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# Chemical determination of silica in seagrass leaves reveals two operational silica pools in *Zostera marina*

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**Abstract** Silicon is a major driver of global primary productivity and CO<sub>2</sub> sequestration, and is a beneficial element for the growth and environmental stress mitigation of many terrestrial and aquatic plants. However, only a few studies have examined the occurrence of silicon in seagrasses, and its function within seagrass ecosystems and the role of seagrasses in silicon cycling remain largely unexplored. This study uses for the first time two methods, the wet-alkaline digestion and the hydrofluoric acid digestion, to quantify silicon content in seagrass leaves using the species *Zostera marina* and elaborates on the potential role of silicon in seagrass biogeochemistry

and ecology, as well as the role of seagrass ecosystems as a silicon reservoir. The results revealed that seagrass leaves contained 0.26% silicon:dry-weight, which is accumulated in two forms of silica: a labile form digested with the alkaline method and a resistant form digested only with acid digestion. These findings support chemical digestions for silicon quantification in seagrass leaves and provide new insights into the impact of seagrasses on the marine silicon cycle. Labile silica will be recycled upon leaf degradation, benefiting siliceous organisms, while refractory silica will contribute to the ecosystem's buried silica stock and coupled carbon sequestration. In the Bay of Brest (France), the seagrass silicon reservoir was estimated at  $0.18 \pm 0.07$  g Si m<sup>-2</sup>, similar to that of benthic diatoms, underscoring the potential role

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of seagrasses in silicon biogeochemistry in the land–ocean continuum, where they might act as a buffer for silicon transport to the ocean.

**Keywords** Biogenic silica · Silicon biogeochemistry · Land–ocean continuum · Seagrasses · *Zostera marina*

## Introduction

Silicon, the second most abundant element in the Earth's crust after oxygen, exists in various forms in terrestrial and aquatic ecosystems. It is present in a dissolved state as silicic acid (dSi) and in a particulate pool that includes lithogenic silica, which originates from terrigenous rocks, and biogenic silica (bSi), the hydrated amorphous form of silica ( $\text{SiO}_2 \cdot n\text{H}_2\text{O}$ ) produced by organisms such as diatoms, plants, sponges, and Rhizaria (Conley 2002; DeMaster 2003; Tréguer et al. 2021). Accurately quantifying the silicon reservoir in terrestrial and aquatic ecosystems is crucial to understand the biogeochemical cycling of this element at regional and global scales, which has significant implications for global primary productivity, carbon cycling, the functioning of marine food webs, and the cycling of other major nutrients, including nitrogen and phosphorus (Struyf et al. 2009; Tréguer et al. 2018).

In plants, silicon is not considered an essential element, but it is beneficial for the growth and development of many terrestrial and aquatic species (Cooke and Leishman 2011; Schoelynck and Struyf 2016; Manivannan et al. 2023). Its accumulation increases plant structural strength, mitigates metal toxicity, and enhances resistance to pathogens and herbivores (Liang et al. 2007; Ma and Yamaji 2008; Etesami and Jeong 2018). Most plants accumulate silicon in the form of solid amorphous silica bodies known as phytoliths (Kameník et al. 2013; Koné et al. 2019), which account for 0.1% to 10.0% of their dry weight (Epstein 1999). Beyond providing structural, physiological and protective benefits, phytoliths make plant tissues more resistant to degradation after plant death, thereby enhancing carbon sequestration (Parr and Sullivan 2005; Song et al. 2016). Furthermore, deposition and burial of phytoliths after plant death constitute the main source of bSi in sediments of terrestrial and many aquatic habitats, making vegetated

ecosystems important silica sinks (Conley 2002; Struyf et al. 2005).

Vegetated coastal habitats, such as those formed by seagrass meadows, provide crucial ecosystem services related to climate change mitigation, habitat provision, and nutrient cycling (Barbier et al. 2011; Duarte et al. 2013; Holmer 2019). Seagrass meadows are among the most productive ecosystems in the world and play an important role in biogeochemical cycling (Marbà et al. 2006; Mateo et al. 2006). These plants occur in coastal and estuarine ecosystems under the influence of rivers and terrestrial streams, which are the main input of silicon to the ocean (Tréguer et al. 2021). Several studies have reported a positive correlation between seagrass coverage and dSi levels in the water column, but the causes are still enigmatic (Herman et al. 1996; Kamermans et al. 1999). Moreover, other vegetated ecosystems in the land–ocean aquatic continuum, such as salt marshes and mangroves, sequester significant amounts of bSi and control bSi exchange between terrestrial and marine environments (Carey and Fulweiler 2014; Elizondo et al. 2021), raising the question of whether seagrasses might also interplay in the biogeochemical cycling of silicon.

