Plant Soil (2012) 352:157–171
DOI 10.1007/s11104-011-0986-5

REGULAR ARTICLE

In situ biogenic silica variations in the invasive salt marsh plant, *Spartina alterniflora:* A possible link with environmental stress

Jérémy Querné · Olivier Ragueneau · Nathalie Poupart

Received: 11 February 2011 / Accepted: 29 August 2011 / Published online: 1 October 2011

Abstract
Aims Higher plants are an understudied component of the global silicon cycle; they absorb silicic acid (dSi) which is stored as biogenic silica ($bSiO_2$). Si is believed to alleviate physical, chemical, and biological stresses such as storms, high salinity, heavy metal toxicity, grazing, and disease. We investigated a Si-accumulating invasive species growing in the tidal marshes of the Bay of Brest (France), viz., *Spartina alterniflora*. Our objectives were to determine (1) where and when $bSiO_2$ accumulates in the plant during its life cycle, (2) whether this accumulation varies with abiotic factors: wave action, estuarine salinity, and duration of immersion, and (3) if the accumulation was limited by dSi availability in marsh porewater.
Methods A 2 years field survey permitted to sample plants which were analyzed for there $bSiO_2$ concentrations. Sediment cores were sampled seasonally and the dSi concentrations in the porewater were measured from 0 to 10 cm.
Results $bSiO_2$ accumulated more in mature leaves than in other organs. There was a strong linear relationship between $bSiO_2$ concentration and plant length. $bSiO_2$ concentrations did not increase, but rather decreased as a function of exposure to the three abiotic factors tested. dSi availability was not significantly different for each of the tested sites and dSi profiles did not exhibit huge losses in the root zone.
Conclusions Our evidence suggests that dSi availability did not seem to be a limiting factor. $bSiO_2$ did not increase with increasing abiotic stresses but was strongly correlated with growth. Hence, *S. alterniflora* is likely to have other adaptive strategies for dealing with environmental stressors but it did not exclude the possible role of Si in alleviating these stresses. If this is the case, there remain intriguing questions about Si uptake, its availability, and its role in silicification and growth.

Keywords Abiotic factors · Biogenic silica · Duration of immersion · Estuarine salinity · Spartina alterniflora · Wave activity

Responsible Editor: Jian Feng Ma.

J. Querné (✉) · O. Ragueneau
LEMAR UMR6539, U.B.O, Institut Universitaire Européen de la Mer, I.U.E.M,
Université de Bretagne Occidentale,
place Nicolas Copernic,
29280 Plouzane, France
e-mail: jeremy.querne@univ-brest.fr

J. Querné · N. Poupart
LEBHAM EA3877, U.B.O, Institut Universitaire Européen de la Mer, I.U.E.M, Université de Bretagne Occidentale,
place Nicolas Copernic,
29280 Plouzane, France

J. Querné
GEOMER UMR6554, U.B.O,
Institut Universitaire Européen de la Mer, I.U.E.M,
Université de Bretagne Occidentale,
place Nicolas Copernic,
29280 Plouzane, France

Introduction

Silicon is the second most abundant element in soils but has only recently been considered as an essential nutrient for plants (Epstein 1994; Epstein 1999; Epstein 2009). Silicon has beneficial effects on the growth of many plant species, especially monocotyledonous angiosperms including rice, sugarcane, and some members of the Cyperaceae (Epstein 1994; 1999; Liang et al. 2007). These findings motivated Epstein and Bloom (2005) to enlarge the definition of "essential nutrient" beyond previous understanding (Arnon and Stout, 1939) to include silicon as an essential element. These authors defined an essential element as (1) part of a molecule that is an intrinsic component of the structure or metabolism of plants, or (2) one which when deficient leads to plant abnormalities in growth, development, or reproduction.

Plants absorb silicon from soil solutions via the roots in a dissolved form (dSi), i.e., as aqueous monosilicic acid (H_4SiO_4) (Epstein 1994). It is transported in the transpiration stream through the xylem to the aerial organs and irreversibly deposited as amorphous silica, which is referred to as biogenic silica ($bSiO_2$) (Raven 2003; Ma and Yamaji 2006). These silica bodies in plant tissues are commonly termed "opal phytoliths" (Epstein 1994). There are large variations in the distribution of $bSiO_2$ in plants; some have highly silicified roots (Piperno 1988; Piperno 2006) while others accumulate silica in reproductive structures (Ma and Takahashi 2002). Most of the mechanisms involved are still unknown, but it appears that $bSiO_2$ is deposited in highest concentrations within organs that lose water in great quantities through evapotranspiration (Street-Perrott and Barker 2008).

Higher plants can be classified in categories by their silicon accumulation behavior. Plants accumulating $bSiO_2$ in their tissues to >1% of dry weight (DW) are termed Si-accumulators. Those that accumulate $bSiO_2$ to 1–0.5% of DW are termed intermediate accumulators, and plants accumulating <0.5% of DW are termed Si-excluders (Ma and Takahashi 2002). Most of the Si-accumulators are monocotyledonous and members of the Cyperaceae and Poaceae (Ma and Takahashi 2002).

Si has distinct effects in Si-accumulators exposed to abiotic and biotic stresses (Ma 2004; Ma and Yamaji 2006). In rice and barley, silicon is believed to alleviate toxic effects of heavy metals like Cd, Zn, Mn, and Al (Epstein 1994; Epstein 1999; Liang et al. 2007). In the same species, Si can limit physical stress effects like radiation injury, water stresses, drought, freezing, and may also improve resistance to strong winds by increasing stem rigidity (Epstein 1999; Ma and Takahashi 2002; Ma 2004). Si also has a role in defenses against fungi and insects, and provides general protection against herbivory (McNaughton and Tarrants 1983; McNaughton et al. 1985). Among photosynthetic organisms, other siliceous species, including diatoms, deter grazers by accumulating silicon (Smetacek et al. 2004; Pondaven et al. 2007).

Halophytes provide opportunities for studying silica accumulation processes in saline environments. The cordgrass *Spartina alterniflora* (Poaceae) is a halophyte that is subjected to seawater immersion and other physiological stresses that prevail in salt marsh environments. This species is an intermediate Si-accumulator (Norris and Hackney 1999; Hodson et al. 2005; Hou et al. 2010). In a few previous studies, it has been shown that *S. alterniflora* accumulates silica during growth (Norris and Hackney 1999), mostly in mature leaves (Hou et al. 2010). Si helps to alleviate physical and chemical stresses in rice. Rice is so genetically close to cordgrass that *S. alterniflora* RNA is able to hybridize with rice microarrays (Chelaifa et al. 2010). Hence, we propose that silica might also mitigate abiotic stresses in *S. alterniflora* and that silica concentration in plants should be related to elevated abiotic pressures.

In the Bay of Brest (France), *S. alterniflora* is listed as an invasive species threatening protected natives such as *Limonium humile* (UICN red list since 1995) (Tesson et al. 1997; Quéré et al. 2010). Since its probable accidental introduction during World War II, this cordgrass has colonized soft intertidal sediments of the bay, extending from salt marshes to freshwater tidal marshes (Bougault et al. 2004; Bougault et al. 2005; Sparfel et al. 2005; Querné et al. in revision). *S. alterniflora* has replaced original salt marsh vegetation in the bay, which was previously dominated by perennial and herbaceous species belonging mainly to the family Chenopodiaceae, whose members are not Si-accumulators. In a larger study focusing on the impact of this invasion on the coastal ecosystem of the bay, we identified abiotic factors controlling the growth and production of *S. alterniflora*, viz., wave activity, salinity, and duration of immersion (Querné et al. in revision).

Our first objective of the present study was to describe, through field experiments and survey, where and when in its life cycle *S. alterniflora* accumulates $bSiO_2$. For this, we studied intra-plant and temporal variations in $bSiO_2$.

Our second objective was to determine whether *S. alterniflora* plants subjected to (1) higher wave action and (2) longer time of immersion would increase rigidity by accumulating more $bSiO_2$ and (3) whether plants subjected to higher salinities would increase their $bSiO_2$ to cope with increased salt stress.

Our last objective was to examine the availability of dSi in porewater marsh sediment and to determine if it could limit the accumulation of $bSiO_2$ in *S. alterniflora*.

