, S. (2017). A global map of saltmarshes. *Biodiversity Data Journal*, 5, e11764. <u>https://doi.org/10.3897/bdj.5.e11764</u>

- Meheruna, A., & Akagi, T. (2006). Role of fine roots in the plant-induced weathering of andesite for several plant species. *GEOCHEMICAL JOURNAL*, 40(1), 57-67. <u>https://doi.org/10.2343/geochemj.40.57</u>
- Middelburg, J. J., Nieuwenhuize, J., Lubberts, R. K., & van de Plassche, O. (1997). Organic Carbon Isotope Systematics of Coastal Marshes. *Estuarine, Coastal and Shelf Science*, 45(5), 681-687. <u>https://doi.org/https://doi.org/10.1006/ecss.1997.0247</u>
- Middelburg, J. J., Soetaert, K., & Hagens, M. (2020). Ocean Alkalinity, Buffering and Biogeochemical Processes. *Reviews of Geophysics*, 58(3). <u>https://doi.org/10.1029/2019rg000681</u>
- Millero, F. J. (1979). The thermodynamics of the carbonate system in seawater. *Geochimica et Cosmochimica Acta*, 43(10), 1651-1661. <u>https://doi.org/https://doi.org/10.1016/0016-7037(79)90184-4</u>
- Mittmann-Goetsch, J., Wilson, M., Jensen, K., & Mueller, P. (2024). Wetland roots as soil reducers Insights from a Wadden Sea salt-marsh study. Research Square Platform LLC. https://dx.doi.org/10.21203/rs.3.rs-3934063/v1
- Moffett, K. B., & Gorelick, S. M. (2016). Relating salt marsh pore water geochemistry patterns to vegetation zones and hydrologic influences. *Water Resources Research*, *52*(3), 1729-1745. https://doi.org/10.1002/2015wr017406
- Morris, J. T., Sundareshwar, P. V., Nietch, C. T., Kjerfve, B., & Cahoon, D. R. (2002). Response of coastal wetlands to rising sea level. *Ecology*, 83(10), 2869-2877. <u>https://doi.org/https://doi.org/10.1890/0012-9658(2002)083[2869:ROCWTR]2.0.CO;2</u>
- Mucci, A. (1983). The solubility of calcite and aragonite in seawater at various salinities, temperatures, and one atmosphere total pressure. *American Journal of Science*, 283(7), 780-799. <u>https://doi.org/10.2475/ajs.283.7.780</u>
- Mueller, P. (2017). Global Change and Land Use Effects on Carbon Turnover in Tidal Wetlands [Dissertation]. <u>https://ediss.sub.uni-hamburg.de/handle/ediss/7376</u>
- Mueller, P., Granse, D., Nolte, S., Weingartner, M., Hoth, S., & Jensen, K. (2020). Unrecognized controls on microbial functioning in Blue Carbon ecosystems: The role of mineral enzyme stabilization and allochthonous substrate supply. *Ecology and Evolution*, 10(2), 998-1011. <u>https://doi.org/10.1002/ece3.5962</u>
- Mueller, P., Jensen, K., & Megonigal, J. P. (2016). Plants mediate soil organic matter decomposition in response to sea level rise. *Global Change Biology*, 22(1), 404-414. <u>https://doi.org/10.1111/gcb.13082</u>

- Mueller, P., Kutzbach, L., Mozdzer, T. J., Jespersen, E., Barber, D. C., & Eller, F. (2023). Minerogenic salt marshes can function as important inorganic carbon stores. *Limnology and Oceanography*, 68(4), 942-952. <u>https://doi.org/10.1002/lno.12322</u>
- National Academies of Sciences, E., & Medicine. (2022). A Research Strategy for Ocean-based Carbon Dioxide Removal and Sequestration. *The National Academies Press*. <u>https://doi.org/doi:10.17226/26278</u>

Nellemann, C. (2009). Blue carbon. A UNEP rapid response assessment.