The elemental composition of seagrass tissues, particularly leaves, has been extensively studied for macronutrients such as nitrogen and phosphorus as drivers of productivity and in relation to nutrient limitation and disturbances like eutrophication (e.g., Duarte 1990; Pedersen and Borum 1992; Quigley et al. 2020). However, information on silicon content in seagrasses remains limited, with only few studies quantifying the amount of silicon in seagrass leaves to date (e.g., Herman et al. 1996; Vonk et al. 2018; Rondevaldova et al. 2023). Silicon content in the leaves of *Zostera marina* was measured along a dSi gradient within an estuarine ecosystem in The Netherlands to evaluate the effect of dSi concentrations in the water column on seagrass bed depletion over a 20-year period (Herman et al. 1996). Further studies have quantified silicon content in two seagrass species (*Enhalus acoroides* and *Halophila ovalis*) in the Philippines (Rondevaldova et al. 2023) and across four seagrass families as part of a global multi-elemental database (Vonk et al. 2018). In these studies, silicon content, in the form of silica, was determined using acid digestion followed by atomic absorption spectrophotometry or inductively coupled plasma mass

spectrometry, techniques that are relatively unconventional compared to those commonly employed in plant silica research (Sauer et al. 2006). The limited data on seagrass silicon content and the use of unconventional methods to determine it prevent an assessment of the function of silicon in seagrass ecophysiology and biogeochemistry.

We hypothesized that seagrasses may contain a significant amount of silicon in their tissues and thus could impact the silicon cycle of marine ecosystems. To do a first estimate of whether seagrasses are a silicon reservoir in the land–ocean continuum and coastal ecosystems, we first assessed two commonly used methods for measuring bSi in marine organisms, seawater, sediments, and terrestrial plants—the wet-alkaline digestion with sodium hydroxide (NaOH) or sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) and the acid digestion with hydrofluoric acid (HF) (DeMaster 1981; Ragueneau et al. 2005b; Kraska and Breitenbeck 2010; Maldonado et al. 2019)—to quantify bSi content in seagrasses. Specifically, we focused on quantifying bSi in the leaves of the species *Z. marina*, a widespread circumpolar seagrass in the Northern Hemisphere (Moore 2006), to compare these two conventional methods in bSi studies with those used previously (Herman et al. 1996; Vonk et al. 2018; Rondevaldova et al. 2023). We evaluated whether it exists differences between the results obtained with each of these methods, which could suggest the existence of distinct functional groups of bSi in seagrass tissues. We then did a first estimate of the silicon reservoir of *Z. marina* in a temperate estuarine bay (Bay of Brest, France), one of the best-studied coastal ecosystems in terms of structure and ecosystem functioning, including silicon biogeochemistry (e.g., Chauvaud et al. 2000; Ragueneau et al. 2005a; Laruelle et al. 2009). The silicon reservoir of *Z. marina* in the bay was discussed within the regional silicon reservoirs and compared to those previously published for other cohabiting silicifying organisms (i.e., benthic diatoms and sponges; Leynaert et al. 2011; López-Acosta et al. 2022) and riverine sources (Ragueneau et al. 2005a). Ultimately, our findings aim to contribute to the ecological discussion regarding the impact of bSi accumulation by seagrasses from the leaves to the ecosystem scale.

## Methods

### *Study site and sample preparation*

Fieldwork was conducted in November 2021 in Lanvéoc, France (48° 17' 34.973'' N, 4° 27' 32.122'' W), a sandy area located on the south coast of the Bay of Brest (NE Atlantic; Fig. 1). Seagrass beds of *Z. marina* cover a total surface area of 1.00 km<sup>2</sup> in the Bay of Brest (Auby et al. 2018). At the sampling location, the seagrass *Z. marina* forms a meadow of 0.23 km<sup>2</sup> at water depth between 1.5 to 5 m (Fig. 1). Lanvéoc's meadow is representative of the seagrass beds found in the Bay of Brest, which are very narrow in the vertical dimension (from the top to the bottom of the foreshore) but spread along the coastline with a percentage coverage ranging from 5 to 25% (Auby et al. 2018).

Fifty-eight shoots of *Z. marina* were randomly collected by hand during low tide at a high-coefficient tide (Fig. 1) and immediately transported to the Laboratory of Environmental Marine Sciences (LEMAR, Plouzané, France) in a cooler. In the laboratory, the seagrass shoots were cleaned, and the leaves were sorted and measured for length and width to determine their biometric features, with data available in Supplementary Information 1. For the determination of bSi, young leaves (2nd and 3rd newly formed leaves) were selected, carefully cleaned of epiphytes with a razor blade, rinsed with Milli-Q water, and dried at 60 °C for 48 h. The dried leaves were then ground together to a fine powder using a ball mill (MM400, Retsch), and the required amount of sample for the different analytical methods was weighed directly into digestion tubes on a precision balance (XS105 Analytical Balance, Mettler Toledo). This methodological approach included technical replicates to enable a comprehensive assessment of the accuracy and performance of the analytical methods, which constituted the main objective of this study.

### *Digestion protocols*

**Hydrofluoric acid digestion** A 48-h digestion with hydrofluoric acid (HF) is commonly used for the digestion of bSi in siliceous organisms, including plants (Saito et al. 2005; Ragueneau et al. 2005b; Maldonado et al. 2010). This acid dissolves the SiO<sub>2</sub> complex, whether of lithogenic or biogenic origin. This

chemical property makes it an effective acid for the measurement of resistant types of bSi, whose properties are similar to those of lithogenic silica.