Materials and methods

Sampling strategy and study sites

The Bay of Brest is a shallow (mean depth 8 m) semi-enclosed, macrotidal coastal system with an area of 180 km². It is connected to the Atlantic Ocean by a deep (40 m), narrow (1.8 km wide) strait (Fig. 1). The Aulne and Elorn rivers carry 80% of total freshwater draining into the bay. Maximum tidal amplitude is 8 m during spring tides; the mean tidal range during the study was 4.13 m (SHOM, Service of Hydrography and Oceanography of the French Navy). A semidiurnal tidal action enhances mixing of the water masses (Chauvaud et al. 2000). The dominant winds are southwesterly (MeteoFrance).

The study was carried out from June 2006 to December 2008 on tidal marshes dominated by *S. alterniflora* and located 16 km from Brest. Experiments were conducted on three representative marshes with similar muddy sediments. In these selected sites, *S. alterniflora* has spread on estuarine marshes from upstream freshwater tidal marshes to downstream salt marshes. Cordgrass in the Bay of Brest occurs across the complete salinity gradient from freshwater to seawater. It has also spread from low marshes frequently flooded at high tide to high marshes that are only exceptionally flooded at spring tides.

To study the effect of the wave action on the $bSiO_2$ concentration of *S. alterniflora*, three stations were selected. One was designated the protected marsh (PM; 48°20′40 N, 4°16′54 W). This marsh was not oriented towards prevailing winds and was therefore sheltered from storms and wave action (Fig. 1). The two other stations, designated Marsh 1 (M1; 48°21′ 28 N, 4°19′20 W) and Marsh 2 (M2; 48°21′10 N, 4° 20′51 W) were on open, riverside marshes exposed to prevailing southerly winds and therefore exposed to storms and wave action. Plants at PM, M 1 and M 2 were harvested monthly. The 3 sites experienced the same salinities (29.6 in the river and 25.6 in soil) and were located at the same tidal elevation (1.5 m). To confirm the wave exposure on the marshes studied, we used a modeling approach that computed wave heights at the mouth of each marsh (Querné et al. in revision) (Table 1).

To study the effect of salinity on $bSiO_2$ concentration in *S. alterniflora*, we selected three stations across an estuarine salinity gradient located at M1 (Fig. 1). These stations were located at the same tidal elevation (1.5 m), and were designated "downstream at high salinity" (29.6 in the river and 25.6 in soil), "midstream at mid salinity" (18.5 in the river and 21.4 in soil) and "upstream at low salinity" (0.5 in the river and 10.1 in soil). Plants at each station were harvested seasonally in March, June, September, and December 2008 (Table 1).

To study the effect of duration of immersion on $bSiO_2$ concentration in *S. alterniflora*, we selected sampling stations at different tidal elevations (in the downstream sector of M1). Salinities were similar among these stations (29.6 in the river and 25.6 in soil) (Fig. 1). The stations were located along a 100 m transect deployed perpendicularly to the river and were designated: "low" (1.1 m elevation), "middle" (2 m), and "high" (3 m). Plants at these stations were harvested seasonally in March, June, September, and December 2008. The low site was located on the low marsh where there were a protracted periods of immersion (5.8 h at spring tide, 5.18 h at a mean high tide and 2.75 h at neap tide), the middle site was located on the mid marsh where immersion times were of intermediate duration (4.75 h at spring tides, 3.87 h at mean high tide, 0 h at neap tide). The high site was located on high marsh where there were short durations of immersion (3.00 h at spring tide, 1.88 h at mean high tide, 0 h at neap tide) (Table 1). Descriptions of the stations and of the methodologies for measuring river salinity, soil salinity, tidal elevation, and duration of immersion are further detailed in Querné et al. (in revision).

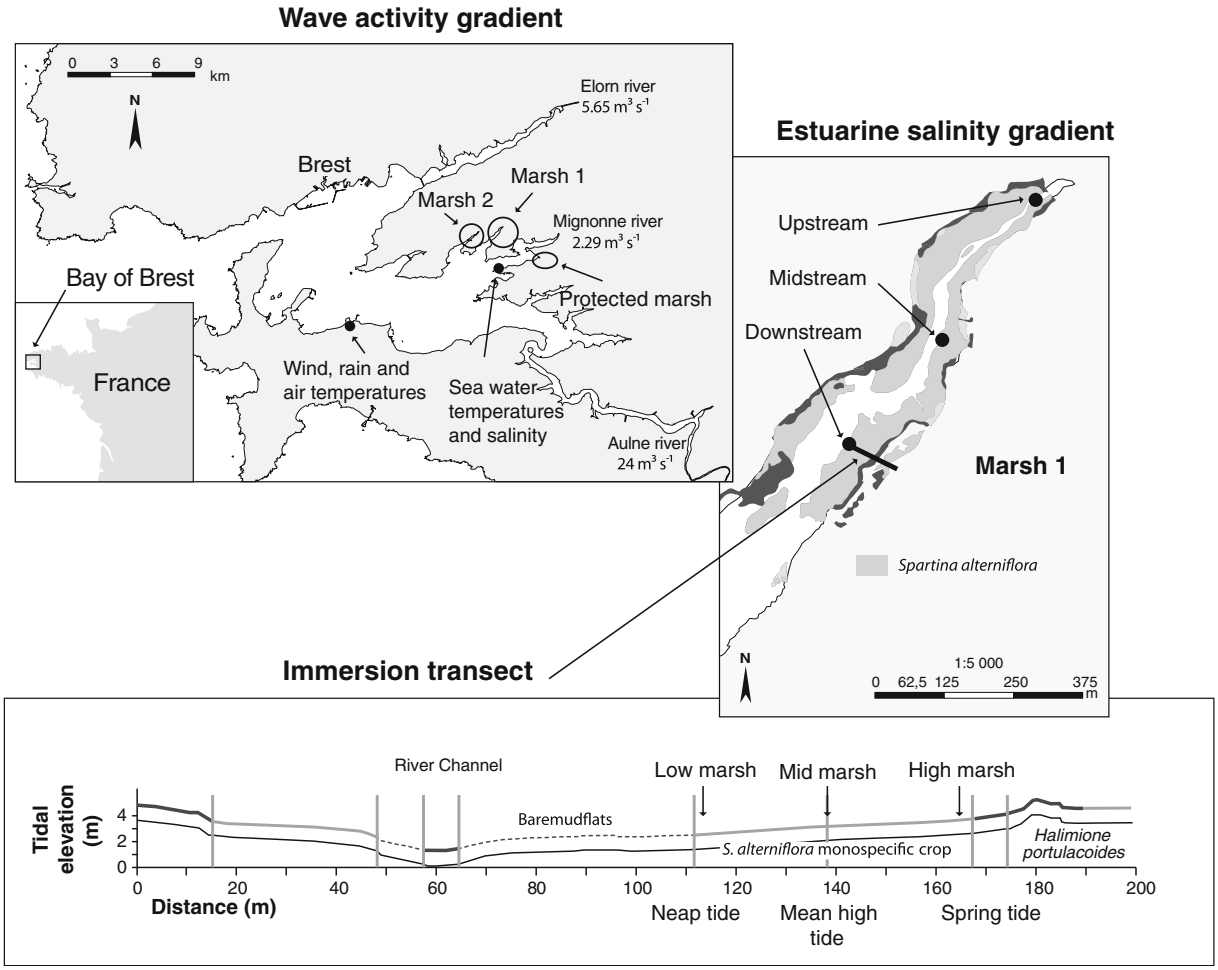

Fig. 1 Location of the Bay of Brest on the Atlantic coastline of France showing in the top left panel, the three salt marshes studied (Marsh 1, 2, and Protected marsh, spanning a gradient of wave action); the top right panel depicts sampling sites (upstream, midstream, and downstream sites with salinities of 0, 18.5, and 30, respectively) on Marsh 1 across an estuarine salinity gradient; the lower panel shows sampling sites (low, mid, and high marsh) on Marsh 1, across an immersion gradient

Table 1 Summary of the sampling design with their names, code names, the factors that was tested on theses sites, and the conditions concerning the tested factors. The given duration of immersion were calculated for an mean high tide represented by a 6.95 m water amplitude (Querné et al. in revision)

Sampling site	Code Name	Factor tested	Condition
Marsh 1	M1	Wave activity	Open
Marsh 2	M2		Open
Protected Marsh	PM		Protected
Marsh 1	Downstream	Salinity	Salt marsh
	Midstream		Brackish marsh
	Upstream		Freshwater tidal marsh
Marsh 1	Low marsh	Immersion	5 h 11 min
	Mid marsh		3 h 52 min
	High marsh		1 h 53 min

Sample treatment

Samples of aboveground material were collected from different plots deployed in monospecific stands of *S. alterniflora*. Aboveground *S. alterniflora* was harvested at each station on each sampling day by clipping vegetation at the sediment surface in three adjacent replicate plots of 0.0625 m^2. Minimal areas and optimal numbers of replicates were determined for each sampling station using density as a variable (Querné et al. in revision). All standing living and dead culms, and litter were removed and inserted into pre-labeled plastic bags for transport to the laboratory. Dead material, which was identified by yellowish or brownish coloration, was separated from living material (Darby and Turner 2008), cleaned and oven dried at 60°C to constant mass. Each living plant was cleaned, frozen, and then freeze-dried. Materials were weighed to the nearest 0.01 g to give values of living and dead biomass. Previously live dried material of aerial plant parts (leaves, stems, flowers, and seeds) were milled to powder and homogenized.