- Nguyen, C., Todorovic, C., Robin, C., Christophe, A., & Guckert, A. (1999). Continuous monitoring of rhizosphere respiration after labelling of plant shoots with <sup>14</sup>CO<sub>2</sub>. *Plant and Soil*, *212*(2), 189-199. <u>https://doi.org/10.1023/a:1004681528074</u>
- Norbisrath, M., Pätsch, J., Dähnke, K., Sanders, T., Schulz, G., Van Beusekom, J. E. E., & Thomas, H. (2022). Metabolic alkalinity release from large port facilities (Hamburg, Germany) and impact on coastal carbon storage. *Biogeosciences*, 19(22), 5151-5165. <u>https://doi.org/10.5194/bg-19-5151-2022</u>
- Odum, E. P. (1968). Energy Flow in Ecosystems: A Historical Review. *American Zoologist*, 8(1), 11-18. <u>https://doi.org/10.1093/icb/8.1.11</u>
- Ouyang, X., & Lee, S. Y. (2014). Updated estimates of carbon accumulation rates in coastal marsh sediments. *Biogeosciences*, 11(18), 5057-5071. <u>https://doi.org/10.5194/bg-11-5057-2014</u>
- Parkhurst, D. L., & Appelo, C. A. J. (2013). Description of input and examples for PHREEQC version 3: a computer program for speciation, batch-reaction, one-dimensional transport, and inverse geochemical calculations [Report](6-A43). (Techniques and Methods, Issue. U. S. G. Survey. <u>http://pubs.er.usgs.gov/publication/tm6A43</u>
- Phillips, D. L., Newsome, S. D., & Gregg, J. W. (2005). Combining sources in stable isotope mixing models: alternative methods. *Oecologia*, 144(4), 520-527. <u>https://doi.org/10.1007/s00442-004-1816-8</u>
- Pörtner, H.-O., Roberts, D. C., & H. Adams, I. A., C. Adler, R. Adrian, P. Aldunce, E. Ali, R. Ara Begum, B. Bednar-Friedl, R. Bezner Kerr, R. Biesbroek, J. Birkmann, K. Bowen, M.A. Caretta, J. Carnicer, E. Castellanos, T.S. Cheong, W. Chow, G. Cissé, S. Clayton, A. Constable, S. Cooley, M.J. Costello, M. Craig, W. Cramer, R. Dawson, D. Dodman, J. Efitre, M. Garschagen, E.A. Gilmore, B. Glavovic, D. Gutzler, M. Haasnoot, S. Harper, T. Hasegawa, B. Hayward, J.A. Hicke, Y. Hirabayashi, C. Huang, K. Kalaba, W. Kiessling, A. Kitoh, R. Lasco, J. Lawrence, M.F. Lemos, R. Lempert, C. Lennard, D. Ley, T. Lissner, Q. Liu, E. Liwenga, S. Lluch-Cota, S. Löschke, S. Lucatello, Y. Luo, B. Mackey, K. Mintenbeck, A. Mirzabaev, V. Möller, M. Moncassim Vale, M.D. Morecroft, L. Mortsch, A. Mukherji, T. Mustonen, M. Mycoo, J. Nalau, M. New, A. Okem (South Africa), J.P. Ometto, B. O'Neill, R. Pandey, C. Parmesan, M. Pelling, P.F. Pinho, J. Pinnegar, E.S. Poloczanska,

A. Prakash, B. Preston, M.-F. Racault, D. Reckien, A. Revi, S.K. Rose, E.L.F. Schipper, D.N. Schmidt, D. Schoeman, R. Shaw, N.P. Simpson, C. Singh, W. Solecki, L. Stringer, E. Totin, C.H. Trisos, Y. Trisurat, M. van Aalst, D. Viner, M. Wairu, R. Warren, P. Wester, D. Wrathall, and Z. Zaiton Ibrahim. (2022). Technical Summary: Climate Change 2022: Impacts, Adaptation, and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. https://doi.org/10.1017/9781009325844.002

- Ramnarine, R., Wagner-Riddle, C., Dunfield, K. E., & Voroney, R. P. (2012). Contributions of carbonates to soil CO<sub>2</sub> emissions. *Canadian Journal of Soil Science*, 92(4), 599-607. <u>https://doi.org/10.4141/cjss2011-025</u>
- Raymond, P. A., Bauer, J. E., & Cole, J. J. (2000). Atmospheric CO<sub>2</sub> evasion, dissolved inorganic carbon production, and net heterotrophy in the York River estuary. *Limnology and Oceanography*, 45(8), 1707-1717. <u>https://doi.org/10.4319/lo.2000.45.8.1707</u>
- Raymond, P. A., & Hamilton, S. K. (2018). Anthropogenic influences on riverine fluxes of dissolved inorganic carbon to the oceans. *Limnology and Oceanography Letters*, 3(3), 143-155. <u>https://doi.org/10.1002/lol2.10069</u>
- Reaves, C. M. (1986). Organic matter metabolizability and calcium carbonate dissolution in nearshore marine muds. *Journal of Sedimentary Research*, *56*(4), 486-494. <u>https://doi.org/10.1306/212f8963-2b24-11d7-8648000102c1865d</u>
- Reddy, A. R., Rasineni, G. K., & Raghavendra, A. S. (2010). The impact of global elevated CO<sub>2</sub> concentration on photosynthesis and plant productivity. *Current Science*, 46-57.
- Reents, S., Mueller, P., Tang, H., Jensen, K., & Nolte, S. (2021). Plant genotype determines biomass response to flooding frequency in tidal wetlands. *Biogeosciences*, 18(2), 403-411. <u>https://doi.org/10.5194/bg-18-403-2021</u>
- Reithmaier, G. M. S., Cabral, A., Akhand, A., Bogard, M. J., Borges, A. V., Bouillon, S., Burdige, D. J., Call, M., Chen, N., Chen, X., Cotovicz, L. C., Eagle, M. J., Kristensen, E., Kroeger, K. D., Lu, Z., Maher, D. T., Pérez-Lloréns, J. L., Ray, R., Taillardat, P., . . . Santos, I. R. (2023). Carbonate chemistry and carbon sequestration driven by inorganic carbon outwelling from mangroves and saltmarshes. *Nature Communications*, *14*(1). <u>https://doi.org/10.1038/s41467-023-44037-w</u>
- Reithmaier, G. M. S., Ho, D. T., Johnston, S. G., & Maher, D. T. (2020). Mangroves as a Source of Greenhouse Gases to the Atmosphere and Alkalinity and Dissolved Carbon to the Coastal Ocean: A Case Study From the Everglades National Park, Florida. *Journal of Geophysical Research: Biogeosciences*, 125(12). <u>https://doi.org/10.1029/2020jg005812</u>