Three masses of powder of *Z. marina* leaves (5, 10, and 15 mg) were digested following the HF digestion method (Ragueneau et al. 2005b) to (1) evaluate the reproducibility of our measurements, and (2) determine the bSi content within *Z. marina* samples from the Bay of Brest. This also allowed to establish the optimal powder mass necessary for the wet-alkaline digestion method. For this digestion, 5 mL of 2.9N HF (10%) were added into 15-mL polymethylpentene centrifuge tubes (TPX, Nalgene) with *Z. marina* leaves. Five replicates were used for each mass of *Z. marina* evaluated. Three additional tubes without leaves were considered as experimental blanks. The tubes were tightly covered with a cap and kept under a fume hood at room temperature for 48 h to allow the digestion of bSi. After 48 h, each tube was centrifuged at 2500 rpm for 5 min. Then, 0.5 mL of sample was pipetted into a 15-mL polypropylene centrifuge tube (Falcon) and neutralized by adding 14.5 mL of a saturated solution of boric acid ( $\text{H}_3\text{BO}_3$ , 60 g/L). The neutralized samples were kept at room temperature until subsequent analyses for silicon determination (see below), which were carried out no later than 48 h after acid neutralization.

**Wet-alkaline digestion** We also tested the wet-alkaline digestion method, which is widely used to quantify bSi of siliceous organisms (Meunier et al. 2014; Maldonado et al. 2019) in different matrices, such as marine sediments (DeMaster 1981; Conley 1998; Kamatani and Oku 2000) or suspended particulate matter in seawater (Krausse et al. 1983; Ragueneau et al. 2005b), and has proved to completely dissolve bSi particle types at the solid:solution ratio applied (Saccone et al. 2007). This relies on the difference between the rapid dissolution of bSi and the slower release of silica from the coexisting clay minerals (i.e., lithogenic silica). The digestion time depends on the reagent used (NaOH,  $\text{Na}_2\text{CO}_3$ ) and the digested materials, which may consist of bSi that is more or less resistant to the alkaline reagent (Kamatani and Oku 2000; Zhu et al. 2023). Wet-alkaline digestion has never been used on seagrass tissues, so a kinetic assay was conducted in a strong alkaline solution (i.e., 0.5 M NaOH) to determine the time necessary to completely digest bSi, i.e., when the bSi concentration reaches a

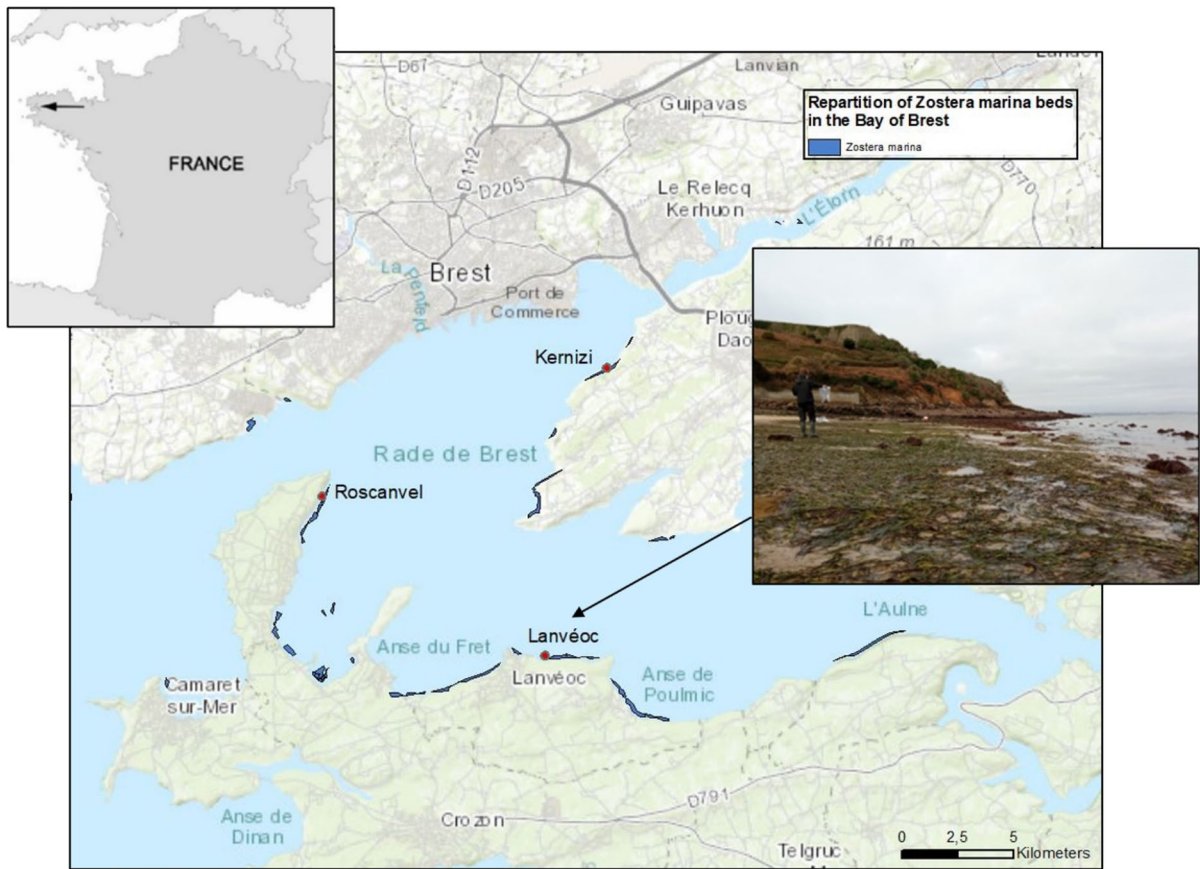
plateau. Ten sampling times over 72 h were used to monitor the concentration of digested bSi in the samples.