To measure the distribution of $bSiO_2$ in specimens, entire plants (n=6, with triplicate measurements for each organ) were collected with their rhizomes from the same plots on July 2006 at PM, M1 and M2 (29.6 salinity, 1.1 m tidal elevation). Young leaves, mature leaves, bases of stems and roots were excised and subjected to the treatment detailed above.

Sediment cores (diameter 8.6 cm, length 30 cm) were sampled seasonally for each stations and brought back to the laboratory in a coolbox to extract the porewater within a 2 h delay. Core samples were sliced every centimeter from 0 to 10 cm depth. Each slice was centrifuged (15 min at 3,500 rpm), and the obtained supernatant was collected, filtered (0.45 μm Millex), and stocked at 4°C until analysis.

Plants biogenic silica and porewater dissolved silica analysis

The alkaline Na_2CO_3 digestion method was used for the measurements of the biogenic silica in *S. alterniflora* (Saccone et al. 2006; Struyf et al. 2005). Thirty milligrams of plant powder were digested in 50 ml of Na_2CO_3 (5%) at 85°C for 4 h until total digestion of the phytoliths was achieved. To avoid pH problems with the reagents, digest solutions were neutralized to pH 7 with HCl (10%) and diluted with ultrapure water. In the diluted solution, silicates were measured by the molybdate colorimetric method using an autoanalyzer (Bran+Luebe) (Brewer and Riley 1966). Dissolved silica in the porewater samples were also analyzed using the molybdate colorimetric method on the autoanalyzer.

Statistical analysis and calculations

The dependent variable were *S. alterniflora* $bSiO_2$ and porewater dSi concentrations. The following were explanatory variables: plant organ, sampling date, wave action at stations "PM, M1, and M2," salinity at stations "downstream, middle stream, and upstream," and duration of immersion at stations "low, middle, and high marsh." Statistical analyses were performed with STATGRAPICS Plus 5.1 software. Homogeneity of variances was tested with Levene's test. $bSiO_2$ concentrations were compared by ANOVA after meeting assumptions of independence, normality, and homoscedasticity. Fisher's LSD test was used after ANOVA for multiple comparisons among means.

The variables selected to represent growth and production were stem length (cm), stem density (m^{-2}), living biomass (g dry weight (DW) m^{-2}) and net aerial primary production (NAPP) (g DW m^{-2} yr^{-1}) (Querné et al. in revision). NAPP was estimated using the Smalley (1959) method and considered changes through sampling intervals in both living and dead plant masses; however decomposition and tidal flush out of litter were not taken into account. The methodology and the NAPP data are further detailed in Querné et al. (in revision).

We calculated an uptake rate for dSi (μmol dSi g^{-1} DW $month^{-1}$) over the productive period using measured $bSiO_2$ concentrations in plants. The productive period here was define as the period of the year where plants length increase, in general between February and September (Querné et al. in revision). The uptake rate was calculated as the difference between the maximum $bSiO_2$ concentration (t) and the minimal $bSiO_2$ concentration (t_{-1}) divided by the number of months between t and t_{-1} and by the molar mass of $bSiO_2$. The uptake rate was calculated at each station during the productive period in 2008.

DSi stocks (mmol m^{-2}) were calculated using dSi porewater concentrations, an average porosity of 0.8, integrated from 0 to 10 cm depth. Annual means were calculated for each stations in 2008.

PCA (Principle Component Analysis) and ANOVA analyses were used to test the correlation between $bSiO_2$ in plants, dSi stocks in porewater (this study), plant length, stem densities, living biomasses, wave height, soil salinity, and tidal elevation (Querné et al. in revision). Linear correlation were tested for all variables. Data from all our sampling period were selected (from 2006 to 2008) and reached 114 observations. Tidal elevation was selected to discriminate data along the immersion gradient. NAPP, and dSi uptake were not selected for the analysis due to lack of data along the sampling period. Plant length, stem densities and living biomasses were used to monitor plant production. Statistical analyses were performed with STATGRAPICS Plus 5.1 software. Variables close to one had the strongest influence on the component and variables close to zero have limited influence. Parallel vectors were strongly correlated and squared vectors were not. Vectors with opposite directions showed a negative correlation.

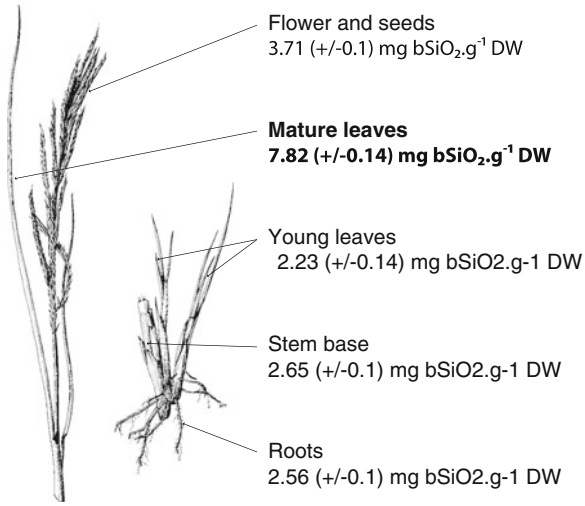

Fig. 2 Biogenic silica ($bSiO_2$) concentrations of different above- and belowground organs of *Spartina alterniflora* (plants harvested in June 06). Values represent mean ± SE. (Image from University of Florida, 1998, Center for Aquatic and Invasive Plants)

Results

Intra-plant variation in $bSiO_2$ concentration

We measured $bSiO_2$ concentration in different plant organs (Fig. 2). The lowest $bSiO_2$ concentrations were found in the bases of young leaves, roots, and in the bases of the stems; these were significantly lower than flower and seed values (ANOVA, $F_{(4;\ 19)}=291.99$, $p<0.05$). The mature leaves were significantly more highly silicified than other organs (ANOVA, $F_{(4;\ 19)}=291.99$, $p<0.05$).

Temporal variation in $bSiO_2$ concentrations

$bSiO_2$ concentrations of *S. alterniflora* aerial parts were measured monthly over 2.5 years at PM (Fig. 3). $bSiO_2$ concentration was strongly influenced by plant development and phenology. Low $bSiO_2$ concentrations (2–4 mg g^{-1} DW) were found in winter (February 2007, January 2008 and December 2008) before the beginning of the growth season. $bSiO_2$ concentrations increased through the vegetative growth season, and reached a maximum in summer (July 2007, August 2007, September 2007, and June 2008) when plants reached the fructification stage (6–10 mg g^{-1} DW) (ANOVA, $F_{(21;\ 44)}=12.73$, $p<0.05$). $bSiO_2$ concentrations decreased through fall when culm death occurred, reaching the lowest values in winter. The same pattern was observed at all stations (data not shown).

Maxima were statistically different at site PM between the summers of 2007 and 2008, (ANOVA, $F_{(21;\ 44)}=12.73$, $p<0.05$). Yet, the maxima did not occur in the same month in the 2 years. For example, at PM, maximum $bSiO_2$ concentration occurred in September in 2007 and in June in 2008 (Fig. 3). At the other study sites, there were also year-to-year variations in $bSiO_2$ concentrations and in the timing of the maxima.