- Reithmaier, G. M. S., Johnston, S. G., Junginger, T., Goddard, M. M., Sanders, C. J., Hutley, L. B., Ho, D. T., & Maher, D. T. (2021). Alkalinity Production Coupled to Pyrite Formation Represents an Unaccounted Blue Carbon Sink. *Global Biogeochemical Cycles*, 35(4). <u>https://doi.org/10.1029/2020gb006785</u>
- Ren, L., Jensen, K., Porada, P., & Mueller, P. (2022). Biota-mediated carbon cycling—A synthesis of biotic-interaction controls on blue carbon. *Ecology Letters*, 25(2), 521-540. <u>https://doi.org/10.1111/ele.13940</u>
- Renforth, P., & Henderson, G. (2017). Assessing ocean alkalinity for carbon sequestration. *Reviews of Geophysics*, 55(3), 636-674. <u>https://doi.org/https://doi.org/10.1002/2016RG000533</u>
- Říhová, D., Janovský, Z., Horsák, M., & Juřičková, L. (2018). Shell decomposition rates in relation to shell size and habitat conditions in contrasting types of Central European forests. *Journal of Molluscan Studies*, 84(1), 54-61. <u>https://doi.org/10.1093/mollus/eyx048</u>
- Robinson, C. E., Xin, P., Santos, I. R., Charette, M. A., Li, L., & Barry, D. A. (2018). Groundwater dynamics in subterranean estuaries of coastal unconfined aquifers: Controls on submarine groundwater discharge and chemical inputs to the ocean. *Advances in Water Resources*, 115, 315-331. <u>https://doi.org/https://doi.org/10.1016/j.advwatres.2017.10.041</u>
- Romero-Mujalli, G., Hartmann, J., & Börker, J. (2019a). Temperature and CO<sub>2</sub> dependency of global carbonate weathering fluxes – Implications for future carbonate weathering research. *Chemical Geology*, 527, 118874. <u>https://doi.org/10.1016/j.chemgeo.2018.08.010</u>
- Romero-Mujalli, G., Hartmann, J., Börker, J., Gaillardet, J., & Calmels, D. (2019b). Ecosystem controlled soil-rock pCO<sub>2</sub> and carbonate weathering – Constraints by temperature and soil water content. *Chemical Geology*, 527, 118634. <u>https://doi.org/https://doi.org/10.1016/j.chemgeo.2018.01.030</u>
- Saadaoui, Y., Porada, P., & Müller, P. (in prep). Effects of plant-microbe interactions on the carbon balance of estuarine marshes estimated by a process-based model. In U. o. Hamburg (Ed.).
- Saderne, V., Fusi, M., Thomson, T., Dunne, A., Mahmud, F., Roth, F., Carvalho, S., & Duarte, C. M. (2020). Total alkalinity production in a mangrove ecosystem reveals an overlooked Blue Carbon component. *Limnology and Oceanography Letters*. <u>https://doi.org/10.1002/lol2.10170</u>
- Saderne, V., Fusi, M., Thomson, T., Dunne, A., Mahmud, F., Roth, F., Carvalho, S., & Duarte, C. M. (2021). Total alkalinity production in a mangrove ecosystem reveals an overlooked Blue Carbon component. *Limnology and Oceanography Letters*, 6(2), 61-67. <u>https://doi.org/10.1002/lol2.10170</u>