For this digestion, 40 mL of 0.5 M NaOH was added to 50-mL fluorinated ethylene propylene centrifuge tubes (FEP, Nalgene), 5 tubes containing each 80 mg of *Z. marina* powder and 3 tubes without powder, which were considered as experimental blanks. The samples were immediately placed in a shaking water bath (Julabo SW22) preheated to 85 °C with an oscillation frequency of 52 rpm. At each time interval (1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 24 h, 48 h, 72 h), an aliquot of 1 mL was taken from the FEP tubes after centrifugation at 1,500 rpm for 5 min and neutralized using 0.625 mL of 1.0 M hydrochloric acid solution into a 15-mL polypropylene centrifuge tube (Falcon). The neutralized samples were then diluted with 8.375 mL of Milli-Q water and stored in a refrigerator (4 °C) until subsequent analysis for silicon determination. The maximum bSi digested corresponded to the quantity of bSi retrieved when the plateau was reached, indicating that all the bSi in the sample was digested and released into the milieu. At the end of the alkaline digestion, the digested material was rinsed 3 times with Milli-Q water after centrifugation at 1,500 rpm for 5 min before being dried (60 °C, > 48 h) for subsequent HF digestion, following the protocol described above. Subsequent HF digestion was carried out to ensure that the wet-alkaline solution recovered all bSi present in the samples.

#### Silicon determination

Dissolved silica in digested samples, either in 2.9N HF or in 0.5 M NaOH, was analyzed according to an improved analytical method of the molybdate blue method (Aminot and Kerouel 2007) on an AA3 HR Autoanalyzer (SEAL Analytical) that allows the automatic measurement of nutrient concentrations in solution. Silicon calibration standards were prepared using a Silicon Standard Solution of ammonium fluorosilicate from Merck (traceable to SRM from NIST acidic,  $(\text{NH}_4)_2\text{SiF}_6$  in  $\text{H}_2\text{O}$  1000 mg Si  $\text{L}^{-1}$  Certipur®) in the same matrices as the samples. For samples digested with HF, the matrix consisted of 0.5 parts 2.9N HF and 14.5 parts 60 g  $\text{L}^{-1}$   $\text{H}_3\text{BO}_3$ . For samples digested using wet-alkaline digestion, the matrix consisted of 1 part 0.5 M NaOH, 0.6 parts 1 M HCl and 8.4 parts Milli-Q Water.





**Fig. 1** Distribution of *Zostera marina* beds in the Bay of Brest (France). GPS data were collected on <https://cms.geobretagne.fr> (2018) and computed in ArcMap (ArcGIS Desktop version 10.8). The picture on the right shows a general view of

Lanvéoc's meadow composed of *Z. marina* seagrass during a high-coefficient low tide, where samples were collected for this study

The bSi content in the leaves of *Z. marina* was expressed as dry weight content (Si%) using Eq. (1):

assessed (i.e., 5 mg, 10 mg, and 15 mg) were evaluated with the one-way analysis of variation (ANOVA)

$$Si\% = \frac{([Si](\mu M) * 10^{-6}) * Volume\ digestor(L) * Dilution\ factor * 28.0855\ (g\ mol^{-1}) * 100}{Mass\ sample(g)} \quad (1)$$

where  $28.0855\ g\ mol^{-1}$  is the silicon atomic mass. Metadata and tracked calculations of bSi content in leaves of *Z. marina* are available in Supplementary Information 2.

#### Statistical analysis

The differences between the average Si% of *Z. marina* obtained after acid digestion in 2.9N HF at each mass

test using the R Stats package (R Core Team 2021). The bSi extracted with the wet-alkaline digestion at each sampling time were represented with SigmaPlot 15.0 (Systat Software Inc.) and linear and non-linear models were tested to determine the best fitting kinetic model using the Kinetic Module of the SigmaPlot software. One-way ANOVA and Tukey's honestly significant difference (Tukey's HSD) tests were then performed between each time point to compare pairwise time points and analyze the curve tendency

using the Regression Wizard of Sigmaplot. Finally, the differences between the average Si% in *Z. marina* obtained at the end of each digestion (i.e., wet-alkaline digestion, HF digestion, and the sum of wet-alkaline digestion plus the subsequent HF digestion) were also evaluated with a one-way ANOVA test and an a posteriori pairwise Tukey's HSD test. Before performing any parametric test, data were assessed for normality using the Shapiro–Wilk test and for homoscedasticity with Levene's test, using the R Stats package (R Core Team 2021).

### Seagrass silicon stock at the regional level

At the Bay of Brest, *Z. marina* occupies a total surface of 1.00 km<sup>2</sup>, of which 95% is located in three seagrass meadows: Kernizi, Roscanvel and Lanvéoc (Fig. 1). To estimate the silicon stock of the meadows of *Z. marina* in the Bay of Brest, we used data of biomass of *Z. marina* leaves per m<sup>2</sup> from the three main meadows of the bay. Data for the Kernizi and Roscanvel meadows were obtained from existing literature (Auby et al. 2018; Boyé et al. 2022) and the REBENT monitoring programme (<http://www.rebent.org/>), and that for the Lanvéoc meadow was determined in this study using the same methodology as for the other meadows (Auby et al. 2018). Shoots were collected using 0.1m<sup>2</sup> quadrats randomly positioned within the seagrass meadows (n=5). Shoots within each quadrat were sampled to determine the biomass of epiphyte-cleaned leaves after 48 h of desiccation at 60 °C. This data, along with the silicon content (Si%) measured in this study, were used to estimate the silicon reservoir in leaves of *Z. marina* at the different meadows and at the bay as a whole. Note that our approach to estimating the silicon stock in *Z. marina* for the bay does not account for potential intra-annual variability.