Effect of the wave action on $bSiO_2$ concentrations

We compared $bSiO_2$ concentrations at stations M1, M2, and PM among spring, summer, fall, and winter 2008 (Fig. 4). There were no significant differences in $bSiO_2$ concentrations among the 3 study stations in fall and winter 2008 (ANOVA, $F_{(2;\ 18)}=1.29$, $p>0.05$ and ANOVA, $F_{(2;\ 18)}=2.49$, $p>0.05$, respectively). In spring 2008, the concentrations at PM and M1 were significantly higher than at M2 (ANOVA, $F_{(2;\ 24)}=6.18$, $p<0.05$). In summer 2008, $bSiO_2$ concentration was statistically higher at PM than at M2 (ANOVA, $F_{(2;\ 24)}=4.12$, $p<0.05$). We originally postulated that plants at M1 and M2, which are subjected to wave action, would have higher $bSiO_2$ concentrations than

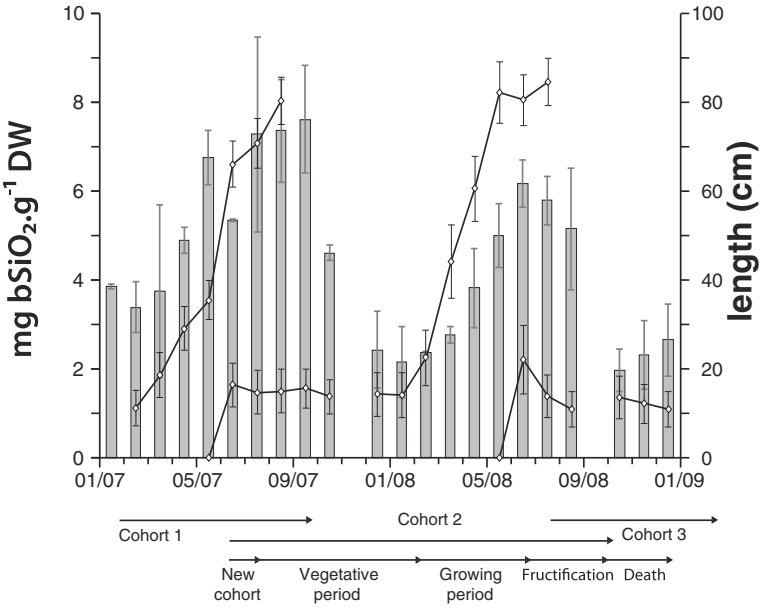

Fig. 3 Temporal variation in (1) biogenic silica ($bSiO_2$) concentration (*bars*) in the aerial parts of *Spartina alterniflora* harvested from the protected marsh (PM) in 2007 and 2008, and (2) mean stem length (*curve*). The life cycle of the plant and different cohorts are presented below the x-axis (Querné et al. in revision). Values represent mean ± SE

plants at station PM, which was less exposed. Our results contradict this postulate.

Effect of estuarine salinity on $bSiO_2$ concentrations

S. alterniflora $bSiO_2$ concentration was examined as a function of estuarine salinity (Fig. 5). In March 2008, the $bSiO_2$ concentration at the midstream station was significantly higher than that of the downstream and upstream stations (ANOVA, $F_{(2;\ 6)}=11.67$, $p<0.05$). In June 2008, there was a significant difference between the midstream station and the downstream and upstream stations (ANOVA, $F_{(2;\ 5)}=10.26$, $p<0.05$). In September 2008 and December 2008, there were no significant differences in $bSiO_2$ concentrations among stations (ANOVA, $F_{(2;\ 6)}=2.09$, $p>0.05$ and ANOVA, $F_{(2;\ 5)}=2.09$, $p>0.05$, respectively). Plants at the midstream station (exposed to a salinity of 18.5) had a higher $bSiO_2$ concentration during growing season than plants subjected to other salinities. Hence, $bSiO_2$ concentrations did not increase with increasing salinity.

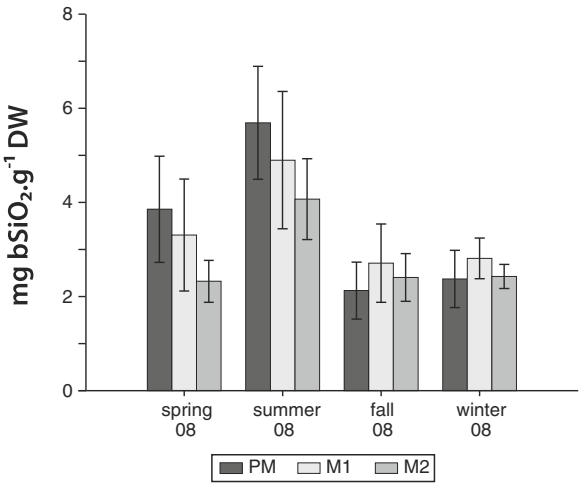

Fig. 4 Seasonal variations in biogenic silica ($bSiO_2$) concentration of the aerial parts of *Spartina alterniflora* measured in 2008 at Marsh 1 (M1) and Marsh 2 (M2), which are exposed to wave action and at the Protected Marsh (PM), which is protected from wave action. Values represent mean ± SE

Effect of duration of immersion on $bSiO_2$ concentrations

$bSiO_2$ concentrations were examined as a function of elevation on the marsh (i.e., different durations of immersion). There was a trend of decreasing $bSiO_2$ concentrations from low to high elevations (Fig. 6). In June 2008, the mean $bSiO_2$ concentrations of all stations were statistically higher than in other months (ANOVA, $F_{(3;\ 28)}=9.89$, $p<0.05$). In June 2008, there was a significant difference between $bSiO_2$ concentrations on the mid marsh and at the

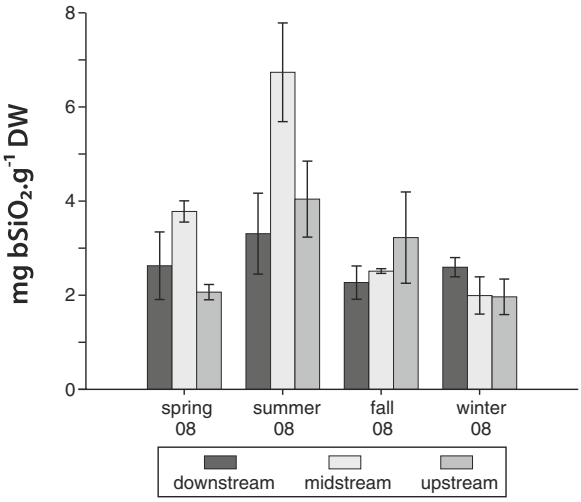

Fig. 5 Seasonal variation in biogenic silica ($bSiO_2$) concentration in the aerial parts of *Spartina alterniflora* measured in 2008 at stations subjected to different salinities across an estuarine gradient at Marsh 1: downstream (river salinity, 29.6 and soil salinity, 25.6), mid stream (river salinity, 18.5 and soil salinity, 21.4) and upstream (river salinity, 0.5 and soil salinity, 10.1). Values represent mean ± SE

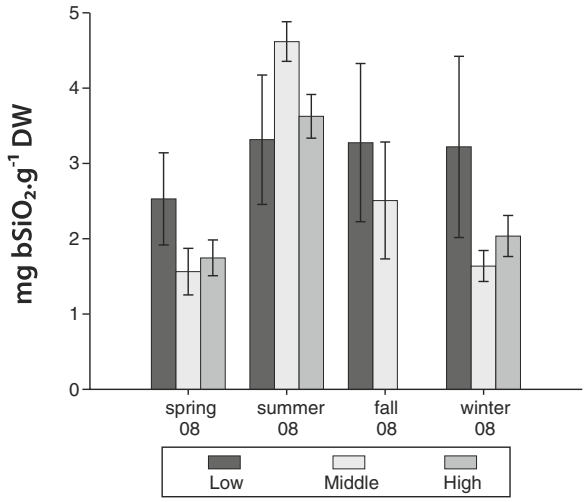

Fig. 6 Seasonal variations in biogenic silica ($bSiO_2$) concentrations of aerial parts of *Spartina alterniflora* in 2008 at stations of different tidal elevation, and hence, different durations of immersion across the immersion gradient on Marsh 1: low (1.1 m; 5.8 h at spring tide, 5.18 h at mean high tide, and 2.75 h at neap tide), middle (2 m; 4.75 h at spring tide, 3.87 h at mean high tide, and 0 at neap tide), high (3 m; 3.0 h at spring tide, 1.88 h at mean high tide, and 0 at neap tide) and pool (4 m; 1.12 h at spring tide). Values represent mean ± SE

low and high stations (ANOVA, $F_{(2;\ 5)}=5.88$, $p<0.05$) (Fig. 6). In September 2008, there were no significant differences in $bSiO_2$ concentrations among stations (ANOVA, $F_{(1;\ 4)}=1.03$, $p>0.05$) (Fig. 6). The $bSiO_2$ concentration of the low station did not vary significantly through year, and there was no evidence of a peak in concentration (ANOVA, $F_{(3;\ 7)}=0.42$, $p>0.05$). Plants in the mid marsh (with an intermediate duration of immersion) had largest increases in $bSiO_2$ through the year, with a maximum in June 2008 during the growth period. We had previously postulated that highest $bSiO_2$ concentrations would occur in plants at the low site, which was subjected to longest durations of flooding, but this was not the case.