- Saderne, V., Geraldi, N. R., Macreadie, P. I., Maher, D. T., Middelburg, J. J., Serrano, O.,
  Almahasheer, H., Arias-Ortiz, A., Cusack, M., Eyre, B. D., Fourqurean, J. W., Kennedy, H.,
  Krause-Jensen, D., Kuwae, T., Lavery, P. S., Lovelock, C. E., Marba, N., Masqué, P., Mateo,
  M. A., . . . Duarte, C. M. (2019). Role of carbonate burial in Blue Carbon budgets. *Nature Communications*, 10(1). https://doi.org/10.1038/s41467-019-08842-6
- Salimi, S., Almuktar, S. A. A. A. N., & Scholz, M. (2021). Impact of climate change on wetland ecosystems: A critical review of experimental wetlands. *Journal of Environmental Management*, 286, 112160. <u>https://doi.org/10.1016/j.jenvman.2021.112160</u>
- Salomons, W., & Mook, W. G. (1976). Isotope Geochemistry of Carbonate Dissolution and Reprecitation in Soils. Soil Science, 122(1), 15-24. <u>https://journals.lww.com/soilsci/Fulltext/1976/07000/ISOTOPE\_GEOCHEMISTRY\_OF\_CA\_RBONATE\_DISSOLUTION\_AND.3.aspx</u>
- Santner, J., Larsen, M., Kreuzeder, A., & Glud, R. N. (2015). Two decades of chemical imaging of solutes in sediments and soils – a review. *Analytica Chimica Acta*, 878, 9-42. <u>https://doi.org/https://doi.org/10.1016/j.aca.2015.02.006</u>
- Santos, I. R., Burdige, D. J., Jennerjahn, T. C., Bouillon, S., Cabral, A., Serrano, O., Wernberg, T., Filbee-Dexter, K., Guimond, J. A., & Tamborski, J. J. (2021). The renaissance of Odum's outwelling hypothesis in 'Blue Carbon' science. *Estuarine, Coastal and Shelf Science*, 255, 107361. <u>https://doi.org/https://doi.org/10.1016/j.ecss.2021.107361</u>
- ŠantRůČková, H., Bird, M. I., & Lloyd, J. (2000). Microbial processes and carbon-isotope fractionation in tropical and temperate grassland soils. *Functional Ecology*, 14(1), 108-114. <u>https://doi.org/https://doi.org/10.1046/j.1365-2435.2000.00402.x</u>
- Schwichtenberg, F., Pätsch, J., Böttcher, M. E., Thomas, H., Winde, V., & Emeis, K.-C. (2020). The impact of intertidal areas on the carbonate system of the southern North Sea. *Biogeosciences*, 17(16), 4223-4245. <u>https://doi.org/10.5194/bg-17-4223-2020</u>
- Senna, M. (1989). Determination of Effective Surface Area for the Chemical Reaction of Fine Particulate Materials. *Particle & amp; Particle Systems Characterization*, 6(1-4), 163-167. <u>https://doi.org/10.1002/ppsc.19890060127</u>
- Sippo, J. Z., Maher, D. T., Tait, D. R., Holloway, C., & Santos, I. R. (2016). Are mangroves drivers or buffers of coastal acidification? Insights from alkalinity and dissolved inorganic carbon export estimates across a latitudinal transect. *Global Biogeochemical Cycles*, 30(5), 753-766. https://doi.org/10.1002/2015gb005324
- Song, S., Bellerby, R. G. J., Wang, Z. A., Wurgaft, E., & Li, D. (2023). Organic Alkalinity as an Important Constituent of Total Alkalinity and the Buffering System in River-To-Coast Transition Zones. *Journal of Geophysical Research: Oceans*, 128(8). <u>https://doi.org/10.1029/2022jc019270</u>

- Spatti Júnior, E. P., da Conceição, F. T., Fernandes, A. M., Sardinha, D. d. S., Menegário, A. A., & Moruzzi, R. B. (2019). Chemical weathering rates of clastic sedimentary rocks from the Paraná Basin in the Paulista Peripheral Depression, Brazil. *Journal of South American Earth Sciences*, 96, 102369. <u>https://doi.org/10.1016/j.jsames.2019.102369</u>
- Spivak, A. C., Sanderman, J., Bowen, J. L., Canuel, E. A., & Hopkinson, C. S. (2019). Global-change controls on soil-carbon accumulation and loss in coastal vegetated ecosystems. *Nature Geoscience*, 12(9), 685-692. <u>https://doi.org/10.1038/s41561-019-0435-2</u>
- Stagg, C. L., Osland, M. J., Moon, J. A., Feher, L. C., Laurenzano, C., Lane, T. C., Jones, W. R., & Hartley, S. B. (2021). Extreme Precipitation and Flooding Contribute to Sudden Vegetation Dieback in a Coastal Salt Marsh. *Plants*, 10(9), 1841. <u>https://doi.org/10.3390/plants10091841</u>
- Stumm, W., & Morgan, J. J. (1981). Aquatic chemistry an introduction chemical equilibria in natural water (2nd ed.). Wiley-Interscience Publication. <u>https://books.google.de/books?id=zPROAAAAMAAJ</u>
- Subhas, A. V., Rollins, N. E., Berelson, W. M., Dong, S., Erez, J., & Adkins, J. F. (2015). A novel determination of calcite dissolution kinetics in seawater. *Geochimica et Cosmochimica Acta*, 170, 51-68. <u>https://doi.org/10.1016/j.gca.2015.08.011</u>
- Sulpis, O., Agrawal, P., Wolthers, M., Munhoven, G., Walker, M., & Middelburg, J. J. (2022). Aragonite dissolution protects calcite at the seafloor. *Nature Communications*, 13(1). <u>https://doi.org/10.1038/s41467-022-28711-z</u>
- Sulpis, O., & Middelburg, J. J. (2023). Inorganic blue carbon sequestration. *Nature Sustainability*. <u>https://doi.org/10.1038/s41893-023-01143-3</u>
- Sun, Y., Wang, C., Chen, H. Y. H., Luo, X., Qiu, N., & Ruan, H. (2021). Asymmetric responses of terrestrial C:N:P stoichiometry to precipitation change. *Global Ecology and Biogeography*, 30(8), 1724-1735. <u>https://doi.org/10.1111/geb.13343</u>
- Tang, H., Liebner, S., Reents, S., Nolte, S., Jensen, K., Horn, F., & Mueller, P. (2021). Plant genotype controls wetland soil microbial functioning in response to sea-level rise. *Biogeosciences*, 18(23), 6133-6146. <u>https://doi.org/10.5194/bg-18-6133-2021</u>
- Thaysen, E. M., Jacques, D., Jessen, S., Andersen, C. E., Laloy, E., Ambus, P., Postma, D., & Jakobsen, I. (2014). Inorganic carbon fluxes across the vadose zone of planted and unplanted soil mesocosms. *Biogeosciences*, 11(24), 7179-7192. <u>https://doi.org/10.5194/bg-11-7179-2014</u>