## Results

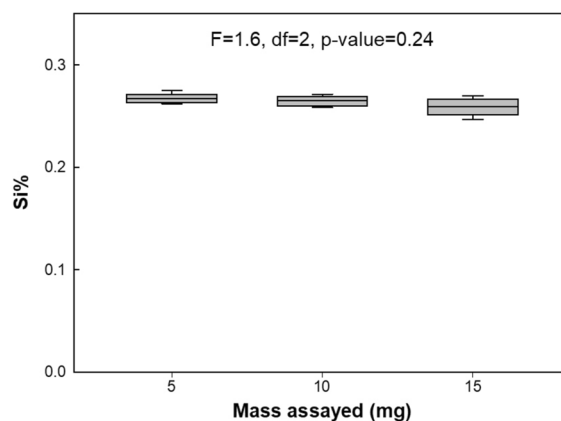
### Assessment of silica extraction methods on seagrass leaves

Prior to evaluating the performance of hydrofluoric acid digestion and wet-alkaline digestion methods in the extraction of bSi from seagrass samples, preliminary tests were conducted to determine the

applicability of these methods to seagrass leaves and whether any adjustments were necessary.

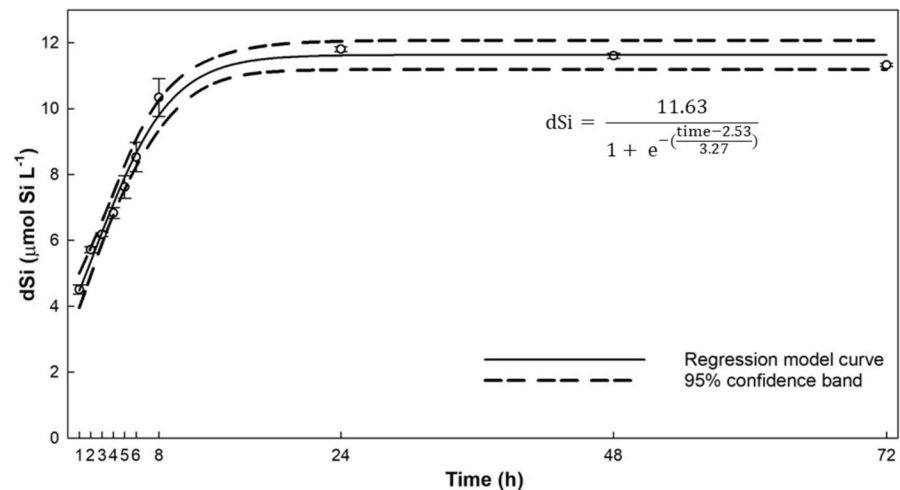
The evaluation of the reproducibility of bSi extraction using the acid digestion method with 15 mL of 2.9N HF showed no significant differences between the three masses examined (F=1.6, df=2, p-value=0.24; Fig. 2). This finding indicates that small dry weight masses of seagrass, as low as 5 mg, are suitable for determining bSi content in leaves of *Z. marina*.

The time required for complete digestion of bSi using wet-alkaline digestion varies depending on the origin and structural complexity of the bSi. To determine the necessary digestion time for seagrass bSi in 0.5 M NaOH, a kinetic assay was conducted to identify when the dSi concentration reached a plateau, i.e., the digestion was completed. Experimental data showed that dSi concentration within the digestion tubes (n=5) increased linearly during the first 8 h, then reaching a plateau at 24 h, with a mean dSi concentration of  $11.81 \pm 0.06 \mu\text{mol Si L}^{-1}$ , beyond which no further increase was observed (Fig. 3). This was corroborated by the kinetic model describing the digestion process, which followed a saturable sigmoidal sigmoid function of 3-parameter ( $R^2=0.989$ , p-value < 0.01; Fig. 3). This model also confirmed that the plateau was reached at 24 h, with a maximum modelled dSi concentration of  $11.63 \pm 0.19 \mu\text{mol Si L}^{-1}$ . These results indicated that digestion of *Z. marina* bSi in 0.5 M NaOH was complete at 24 h.