Availability of dSi in marsh sediment porewaters

Annual dSi profiles in the porewater of the selected marsh showed an increase with depth on all the studied sites (Fig. 7a,b,c). A high variability in dSi profiles was explained by a seasonal trend showing higher dSi concentrations in fall profiles but was not statistically significant (data not shown). At M1, annual dSi profile did not show significant differences with depth (ANOVA, $F_{(9;29)}=1.58$, $p>0.05$) (Fig. 7a).

The PM site had a dSi profile with a significantly lower concentration at −1 cm and higher concentrations at −8, −9 and −10 cm (ANOVA, $F(9;29)=6.58$, $p<0.05$) (Fig. 7a). The downstream dSi profile did not show any significant variations with depth (ANOVA, $F_{(9;29)}=1.58$, $p>0.05$), the midstream dSi profile had a statistically lower dSi concentration at −1 cm (ANOVA, $F_{(9;29)}=4.15$, $p<0.05$), and the upstream dSi profile did not show significant differences in dSi concentrations with depth (ANOVA, $F_{(9;29)}=1.57$, $p>0.05$) (Fig. 7b). The low marsh dSi profile exhibited a significantly lower dSi concentration in at −1 cm (ANOVA, $F_{(9;29)}=4.15$, $p<0.05$), the mid marsh dSi profile had also a significantly lower dSi concentration at −1 cm (ANOVA, $F_{(9;28)}=3.3$, $p<0.05$), and the high marsh dSi profile showed a different trend where the dSi concentrations from −1 to −4 cm were significantly lower then dSi concentrations measured from −7 to −10 cm (ANOVA, $F_{(9;30)}=4.45$, $p<0.05$) (Fig. 7c). Any of these dSi profiles showed a strong significant decrease in dSi concentration in the porewater that could have been imputed to *S. alterniflora* roots except for the depletion on the high marsh profile.

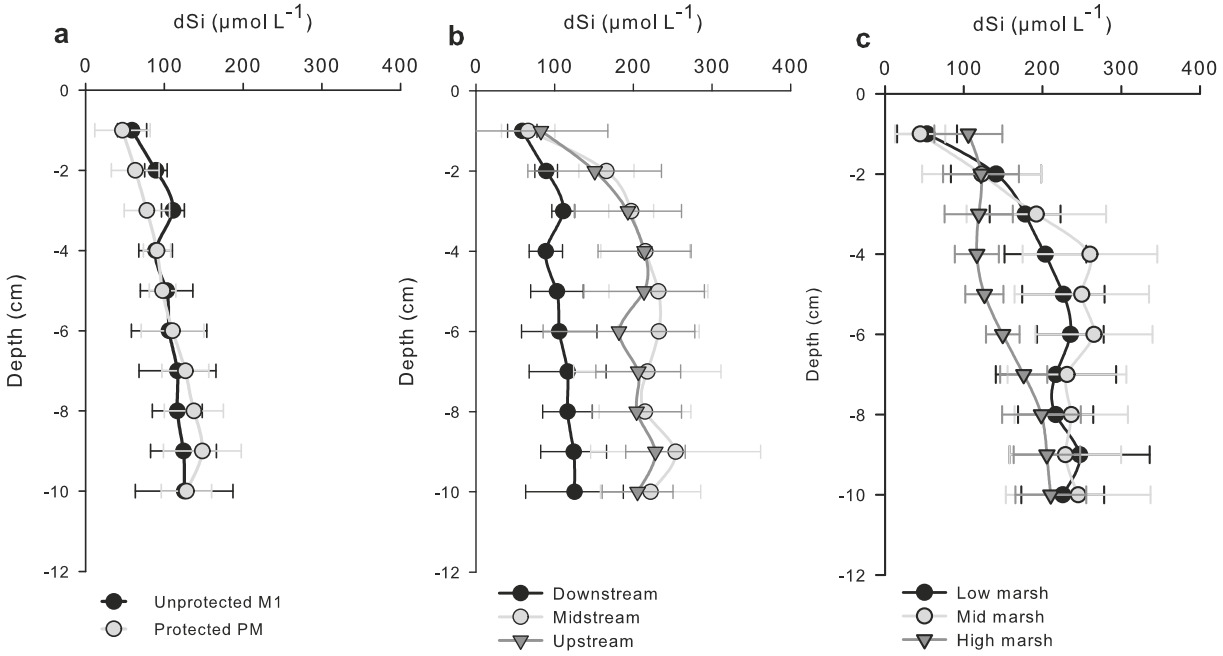

Fig. 7 Annual mean porewater dissolved silica (DSi) profiles (µmol L^{-1}) from 0 to 10 cm depth as a function of wave activity (protected PM and unprotected M1 sites) (**a**), salinity (downstream, midstream, upstream) (**b**), and tidal elevation (low marsh, mid marsh, high marsh) (**c**) for the year 2008

Plant $bSiO_2$, porewater dSi stocks, and dSi uptake variations in relation to plant height, density, live biomass, and NAPP along our three gradients.

Maximum $bSiO_2$ concentrations obtained during the productive period in June 2008 were related to plant height (cm), densities (stems m^{-2}), live biomass (g DW m^{-2}), NAPP values (g DW m^{-2} yr^{-1}) (Querné et al. in revision), calculated dSi uptake (µmol dSi g^{-1} DW $month^{-1}$), and calculated dSi stocks in porewater (mmol dSi m^{-2}) in the corresponding period of the year (i.e., growth period in summer) (Table 2).

Data from PM, M1, and M2 were compared to demonstrate effects of wave exposure on production and silicification. PM was the most protected station, and had plants with the highest NAPP value and $bSiO_2$ concentration (Table 2). There was no significant differences between dSi stocks between PM and M1 (ANOVA, $F_{(1;6)}=0.5$, $p>0.05$) and only little differences between PM and M1 dSi uptake rate (Table 2).

The effects of estuarine salinity on primary production and silicification were demonstrated by relating NAPP in 2008 to the highest $bSiO_2$ concentration across the estuarine salinity gradient in summer 2008. Highest primary production occurred upstream of the marsh at the lowest salinity, whereas the highest $bSiO_2$ concentration and highest uptake rate occurred in plants in the midstream site at a salinity of 18.5 (Table 2). The calculated dSi stocks did not show any significant differences between downstream, midstream and upstream sites (ANOVA, $F_{(2;9)}=2.94$, $p>0.05$) (Table 2). However, dSi stocks showed a trend were the lowest stocks was found downstream and the highest was found at the midstream site, which follows the same trend as NAPP and $bSiO_2$ concentrations (Table 2).

The effects of duration of immersion on primary production and silicification were demonstrated by comparing NAPP in 2008 to $bSiO_2$ concentrations of plants across the immersion gradient. Highest production occurred at the mid marsh station, a shore level with an intermediate duration of immersion. At the mid marsh station, high NAPP resulted from high plant density and a high living biomass. The mid marsh station also had the highest $bSiO_2$ concentration and the highest uptake rate (Table 2). dSi stocks did not showed significant differences between the different stations of the immersion gradient (ANOVA, $F_{(2;9)}=0.87$, $p>0.05$) (Table 2).