- Thomas, H., Schiettecatte, L. S., Suykens, K., Koné, Y. J. M., Shadwick, E. H., Prowe, A. E. F., Bozec, Y., De Baar, H. J. W., & Borges, A. V. (2009). Enhanced ocean carbon storage from anaerobic alkalinity generation in coastal sediments. *Biogeosciences*, 6(2), 267-274. <u>https://doi.org/10.5194/bg-6-267-2009</u>
- Tobias, C., & Neubauer, S. C. (2019). Chapter 16 Salt Marsh Biogeochemistry—An Overview. In G. M. E. Perillo, E. Wolanski, D. R. Cahoon, & C. S. Hopkinson (Eds.), *Coastal Wetlands* (Second Edition) (pp. 539-596). Elsevier. <u>https://doi.org/https://doi.org/10.1016/B978-0-444-63893-9.00016-2</u>
- Van Dam, B. R., Lopes, C., Osburn, C. L., & Fourqurean, J. W. (2019). Net heterotrophy and carbonate dissolution in two subtropical seagrass meadows. *Biogeosciences*, 16(22), 4411-4428. <u>https://doi.org/10.5194/bg-16-4411-2019</u>
- Van Geldern, R., Schulte, P., Mader, M., Baier, A., & Barth, J. A. C. (2015). Spatial and temporal variations of pCO<sub>2</sub>, dissolved inorganic carbon and stable isotopes along a temperate karstic watercourse. *Hydrological Processes*, 29(15), 3423-3440. <u>https://doi.org/10.1002/hyp.10457</u>
- van Veen, J. A., Liljeroth, E., Lekkerkerk, L. J. A., & van de Geijn, S. C. (1991). Carbon Fluxes in Plant-Soil Systems at Elevated Atmospheric CO<sub>2</sub> Levels. *Ecological Applications*, 1(2), 175-181. <u>https://doi.org/https://doi.org/10.2307/1941810</u>
- Verrecchia, E. P. (2011). Pedogenic Carbonates. In (pp. 721-725). Springer Netherlands. https://doi.org/10.1007/978-1-4020-9212-1\_161
- Vicca, S., Goll, D. S., Hagens, M., Hartmann, J., Janssens, I. A., Neubeck, A., Peñuelas, J., Poblador, S., Rijnders, J., Sardans, J., Struyf, E., Swoboda, P., Van Groenigen, J. W., Vienne, A., & Verbruggen, E. (2022). Is the climate change mitigation effect of enhanced silicate weathering governed by biological processes? *Global Change Biology*, 28(3), 711-726. <u>https://doi.org/10.1111/gcb.15993</u>
- Vranken, M., Oenema, O., & Mulder, J. (1990). Effects of tide range alterations on salt marsh sediments in the Eastern Scheldt, S. W. Netherlands. *Hydrobiologia*, 195(1), 13-20. <u>https://doi.org/10.1007/bf00026810</u>
- Waldbusser, G. G., Steenson, R. A., & Green, M. A. (2011). Oyster Shell Dissolution Rates in Estuarine Waters: Effects of pH and Shell Legacy. *Journal of Shellfish Research*, 30(3), 659-669. <u>https://doi.org/10.2983/035.030.0308</u>
- Wang, S. R., Di Iorio, D., Cai, W. J., & Hopkinson, C. S. (2018). Inorganic carbon and oxygen dynamics in a marsh-dominated estuary. *Limnology and Oceanography*, 63(1), 47-71. <u>https://doi.org/10.1002/lno.10614</u>