**Fig. 2** Average ( $\pm$ SD) silicon content (Si%) in *Z. marina* leaves. Three different masses (5, 10, 15 mg) of *Z. marina* with five replicates were digested using 2.9N HF

**Fig. 3** Extraction of dissolved silica (dSi; in  $\mu\text{mol Si L}^{-1}$ ) through time (in hours). Error bars represent standard deviation calculated from five replicates digested using 0.5 M NaOH. The statistics of model's goodness of fit are available in Supplementary Information 3



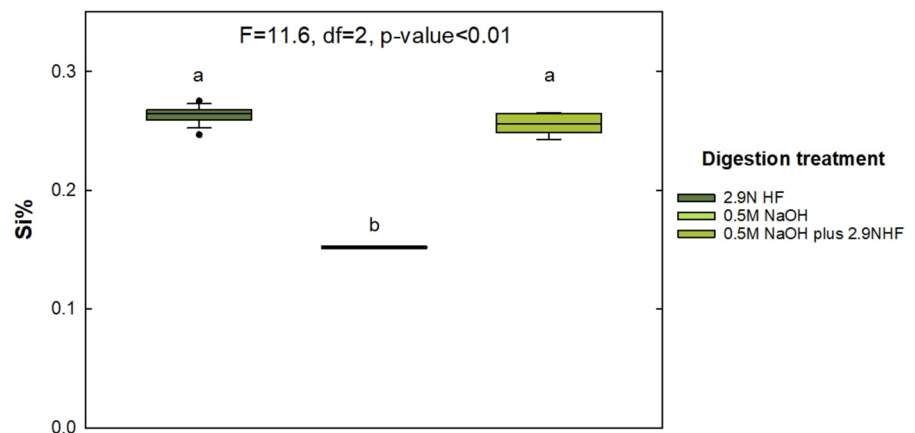
#### Hydrofluoric acid and wet-alkaline digestion comparison

The bSi content extracted using HF digestion and wet-alkaline digestion showed significant differences ( $F=11.6$ ,  $df=2$ ,  $p\text{-value}<0.01$ ; Fig. 4). A pairwise comparison further confirmed significant differences between the bSi content obtained with these two methods ( $p\text{-value}<0.01$ ; Fig. 4). The average bSi content of *Z. marina* leaves measured with the HF digestion method was  $0.26\pm 0.01$  Si% (mean  $\pm$  SD;  $n=15$ ), whereas the amount of silica extracted with 0.5 M NaOH after 24 h of digestion was  $0.15\pm 0.001$  Si% ( $n=5$ ). This amount corresponded to only  $57.70\pm 0.38\%$  of the bSi extracted using 2.9N HF.

Samples digested in 0.5 M NaOH for bSi extraction were afterwards digested in 2.9N HF using the

same method as before. This additional HF digestion continued to extract bSi from *Z. marina* leaf samples (Fig. 4). The bSi content retrieved with the HF digestion conducted after wet-alkaline digestion was  $0.10\pm 0.01$  Si% ( $n=5$ ). The total bSi content retrieved with the wet-alkaline digestion and the subsequent HF digestion was  $0.26\pm 0.01$  Si% ( $n=5$ ), showing no significant differences when compared to the bSi content obtained with only HF digestion ( $p\text{-value}=0.95$ ). These results suggest that *Z. marina* contain two operational silica pools: a labile one, digested by the alkaline method, and a more resistant one, digested only by HF.

**Fig. 4** Average ( $\pm$ SD) silicon content (Si%) of *Zostera marina* extracted using 2.9N HF, 0.5 M NaOH, and the sum of wet-alkaline digestion using 0.5 M NaOH and a subsequent 2.9N HF digestion. Significant differences ( $p<0.05$ ) are indicated with letters according to the results of a one-way ANOVA analysis and the a posteriori pairwise Tukey's HSD test





### Seagrass silicon reservoir at the regional level

Based on the bSi content measured in the leaves of *Z. marina* in this study (0.26 Si%), we estimated the silicon pool in the *Z. marina* meadows in the Bay of Brest (Table 1). The average ( $\pm$ SD) silicon stock within leaves of *Z. marina* ranged from  $0.12 \pm 0.04$  to  $0.18 \pm 0.08$  g Si m<sup>-2</sup>, depending on the meadow (Table 1). The Roscanvel meadow, which constitutes 65% of the total distribution area of *Z. marina* in the Bay of Brest and exhibited the highest leaf biomass, accounted for the largest silicon reservoir in the bay ( $118.9 \pm 50.3 \times 10^3$  g Si). At the bay level, the silicon reservoir in the leaves of *Z. marina* was estimated at  $167.4 \pm 70.0 \times 10^3$  g Si (Table 1).

### Discussion

After examining the performance of digestion with acid and alkaline reagents, chemical digestion with 2.9N HF emerged as the optimal method for determining the total amount of bSi in *Z. marina* leaves. Our results showed that the commonly used wet-alkaline digestion failed to recover all the bSi content in seagrass leaves, unlike HF digestion. Notably, after 24 h, bSi was no longer released from the leaves of *Z. marina* during the wet-alkaline digestion, suggesting the presence of a fraction of bSi within seagrass leaves that is resistant to extraction by 0.5 M NaOH and is only extracted with aggressive HF digestion. This is supported by the results of the HF digestion conducted after the wet-alkaline digestion, which allowed the retrieval of 100% of the bSi

content. These outcomes suggested the existence of two operational pools of bSi in *Z. marina* leaves: a more labile and digestible bSi that is released by wet-alkaline digestion and a more resistant bSi that is only released with 2.9N HF.