Table 2 Mean values of length, density, live biomass, net aerial primary production (NAPP) (from Querné et al. in revision), biogenic silica ($bSiO_2$) concentration in aerial parts of *Spartina alterniflora*, dissolved silica uptake rate (μmol dSi g^{-1} DW $month^{-1}$) during the summer maximum in 2008, and dissolved silica (dSi) mean annual stocks in sediment porewater (mmol dSi m^{-2}) as functions of wave action, salinity and duration of immersion; values represent mean ± SD

Factors tested		Protected Marsh	Marsh 1	Marsh 2
Wave action	Length (cm)	84.7±5.3	63.4±7	59.4±5.6
	Density (stems m^{-2})	485±37	1141±416	608±97
	Biomass (g DW m^{-2})	807±49	443±153	614±56
	NAPP (g DW m^{-2} y^{-1})	1613	987	887
	$bSiO_2$ (mg g^{-1} DW)	6.2±0.5	4.3±0.7	4.4±1.2
	Uptake dSi (μmol g^{-1} DW $month^{-1}$)	13.37	12.15	6.91
	dSi stock (mmol m^{-2})	7.3±0.7	8.1±2.3	No data
		Downstream	Midstream	Upstream
Salinity	Length (cm)	42.5±9	71±5.5	83±9.8
	Density (stems m^{-2})	736±121	565±128	592±254
	Biomass (g DW m^{-2})	695±177	1165±296	1762±369
	NAPP (g DW m^{-2} y^{-1})	721	1408	1774
	$bSiO_2$ (mg g^{-1} DW)	3.3±0.8	6.8±1	4±0.8
	Uptake dSi (μmol g^{-1} DW $month^{-1}$)	6.17	16.44	10.94
	dSi stock (mmol m^{-2})	8.1±2.3	14.4±3.3	12.1±5.0
		Low marsh	Mid marsh	High marsh
Immersion	Length (cm)	42.5±9	36±2	57±8
	density (stems m^{-2})	736±121	1056±105	373±37
	biomass (g DW m^{-2})	695±177	818±166	404±52
	NAPP (g DW m^{-2} y^{-1})	721	831	508
	$bSiO_2$ (mg g^{-1} DW)	3.3±0.8	4.6±0.1	3.6±0.3
	Uptake dSi (μmol g^{-1} DW $month^{-1}$)	6.72	16.94	10.44
	dSi stock (mmol m^{-2})	14.4±3.3	14.4±6.2	11.0±2.4

To test the correlation between plant $bSiO_2$, dSi stocks, plant production, and abiotic factors, a PCA analysis was used (Fig. 8). Three components explained 71.79% of the variability. The three graphs formed by our three components showed the formation of three groups of variables (Fig. 8a,b,c). The first group included plant $bSiO_2$, plant length, and plant living biomasses. Plant $bSiO_2$ and living biomasses were positively correlated but the variability explained was low (ANOVA, $F_{(1;90)}=56.41$, $p<0.01$, correlation coefficient=0.62, $R^2=38.5\%$). Plant $bSiO_2$ and plant length were positively correlated (ANOVA, $F_{(1;103)}=239.92$, $p<0.01$, correlation coefficient=0.84, $R^2=69.96\%$). This relationship was linear and showed a strong correlation of the two variables (Fig. 9). The second group of variables included dSi stocks, wave heights, stem densities, and tidal elevations represented by vectors squared from the plant $bSiO_2$ vector (Fig. 8a and b). The influence of these variables on plant $bSiO_2$ was limited. No correlation was found between plant $bSiO_2$ and dSi stocks, linear and logaritmic relationships could not fit the data (ANOVA, $F_{(1;41)}=0.58$, $p>>0.05$, correlation coefficient=−0.11, $R^2=1.4\%$) (Fig. 8a). A weak negative relationship was found between plant $bSiO_2$ and wave height (ANOVA, $F_{(1;114)}=21.84$, $p<0.01$, correlation coefficient=−0.40, $R^2=16\%$) (Fig. 8b). Weak negative relationships were found between plant $bSiO_2$ and stems densities (ANOVA, $F_{(1;92)}=11.88$, $p<0.01$, correlation coefficient=−0.33, $R^2=11.4\%$) (Fig. 8a and b) and between plant $bSiO_2$ and tidal elevation (ANOVA, $F_{(1;114)}=8.89$, $p<0.01$, correlation coefficient=−0.27, $R^2=7.23\%$) (Fig. 8b and c). The last group was represented by soil salinity

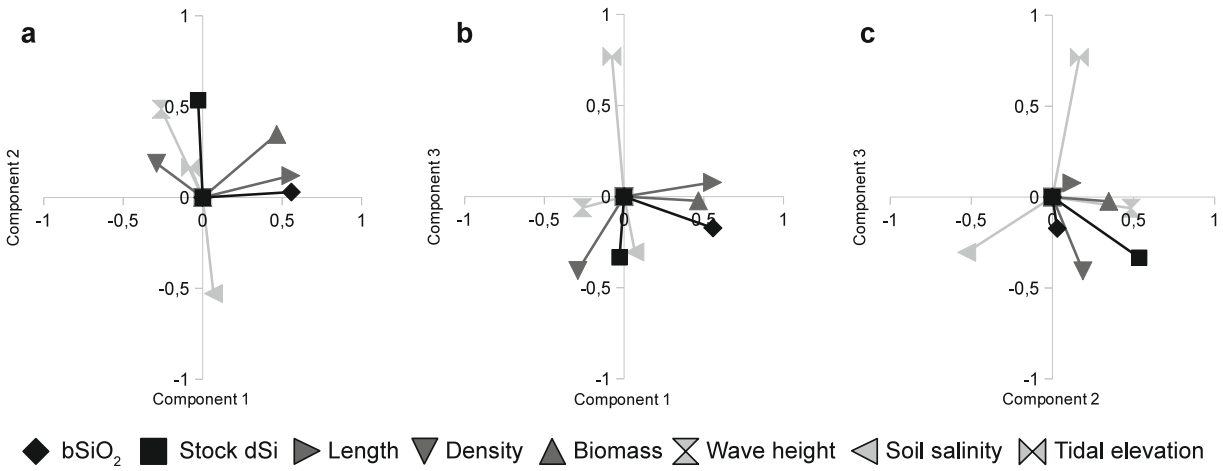

Fig. 8 PCA correlation between plant biogenic silica concentrations ($bSiO_2$), stocks of dissolved silica in sediment porewater (Stock dSi) (this study), plant lengths (Length), stem densities (Density), plant living biomasses (Biomass), wave heights (Wave height), soil salinities (Soil salinity), and tidal elevations (Tidal elevation) (Querné et al. in revision). Three components represent 71.79% of the variability and are represented as three graphs (**a**, **b**, and **c**). The variables close to one have the strongest influence on the component and variables close to zero have limited influences. Parallel vectors are strongly correlated and squared vectors are not. Vectors with opposite directions shows a negative correlation

which was weakly positively correlated to plant $bSiO_2$ (ANOVA, $F_{(1;114)}=10.27$, $p<0.01$, correlation coefficient=0.29, $R^2=8.3\%$) (Fig. 8a,b,c).

Discussion

S. Alterniflora: an intermediate Si-accumulator

Our first objective was to determine where and when biogenic silica accumulated in the plant. $bSiO_2$ measurements on *S. alterniflora* roots, stem base, young leaves, mature leaves, flowers and seeds showed that accumulation was highest in aerial organs, especially in the mature leaves (Fig. 2). These results are consistent with recent experiments on *S. alterniflora* in the Yangtze Estuary (Hou et al. 2010) where whole plant $bSiO_2$ reached 6.71 to 7.97 mg g^{-1} DW. In North Carolina, $bSiO_2$ concentration in *S. alterniflora* was 5–6 mg g^{-1} DW at the mean summer maximum in mature culms (Norris and Hackney 1999). In the present study, the mean summer maximum was 4.63–6.75 mg $bSiO_2$ g^{-1} DW, which represents a variation of 0.5–1.0% in total $bSiO_2$ per unit dry biomass. All of these measures indicate that *S. alterniflora* is an intermediate Si-accumulator rather than a strict Si-accumulator (Ma and Takahashi 2002; Hou et al. 2010). Highest $bSiO_2$ concentration in the Bay of Brest occurred in mature leaves rather than in flower and seeds (Fig. 2). However, samples were collected in July when seeds were not fully mature. In other monocotyledonous plants like rice and wheat, seeds are protected by a husk containing a high proportion of $bSiO_2$ (23.22%) (Sun et al. 2008). Whether such seed protection exists in *S. alterniflora* has yet to be determined.