- Wang, Z. A., & Cai, W.-J. (2004). Carbon dioxide degassing and inorganic carbon export from a marsh-dominated estuary (the Duplin River): A marsh CO<sub>2</sub> pump. *Limnology and Oceanography*, 49(2), 341-354. <u>https://doi.org/10.4319/lo.2004.49.2.0341</u>
- Wang, Z. A., Kroeger, K. D., Ganju, N. K., Gonneea, M. E., & Chu, S. N. (2016). Intertidal salt marshes as an important source of inorganic carbon to the coastal ocean. *Limnology and Oceanography*, 61(5), 1916-1931. <u>https://doi.org/10.1002/lno.10347</u>
- Ward, J. K., Tissue, D. T., Thomas, R. B., And, & Strain, B. R. (1999). Comparative responses of model C3 and C4 plants to drought in low and elevated CO<sub>2</sub>. *Global Change Biology*, 5(8), 857-867. <u>https://doi.org/10.1046/j.1365-2486.1999.00270.x</u>
- Weiss, A. (2013). The silica and inorganic carbon system in tidal marshes of the Elbe estuary, Germany : Fluxes and spatio-temporal patterns.
- Wen, H., Sullivan, P. L., Macpherson, G. L., Billings, S. A., & Li, L. (2021). Deepening roots can enhance carbonate weathering by amplifying CO<sub>2</sub>-rich recharge. *Biogeosciences*, 18(1), 55-75. <u>https://doi.org/10.5194/bg-18-55-2021</u>
- Wolf-Gladrow, D. A., Zeebe, R. E., Klaas, C., Körtzinger, A., & Dickson, A. G. (2007). Total alkalinity: The explicit conservative expression and its application to biogeochemical processes. *Marine Chemistry*, 106(1-2), 287-300. https://doi.org/10.1016/j.marchem.2007.01.006
- Wolf, A. A., Drake, B. G., Erickson, J. E., & Megonigal, J. P. (2007). An oxygen-mediated positive feedback between elevated carbon dioxide and soil organic matter decomposition in a simulated anaerobic wetland. *Global Change Biology*, 13(9), 2036-2044. https://doi.org/10.1111/j.1365-2486.2007.01407.x
- Xie, C., Antić, N., Nadal-Romero, E., Yan, L., Tosti, T., Djogo Mračević, S., Tu, X., & Kašanin-Grubin, M. (2022). The Influences of Climatic and Lithological Factors on Weathering of Sediments in Humid Badland Areas [Original Research]. *Frontiers in Earth Science*, 10. <u>https://doi.org/10.3389/feart.2022.900314</u>
- Yadav, S. K., & Chakrapani, G. J. (2006). Dissolution kinetics of rock-water interactions and its implications. *Current Science*, 90(7), 932-937. <u>http://www.jstor.org/stable/24091948</u>
- Yang, B., Byrne, R. H., & Lindemuth, M. (2015). Contributions of organic alkalinity to total alkalinity in coastal waters: A spectrophotometric approach. *Marine Chemistry*, 176, 199-207. <u>https://doi.org/10.1016/j.marchem.2015.09.008</u>
- Yang, R. M., & Yang, F. (2020). Impacts of Spartina alterniflora invasion on soil inorganic carbon in coastal wetlands in China. *Soil Science Society of America Journal*, 84(3), 844-855. <u>https://doi.org/10.1002/saj2.20073</u>

- Yau, Y. Y., Xin, P., Chen, X., Zhan, L., Call, M., Conrad, S. R., Sanders, C. J., Li, L., Du, J., & Santos, I. R. (2022). Alkalinity export to the ocean is a major carbon sequestration mechanism in a macrotidal saltmarsh. *Limnology and Oceanography*, 67(S2). <u>https://doi.org/10.1002/lno.12155</u>
- Yoshimura, K., Nakao, S., Noto, M., Inokura, Y., Urata, K., Chen, M., & Lin, P. W. (2001). Geochemical and stable isotope studies on natural water in the Taroko Gorge karst area, Taiwan—chemical weathering of carbonate rocks by deep source CO<sub>2</sub> and sulfuric acid. *Chemical Geology*, *177*(3), 415-430. <u>https://doi.org/https://doi.org/10.1016/S0009-</u> 2541(00)00423-X
- Yvon-Durocher, G., Jones, J. I., Trimmer, M., Woodward, G., & Montoya, J. M. (2010). Warming alters the metabolic balance of ecosystems. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365(1549), 2117-2126. <u>https://doi.org/10.1098/rstb.2010.0038</u>
- Zamanian, K., Pustovoytov, K., & Kuzyakov, Y. (2016). Pedogenic carbonates: Forms and formation processes. *Earth-Science Reviews*, 157, 1-17. <u>https://doi.org/https://doi.org/10.1016/j.earscirev.2016.03.003</u>
- Zeebe, R. E., & Wolf-Gladrow, D. (2001). CO<sub>2</sub> in seawater: equilibrium, kinetics, isotopes (Vol. 65). Gulf Professional Publishing.
- Zheng, J., Li, J., Lan, Y., Liu, S., Zhou, L., Luo, Y., Liu, J., & Wu, Z. (2019). Effects of Spartina alterniflora invasion on Kandelia candel rhizospheric bacterial community as determined by high-throughput sequencing analysis. *Journal of Soils and Sediments*, 19(1), 332-344. <u>https://doi.org/10.1007/s11368-018-2002-7</u>
- Zhu, B., & Cheng, W. (2011). 13C isotope fractionation during rhizosphere respiration of C3 and C4 plants. *Plant and Soil*, 342(1-2), 277-287. <u>https://doi.org/10.1007/s11104-010-0691-9</u>
- Zuschin, M., Stachowitsch, M., & Stanton, R. J. (2003). Patterns and processes of shell fragmentation in modern and ancient marine environments. *Earth-Science Reviews*, *63*(1-2), 33-82. https://doi.org/10.1016/s0012-8252(03)00014-x
- Zwolsman, J. J. G., Berger, G. W., & Van Eck, G. T. M. (1993). Sediment accumulation rates, historical input, postdepositional mobility and retention of major elements and trace metals in salt marsh sediments of the Scheldt estuary, SW Netherlands. *Marine Chemistry*, 44(1), 73-94. <u>https://doi.org/10.1016/0304-4203(93)90007-B</u>

# **Summary**

Salt marshes have significant potential to sequester anthropogenic  $CO_2$  emissions and store substantial amounts of organic carbon within their ecosystems. On the other hand, salt marshes serve as the source of DIC and alkalinity export, potentially regulating the carbonate chemistry of the adjacent coastal waters, including oceans. However, most studies have focused on the biotic interactions at the ecosystem or the landscape scale, leaving gaps in understanding the potential impact of rhizosphere processes on modulating the alkalinity generation and influencing inorganic carbon dynamics. Furthermore, several salt marsh studies have been conducted in organic-rich sediments, overlooking the organic-poor type in regions such as the Wadden Sea or the Elbe estuary, where inorganic carbon significantly contributes. Hence, this thesis aimed to improve the understanding of the role of the inorganic carbon within the minerogenic salt marsh soil and salt marsh plant-mediated effects on alkalinity generation.