While the HF digestion method has demonstrated efficacy in determining the total bSi content in *Z. marina* leaves, a sequential digestion involving 24 h in 0.5 M NaOH followed by 48 h in 2.9N HF emerge as a powerful combination of methods to measure the labile and refractory fractions of bSi in seagrass leaves, which is neither possible with the HF digestion method nor with the methods used in previous studies (Herman et al. 1996; Vonk et al. 2018; Rondevaldova et al. 2023). The quantification of these two bSi fractions is crucial for evaluating the impact of seagrass meadows on the marine silicon cycle, as these distinct forms of silica are likely to undergo different incorporations into the biogeochemical cycle of silicon. Presumably, the labile bSi within seagrass leaves will be recycled and become available for utilization by other silicified organisms (e.g., diatoms, Rhizaria, sponges) upon seagrass leaf degradation, whereas the refractory seagrass bSi will merely contribute to the standing stock of silica buried in sediments underneath seagrass meadows. This process would be similar to that of other amorphous silica sources, which have varying degrees of solubility and reactivity to dissolution (Saccone et al. 2007). Further studies are needed to evaluate the role of seagrass ecosystems as either sources or sinks of silicon in the biogeochemical cycle of this element.

The mechanisms of silica deposition and the exact location of silicon accumulation in *Z. marina*

**Table 1** Silicon reservoir in leaves of *Zostera marina* seagrass in the Bay of Brest (France). Area (10<sup>6</sup> m<sup>2</sup>) of each meadow and the total distribution of *Z. marina* for the Bay of Brest was estimated from the latest distribution mapping of *Z. marina* in this habitat (OFB-TBM environment 2021). Average ( $\pm$ SD) leaf biomass (g *Z. marina* m<sup>-2</sup>) of the Kernizi and the Ros-

canvel meadows were calculated elsewhere (Auby et al. 2018; Boyé et al. 2022) and that of Lanvéoc was calculated in this study, together with the silicon content of *Z. marina* leaves (0.26 Si%). See Supplementary Information 2 for raw data and detailed calculations

Seagrass meadow	Meadow surface (10 <sup>6</sup> m <sup>2</sup> )	Leaf biomass (g <i>Z. marina</i> m <sup>-2</sup> )	Silicon reservoir (g Si m <sup>-2</sup> )	Total silicon reservoir (10 <sup>3</sup> g Si)
Kernizi	0.07	46.99 ( $\pm$ 14.37)	0.12 ( $\pm$ 0.04)	8.6 ( $\pm$ 2.6)
Roscanvel	0.65	70.37 ( $\pm$ 29.75)	0.18 ( $\pm$ 0.08)	118.9 ( $\pm$ 50.3)
Lanvéoc	0.23	66.76 ( $\pm$ 28.66)	0.17 ( $\pm$ 0.07)	39.9 ( $\pm$ 17.1)
Bay of Brest	1.00	65.19 ( $\pm$ 27.28)	0.18 ( $\pm$ 0.07)	167.4 ( $\pm$ 70.0)

remain unknown, but several lines of evidence provide insights. Recent studies have documented the presence of phytoliths in the roots, stems, and leaves of *Z. marina* (Rong et al. 2024). If these phytoliths are analogous to those found in terrestrial and freshwater plants, they would completely dissolve during wet alkaline digestion (Saccone et al. 2007; Meunier et al. 2014), consistent with the fraction digested in our study with 0.5 M NaOH. Furthermore, *Z. marina* encodes Slp1, a protein that mediates vesicular transport of silica into the apoplasmic space (Nawaz et al. 2020). The presence of Slp1 suggests that *Z. marina* may use a silica deposition mechanism similar to that observed in the monocot angiosperm *Sorghum bicolor*, in which Slp1 facilitates silica incorporation through vesicular transport rather than through the Lsi1/2 pathway, which is absent in *Z. marina* (Kumar et al. 2020). This alternative pathway likely results in the deposition of silica in amorphous forms within the cell wall or extracellular matrix. Such silica deposits, integrated within the organic matrix and reported as small phytoliths in other plant species, are often resistant to extraction with alkaline digestion (Prychid et al. 2003; Kameník et al. 2013). The tight binding of silica to organic components may explain why some of the bSi in *Z. marina* resists alkaline digestion and requires digestion with 2.9N HF for complete extraction.

Seagrass distribution is globally prevalent in coastal environments, extending from intertidal to subtidal depths in both estuarine and marine ecosystems (Green and Short 2003). In the Bay of Brest, a semi-enclosed coastal ecosystem in the NE Atlantic Ocean with tidal ranges of 2 to 8 m, *Z. marina* mainly occurs in discontinuous patches in the intertidal zone (Auby et al. 2018). This study provides the first estimate of the silicon content of *Z. marina* meadows in the bay, offering a basis for comparison with other silicifiers in the ecosystem. However, this initial estimate does not account for potential variability, such as, for example, changes in silicon content as a function of leaf age. If silica accumulation in seagrass follows patterns observed in terrestrial and freshwater plants, where silicon accumulation tends to increase with leaf age (e.g., Motomura et al. 2008; Querné et al. 2012), it is likely that our estimate represents a conservative or underestimated value of the silicon content in these meadows.