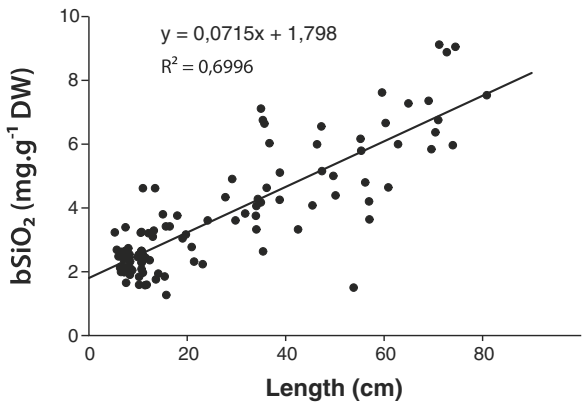

Fig. 9 Linear relationship between plant length (data from Querné et al. in revision) and biogenic silica ($bSiO_2$) concentration, combining all sites and seasons

There was an increase in the $bSiO_2$ concentration of *S. alterniflora* through its developmental cycle beginning in spring and ending in summer (Figs. 3, 4,

5 and 6). Thus, silica accumulation appears to be positively correlated with age (Motomura et al. 2004); young leaves contained less silica than older leaves, in accordance with other experiments on *S. alterniflora* (Norris and Hackney 1999). The $bSiO_2$ concentration of plants in the Bay of Brest was strongly linearly correlated to plant length, which may be regarded as a proxy of plant age (Figs. 8 and 9). Such a relationship did not occur in *S. alterniflora* growing in the Yangtze estuary (Hou et al. 2010), but occured in *Phragmites australis*, *Epilobium hirsutum*, and *Urtica dioica* growing on freshwater tidal marshes in the Schelde estuary (Belgium) (Struyf et al. 2005). A possible limitation of the $bSiO_2$ accumulation by lack of dSi did not seemed to play a major role in this study with the exception of the high marsh (Fig. 7) (Hackney et al., 2002). No significant differences in dSi stocks were found among the tested sites (Table 2), and no strong depletion of dSi was observed in the porewater dSi profiles, except of the high marsh profile were a small depletion occured (Fig. 7). Our results from the Bay of Brest suggest that accumulation of $bSiO_2$ in *S. alterniflora* is more linked to growth and productivity then it is by availability of dSi. In its natural environment, *S. alterniflora* is exposed to a range of abiotic factors that influence its growth and productivity, including wave action, salinity, duration of immersion, microtopography, nutrients, redox conditions, and light (Dame and Kenny 1986; Mendelssohn and Morris 2002). How do these abiotic variables influence silicification in this species?

Is $bSiO_2$ accumulation in *S. alterniflora* related to abiotic pressures?

The accumulation of $bSiO_2$ in plants is believed to alleviate various abiotic and biotic stressors such as pathogens, metal toxicity, drought, and high salinity (Epstein 1994; Epstein 1999; Epstein 2009). The uptake of dSi is also believed to increase plant rigidity, conferring improved resistance to mechanical stresses like storm events (Ma and Takahashi 2002), river currents, and tidal action (Schoelynck et al. 2010).

Across a range of wave action, *S. alterniflora* accumulated the highest $bSiO_2$ concentration and had the highest dSi uptake rate at station PM; lowest values occurred at stations M1 and M2 (Fig. 4, Table 2). NAPP estimates tracked similar trends (Table 2) but there was no differences in the porewater dSi concentrations (Fig. 7) and a weak negative relationship between plant $bSiO_2$ and wave height (Fig. 8). Contrary to our expectations, these results did not demonstrate enhanced silicification under increased abiotic pressure, including high wave action. Plants have a variety of strategies for coping with stress, silicification being only one of them. In *S. alterniflora* (Roland and Douglass 2005; Querné et al. in revision) and *S. anglica* (Swales et al. 2004), strong wave action may limit plant growth and promote production of clones and increased shoot densities. Reduced height may allow plants to minimize wave impact; increases in clone and shoot densities may improve anchorage and reduce excavation by water movement (Roland and Douglass 2005). We found increased stem densities and shorter plants at stations M1 and M2, which were exposed to greatest wave action (Table 2; Querné et al. in revision). The shorter the plants were, the smaller the quantity of $bSiO_2$ they accumulated (Fig. 9). Wetland macrophytes may also increase their rigidity through production of lignin and cellulose (Schoelynck et al. 2010). A complex trade-off between production of lignin and cellulose and accumulation of $bSiO_2$ may also explain reduced silicification at the two stations exposed to high wave action.

We did not detect increased silicification with increasing salinity. However, we observed significant differences in plant $bSiO_2$ concentrations in spring and summer, where midstream plants had the highest $bSiO_2$ concentrations (Fig. 5). NAPP fell with increasing salinity, which is consistent with a common observation of declining growth in *S. alterniflora* with increased salinity (Haines and Dunn 1976; Cavalieri 1983). Laboratory experiments have also demonstrated maximum growth at salinities in the range 0–20 (Haines and Dunn 1976; Linthurst and Seneca 1980; Linthurst and Seneca 1981; Sleimi and Abdelly 2004). We expected silicification to increase downstream, but this was not the case, and over the growth period, both $bSiO_2$ concentration and dSi uptake rate were highest at intermediate salinities (Fig. 4 March to June 2008; Table 2). The trend showed that porewater dSi stocks were higher at the midstream and the upstream sites and was lower at downstream site with the highest salinity, but this difference was not significant (Fig. 7, Table 2). Si addition to culture medium enhance rice tolerance to

salinity by reducing transpiration, thereby reducing osmotic stress as well (Matoh et al. 1986). Silicon may increase root activity, leading to increased uptake of nutrients, which improves mineral balance under salinity stress (Liang et al. 2003). Si also reduces membrane permeability, which again helps plants cope with salinity stress (Liang et al. 2003; Liang et al. 2007). Why then was silicification of *S. alterniflora* reduced at the downstream station? In this particular case, the major effect of salinity was the reduction of plant growth and production which explain why *S. alterniflora* downstream did not accumulate more Si then fully grown plants of the midstream and upstream sites (Table 2, Fig. 9). *S. alterniflora* may well allocate resources to mechanisms other than silicification in order to relieve the stress of abiotic factors and particularly with salinity. As in numerous other halophytes, there are high concentrations of osmoprotector metabolites in the aerial parts of *S. alterniflora*, including glycine-betaine, proline, sugars, and dimethylsulfoniopropionate (DMSP); strategic allocation to these osmolytes may reduce the energy available for growth (Cavalieri 1983; Diggelen et al. 1986). However, accumulation of $bSiO_2$ to limit the effects of increased salinity should be less energy costly than the production of osmoprotector metabolites (Schoelynck et al. 2010). However, the growth of *S. anglica* (a hybrid of *S. alterniflora*) does not increase with addition of dSi to growth media or marsh sediments (De Bakker et al. 1999). In contrast, additions of dSi to growth media have strong, positive effects on the growth of rice (*Zea mays)*, tomato (*Lycopersicon esculentum*), cucumber (*Cucumis sativus*), soybean (*Glycine max*), and other members of the Poaceae (Epstein 1994; Ma and Takahashi 2002). Horsetails (*Equisetum arvense*) require dSi to grow, and they die when it is not added to culture solutions (Epstein 1994; Epstein 1999). To the best of our knowledge, there is no direct evidence of dSi control on the growth of *S. alterniflora*, and further investigations are warranted to determine whether growth, $bSiO_2$ accumulation, or Si uptake are limited by dSi concentrations in sediments.