**Chapter II** investigated the rhizosphere effect on alkalinity generation by simulating soil  $pCO_2$  conditions and identifying the dominant biogeochemical processes responsible for alkalinity in minerogenic salt marsh soil. The results revealed significant positive feedback of elevated soil  $pCO_2$  on TA enrichment, with the maximum TA increase reaching two-fold when soil  $pCO_2$  was raised from 440 to 150,000 µatm. Carbonate mineral dissolution was identified as the possible dominant driving force behind TA generation, with an additional contribution from silicate minerals.

**Chapter III** explored the role of biogenic carbonates in feeding the alkalinity budget. This study indicated that fresh biogenic carbonates on the topsoil might not be relevant to increasing TA production. Instead, soil carbonates might represent the primary source driving mineral dissolution and TA generation in this experiment. This might be related to less water exposure time and larger grain size, causing the shells to be less susceptible to the dissolution process.

**Chapter IV** focused on assessing the effects of salt marsh plants on alkalinity generation. **Experiment I** determined the effect of *Spartina x townsendii* and *Elymus athericus* on alkalinity generation. Both plants significantly enhanced the alkalinity generation, with TA concentrations up to 10-fold higher than non-planted columns. This study and the findings of Chapter II provided evidence that high acidity production from respired CO<sub>2</sub> in the rhizosphere can induce mineral dissolution. However, the relative contribution of rhizosphere-driven alkalinity via mineral dissolution versus anaerobic respiration remains undetermined. **Experiment II** aimed to test the assumption drawn from Chapter III's findings and further explored *Spartina x townsendii*'s role in enhancing biogenic mineral dissolution and alkalinity production. Shell-bearing limestone contributed to mineral dissolution when the powdered minerals were homogeneously mixed with salt marsh soil. *Spartina x townsendii* increased the TA generation; however, adding additional minerals did not enhance alkalinity. This could be linked to the saturation state of the respective carbonate minerals in the outflow water.

In summary, this thesis provided the first empirical evidence of the salt marsh plants' rhizosphere regulating the soil mineral dissolution and the contribution of biogenic carbonates to the alkalinity budget. These findings also challenged the prevailing notion of the unaccountable role of inorganic carbon within the salt marsh, suggesting that carbonate mineral dissolution is likely to be the dominant biogeochemical pathway within the organic-poor minerogenic salt marsh, unlike other organic-rich salt marsh systems. Future studies should also focus on how climate change stressors affect salt marsh biota in terms of alkalinity generation while also incorporating the role of plants in influencing the fate of inorganic carbon in restoration strategies. Additionally, investigating intra- and interspecific variations among plants and their rhizospheres may reveal differential impacts on alkalinity enhancement and the buffering capacity of exported water. Continued research is essential for developing more effective restoration management and climate change mitigation strategies.

# Zusammenfassung

Salzmarschen besitzen erhebliches Potenzial zur Bindung anthropogener CO<sub>2</sub>-Emissionen und haben die Fähigkeit beträchtliche Mengen an organischem Kohlenstoff innerhalb des Ökosystems zu speichern. Des Weiteren stellen Salzmarschen eine Quelle für *DIC* und den Export von Alkalinität dar, wodurch sie die Karbonatchemie angrenzender Küstengewässer, einschließlich der Ozeane, potentiell regulieren können. Die meisten Studien konzentrierten sich jedoch bisher auf die biotischen Interaktionen auf Ökosystem- oder Landschaftsebene, sodass es Lücken im Verständnis der möglichen Auswirkungen von Prozessen der Rhizosphäre auf die Regulierung der Alkalinität und damit auf die anorganische Kohlenstoffdynamik gibt. Darüber hinaus wurden mehrere Studien über Salzmarschen mit hohem Anteil organischer Substanz im Sediment durchgeführt, wobei der an organischer Substanz verarmte Typ in Regionen wie dem Wattenmeer oder dem Elbeästuar, in denen der anorganische Kohlenstoff eine wichtige Rolle spielt, vernachlässigt wurde. Ziel dieser Arbeit war es daher, das Verständnis über die Rolle des anorganischen Kohlenstoffs in mineralischen Salzmarschen und die von Salzmarschpflanzen vermittelten Auswirkungen auf die Alkalinität zu verbessern.

In **Kapitel II** wurde der Rhizosphären-Effekt auf die Alkalinitätsbildung untersucht, indem die  $pCO_2$ -Bedingungen im Boden simuliert und die dominanten biogeochemischen Prozesse identifiziert wurden, die für die Alkalinität in mineralischen Salzmarschböden verantwortlich sind. Die Ergebnisse zeigten eine signifikant positive Wirkung von erhöhtem  $pCO_2$  im Boden auf die TA-Anreicherung, wobei der maximale TA-Anstieg das Zweifache erreichte, wenn der  $pCO_2$ -Wert im Boden von 440 auf 150.000 µatm erhöht wurde. Die Auflösung von Karbonatmineralen wurde als mögliche treibende Kraft der TA-Bildung identifiziert, welche durch die Auflösung von Silikatmineralen ergänzt wurde.