When comparing the average silicon reservoir of seagrasses in the bay ( $0.18 \pm 0.07 \text{ g Si m}^{-2}$ ) with that of benthic diatoms at subtidal depths ( $0.11 \pm 0.09 \text{ g Si m}^{-2}$ ; Grossteffan et al. 2024), both are quite similar. However, intertidal sponges have a significantly higher average silicon reservoir ( $4.3 \pm 4.2 \text{ g Si m}^{-2}$ ; López-Acosta et al. 2022). This large difference is due to the much higher silicon content in sponges (22.34–26.88 Si%, depending on the species present in the intertidal zone of the bay; López-Acosta et al. 2022) compared to *Z. marina* (0.26 Si%). Despite this, sponges and *Z. marina* generally occupy different areas. Sponges primarily grow on rocky substrates, while *Z. marina* grow on sandy and muddy bottoms (Boyé et al. 2022; López-Acosta et al. 2022). Therefore, each likely contributes to silicon cycling in separate zones within the land–ocean continuum of the bay. The potential impact of seagrass distribution on silicon input to the ocean via rivers and terrestrial streams remains to be studied. In the bay, rivers and terrestrial streams are the main source of dSi, supporting about half of the diatom production in this diatom-rich ecosystem (Ragueneau et al. 2005a), and, globally, account for 52% of dSi entering the ocean (Tréguer et al. 2021). As some of the seagrass bSi showed to be reluctant to dissolution, seagrasses might act as a buffer in the transport of dSi to the ocean realm.

To date, only few studies have quantified the bSi content in seagrass tissues. Herman et al. (1996) determined bSi in *Z. marina* leaves from the Rhine-Meuse Estuary (SW Netherlands), finding values ranging from 0.02 to 0.66 Si% depending on location within the estuary, and hypothesizing dSi uptake and leaf bSi content correlate with ambient dSi concentrations. Vonk et al. (2018) measured bSi in seagrass species across four families (Posidoniaceae, Hydrocharitaceae, Cymodoceaceae and Zosteraceae), reporting no significant differences between families and an average bSi content of 0.08 Si%. Within the family Zosteraceae, *Z. marina* exhibited the highest bSi content (0.27 Si%). These values are in agreement with those measured in this study in leaves of *Z. marina* of the Bay of Brest (0.26 Si%), showing that alkaline and acid digestions are suitable for measuring bSi in seagrasses. The most comprehensive study evaluating bSi content of freshwater vegetation, based on 83 different species analyzed with wet-alkaline digestion, showed a bSi content of 0.45

Si% (Schoelynck and Struyf 2016), which is about 5 times higher than the mean content described for seagrasses (0.08 Si%; Vonk et al. 2018) and 1.5 times higher than the mean content measured in *Z. marina* (0.26 Si%; Herman et al. 1996; Vonk et al. 2018, this study).

Silica accumulation is widely recognized as a functional trait in freshwater vegetation, with plants showing adaptations to the prevailing environmental conditions and dominant mechanical forces in their ecosystems (Schoelynck and Struyf 2016). The functional roles of dSi uptake in plants include responses to both biotic (e.g., herbivory) and abiotic (e.g., heavy metals) stresses (Bhat et al. 2019; Acevedo et al. 2021). Additionally, substantial evidence supports a connection between silicon and carbon uptake in freshwater vegetation. In these plants, dSi is assimilated and deposited in the form of solid amorphous silica bodies, known as phytoliths (Kameník et al. 2013; Koné et al. 2019). During phytolith formation, some organic carbon becomes occluded within the phytolith structure (referred to as PhytOC), making it highly resistant to decomposition and thereby facilitating the long-term biogeochemical sequestration of atmospheric CO<sub>2</sub> (Jones and Milne 1963; Parr and Sullivan 2005; Song et al. 2016). Recent findings suggest that this mechanism also occurs in seagrasses (Rong et al. 2024), indicating that the quantification of bSi in seagrass ecosystems could serve as a proxy for carbon sequestration via PhytOC accumulation within seagrass tissues.

Seagrass habitats are being lost at an estimated rate of 7% per year worldwide (Dunic et al. 2021), which has led to conservation and restoration initiatives aimed at mitigating this trend (De Los Santos et al. 2019). In the implementation of these initiatives, the concentration of dSi in seawater is often not measured but can be critical, as low dSi concentration (<15 µM Si) may be a factor in the decline of estuarine seagrass populations (Herman et al. 1996). In the current context of a changing ocean, the global decrease in dSi loads caused by eutrophic conditions and human use of terrestrial waters may lead to dSi depletion in many coastal ecosystems (Ittekkot et al. 2006; Zhang et al. 2020; Taucher et al. 2022). Therefore, understanding the silicon cycle in relation to seagrasses will be essential for addressing the effects of climate change in coastal areas and for the conservation of seagrass ecosystems.

## Conclusion

The findings reported here indicate that the wet-alkaline digestion and hydrofluoric acid digestion methods are valid for seagrass bSi quantification. Additionally, our study highlights the presence of two operational pools of bSi in seagrass leaves: a labile form released by wet-alkaline digestion, and a resistant form that can only be extracted with hydrofluoric acid digestion. These results provide insights into the impact of seagrasses on the biogeochemical cycle of silicon in the land–ocean continuum, as labile silica will be recycled upon leaf degradation, benefiting siliceous organisms living in the coastal environment, while refractory silica will contribute to the buried silica standing stock in the ecosystem. Our study provides a methodological basis for further research on the ecological significance of silicon in seagrass physiological fitness, functional ecology, and biogeochemical dynamics in seagrass ecosystems.

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**Data availability** All data of this study are available within this article and at Supplementary Information 1–3.

#### Declarations

**Competing interests** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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