In **Kapitel III** wurde die Rolle der biogenen Karbonate für die Bodenalkalinität betrachtet. Die Untersuchungen ergaben, dass neue biogene Karbonate, in Form von Muschelschalen, im Oberboden möglicherweise nicht für die Erhöhung der TA-Produktion relevant sind. Stattdessen könnten Bodenkarbonate die primäre Quelle für die Mineralauflösung und TA-Bildung in diesem Versuchsaufbau gewesen sein. Dies könnte mit der kürzeren

Wassereinwirkung und der höheren Korngröße im Boden zusammenhängen, wodurch die Muschelschalen weniger anfällig für den Auflösungsprozess waren.

**Kapitel IV** befasste sich mit dem Einfluss von Salzwiesenpflanzen auf die Bodenalkalinität. In **Versuch I** wurde die Wirkung von *Spartina x townsendii* und *Elymus athericus* auf die Bodenalkalinität bestimmt. Beide Pflanzen erhöhten die Bodenalkalinität erheblich, wobei die TA-Konzentrationen bis zu 10-mal höher waren als in nicht bepflanzten Säulen. Diese Erkenntnisse, sowie die Resultate aus Kapitel II, zeigen, dass eine hohe Säureproduktion aus veratmetem CO<sub>2</sub> in der Rhizosphäre die Auflösung von Mineralen bewirken kann. Der relative Beitrag der Alkalinität in der Rhizosphäre durch Mineralauflösung im Vergleich zur anaeroben Atmung bleibt jedoch unbestimmt. In **Versuch II** wurde die, aus den Ergebnissen von Kapitel III, abgeleitete Annahme überprüft und die Rolle von *Spartina x townsendii* bei der Förderung der biogenen Mineralauflösung und der Alkalinitätsbildung weiter untersucht. Muschelkalk trug zur Mineralauflösung bei, wenn die pulverisierten Minerale homogen mit Salzwiesenboden vermischt wurden. *Spartina x townsendii* erhöhte zwar die TA-Bildung; die Zugabe zusätzlicher Minerale führte jedoch nicht zu einer Erhöhung der Alkalinität. Dies könnte mit dem Sättigungszustand der jeweiligen Karbonatminerale im abfließenden Wasser zusammenhängen.

Zusammenfassend lässt sich sagen, dass diese Arbeit den ersten empirischen Nachweis dafür erbringt, dass die Rhizosphäre der Pflanzen in Salzmarschen die Mineralauflösung im Boden und den Beitrag biogener Karbonate zum Alkalinitätshaushalt reguliert. Diese Ergebnisse stellen zudem die vorherrschende Ansicht der unklaren Rolle des anorganischen Kohlenstoffs in Salzmarschen in Frage und deuten darauf hin, dass die Auflösung von Karbonatmineralen wahrscheinlich der vorherrschende biogeochemische Stoffwechselweg in der, an organischer Substanz verarmten, minerogenen Salzmarsch ist. Künftige Studien sollten daher untersuchen, wie sich Stressfaktoren des Klimawandels auf die Biota der Salzmarschen in Bezug auf Bildung von Alkalinität auswirken. Gleichermaßen sollte die Rolle von Pflanzen hinsichtlich der Dynamik von anorganischem Kohlenstoff in Renaturierungsstrategien einbezogen werden. Darüber hinaus können Untersuchungen der intra- und interspezifischen Variation zwischen Pflanzen und ihren Rhizosphären unterschiedliche Auswirkungen auf die Erhöhung der Alkalinität und die Pufferkapazität der exportierten Wassers aufzeigen. Weitere Forschungsarbeiten sind für die Entwicklung eines wirksameren Renaturierungsmanagements und zur Erarbeitung von Strategien zur Eindämmung des Klimawandels von essentieller Bedeutung.

### **Author contributions statement**

Chapter I: P. Tutiyasarn: visualization; writing the original draft.

**Chapter II**: P. Tutiyasarn.: conceptualization; methodology; investigation; formal analysis; data curation; validation; visualization; writing the original draft. P. Mueller: formal analysis; writing – reviews & editing. G.R. Mujalli: methodology; supervision; writing – reviews & editing. B.V. Dam.: formal analysis, writing – reviews & editing. J. Hartmann: conceptualization; methodology; resources. P. Porada: resources; supervision; writing – reviews & editing.

**Chapter III**: P. Tutiyasarn.: conceptualization; methodology; investigation; formal analysis; data curation; validation; visualization; writing the original draft. G.R. Mujalli: methodology; supervision; writing – reviews & editing. J. Hartmann: conceptualization; methodology; resources. P. Porada: supervision, writing – reviews & editing.

**Chapter IV**: P. Tutiyasarn.: conceptualization; methodology; investigation; formal analysis; data curation; validation; visualization; writing the original draft. P. Mueller: conceptualization; methodology; supervision. P. Porada: resources, supervision, writing – reviews & editing.

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To anyone reading this, I want you to know that you are doing great, no matter what you are going through. I hope my work can inspire you and that my message empowers you to overcome your life challenges.

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