S. alterniflora did not increase $bSiO_2$ accumulation on the low marsh when stressed by immersion. We expected that across the immersion gradient, plants located on the lower part of the marsh would increase rigidity by accumulating $bSiO_2$ (Schoelynck et al. 2010). However the highest $bSiO_2$ concentration (Fig. 6), NAPP, and dSi uptake rate (Table 2) occurred in plants at the mid marsh station with an intermediate duration of immersion. There was no significant differences in dSi stocks along the sites of the immersion gradient (Table 2) and only a small depletion on the high marsh dSi profile from −4 to −7 cm depth (Fig. 7). There was no significant correlation between plant $bSiO_2$ and tidal elevation (Fig. 8). Although plants on the high marsh are the tallest, they were not the most silicified (Table 2). Could this be a result of the dSi depletion in the high marsh profile even if the dSi stocks were not significantly lower? On the low marsh, plant were not the smallest but produced less and had the smallest uptake rate even if there was not significant differences in dSi profiles or stocks (Fig. 7, Table 2). We suggest that the lower $bSiO_2$ accumulation on the low marsh could be due to a limitation of growth and this did not seem to be only caused by immersion (Fig. 8). Other abiotic factors or nutrient limitations like ammonium, phosphate limitations or sulfite toxicity could reduce plant growth on the low marsh and may indirectly reduce $bSiO_2$ accumulation (Mendelssohn and Morris, 2002).

Conclusion

Increased wave action, salinity, and duration of immersion were expected to promote $bSiO_2$ accumulation in *S. alterniflora* when these factors stressed the plants. This was not apparently the case in this study. However, this does not mean that silica did not help *S. alterniflora* to cope with theses stresses. Laboratory experiments which would grow *S. alterniflora* in a Si depleted medium are needed to solve this matter. We observed a significant variation in *S. alterniflora* $bSiO_2$ concentration along the estuarine salinity gradient and along the immersion gradient (Figs. 5 and 6). The limitation of dSi in marsh sediment did not seem to explain the variation in plant $bSiO_2$ accumulation except from the high marsh (Fig. 7, Table 2). In this fied study, plant length and the calculated dSi uptake were the variables explaining most of the $bSiO_2$ accumulation (Table 2, Fig. 9). There was a clear linear relationship between the length of *S. alterniflora* and its $bSiO_2$ concentration (Fig. 9), in contradiction to the only other study on this topic (Hou et al. 2010). These results suggested that *S. alterniflora* could use a passive mechanism for

Si uptake. A positive relationship between available dSi and plant $bSiO_2$ should be expected. In our field study, growth which was the major factor correlated to plant $bSiO_2$, could have concealed the correlation between available dSi and plant $bSiO_2$. Si uptake mechanisms in *Spartina* are still largely unknown and should be further investigated in laboratory controled experiments. Since growth rate and productivity of *S. alterniflora* increase with decreasing latitude (Dame and Kenny 1986; Mendelssohn and Morris 2002), and hence with increasing temperature, we postulate that $bSiO_2$ accumulation will vary similarly (given the relationship between plant length and silicate concentration) (Fig. 9).

If variations in the $bSiO_2$ concentration of *S. alterniflora* are functions of growth and primary production, these variations may also impact the fate of stored $bSiO_2$. *Spartina alterniflora* is a bioengineer species that helps stabilize marshes by fixing sediment (Gleason et al. 1979), even when it is an invader (Callaway and Josselyn 1992). Moreover, marsh sediments are a known pool of $bSiO_2$ (Norris and Hackney 1999). Phytoliths, such as those in cordgrass, are considered to be both sinks and sources of silica in terrestrial environments (Conley 2002; Derry et al. 2005; Farmer et al. 2005) and in tidal marshes (Struyf et al. 2005; Struyf et al. 2007; Jacobs et al. 2008; Struyf and Conley 2009) as they dissolve more quickly than diatom frustule $bSiO_2$ in sediments (Farmer et al. 2005; Struyf et al. 2007). When *S. alterniflora* invades new habitat, like the Bay of Brest, what net effect will this plant have on the retention and export of silica in tidal marshes? The answer will come from further studies.

Acknowledgement The PhD thesis of J. Querné was funded by Brest Metropole Ocean (B.M.O). This study is part of the project ISICO funded by EC2CO (INSU). The authors would like to thank M. Le Goff (LEMAR) and M. A. Poullaouec (LEBHAM) for their help with the field sampling.

References

Arnon DI, Stout PR (1939) The essentiality of certain elements in minute quantity for plants with special reference to copper. Plant Physiol 14:371–375

Bougault C, Hardegen M, Quéré E (2004) Site Natura 2000 n°46: Rade de Brest, Estuaire de l'Aulne. Inventaire et cartographie des habitats terrestres et des espèces végétales d'intérêt communautaire. 175 p

Bougault C, Hardegen M, Quéré E (2005) Site Natura 2000 N° 24: Rivière Elorn. Inventaire et cartographie des habitats terrestres et des espèces végétales d'intérêt communautaire. 175 p

Brewer PG, Riley JP (1966) The automatic determination of silicate-silicon in natural waters with special reference to sea water. Anal Chim Acta 35:514–519

Callaway J, Josselyn M (1992) The introduction and spread of smooth cordgrass (*Spartina alterniflora*) in South San Francisco Bay. Estuar Coast 15:218–226

Cavalieri AJ (1983) Proline and glycinebetaine accumulation by *Spartina alterniflora* Loisel. in response to NaCl and nitrogen in a controlled environment. Oecologia 57:20–24

Chauvaud L, Jean F, Ragueneau O, Thouzeau G (2000) Long-term variation of the Bay of Brest ecosystem: benthic–pelagic coupling revisited. Mar Ecol Prog Ser 200:35–48

Chelaifa H, Monnier A, Ainouche M (2010) Transcriptomic changes following recent natural hybridization and allopolyploidy in the salt marsh species *Spartina townsendii* and *Spartina anglica* (Poaceae). New Phytol 186:161–174

Conley DJ (2002) Terrestrial ecosystems and the global biogeochemical silica cycle. Glob Biogeochem Cycles 16:1121

Dame RF, Kenny PD (1986) Variability of *Spartina alterniflora* primary production in the euhaline North Inlet estuary. Mar Ecol Prog Ser 32:71–80

Darby FA, Turner RE (2008) Below- and Aboveground *Spartina alterniflora* Production in a Louisiana Salt Marsh. Estuar Coast 31:223–231

De Bakker NVJ, Hemminga MA, Van Soelen J (1999) The relationship between silicon availability, and growth and silicon concentration of the salt marsh halophyte *Spartina anglica*. Plant Soil 215:19–27

Derry LA, Kurtz AC, Ziegler K, Chadwick OA (2005) Biological control of terrestrial silica cycling and export fluxes to watersheds. Nature 433:728–731

Diggelen JV, Rozema J, Dickson DMJ, Broekman R (1986) β-3 Dimethylsulphoniopropionate, proline and quaternary ammonium compounds in Spartina anglica in relation to sodium chloride, nitrogen and sulphur. New Phytol 103:573–586

Epstein E (1994) The anomaly of silicon in plant biology. Proc Nat Aca Sci USA 91:11–17

Epstein E (1999) Silicon. Annu Rev Plant Physio 50:641–664

Epstein E (2009) Silicon: its manifold roles in plants. Ann Appl Biol 155:155–160

Epstein E, Bloom AJ (2005) Mineral nutrition of plants: principles and perspectives. Sinauer, Sunderland

Farmer V, Delbos E, Miller J (2005) The role of phytolith formation and dissolution in controlling concentrations of silica in soil solutions and streams. Geoderma 127:71–79

Gleason ML, Elmer DA, Pien NC, Fisher JS (1979) Effects of stem density upon sediment retention by salt marsh cord grass. Spartina alterniflora Loisel. Estuar Coast 2:271

Hackney CT, Cahoon LB, Preziosi C, Norris A (2002) Silicon is the link between tidal marshes and estuarine fisheries: a new paradigm. In: Weinstein MP, Kreeger DA (eds) Concept and controversies in tidal marsh ecology. Kluwer, Dordrecht, pp 543–552

Haines BL, Dunn EL (1976) Growth and resource allocation responses of *Spartina alterniflora* and *Juncus roemerianus* plant stands in a Georgia salt marsh. Ecology 61:303–312

Hodson MJ, White PJ, Mead A, Broadley MR (2005) Phylogenetic variation in the silicon composition of plants. Ann Bot 96:1027–1046

Hou L, Liu M, Yang Y, Ou D, Lin X, Chen H (2010) Biogenic silica in intertidal marsh plants and associated sediments of the Yangtze Estuary. J Environ Sci 22:374–380

Jacobs S, Struyf E, Maris T, Meire P (2008) Spatiotemporal aspects of silica buffering in restored tidal marshes. Estuar Coast Shelf Sci 80:42–52

Liang Y, Chen Q, Liu Q, Zhang W, Ding R (2003) Exogenous silicon (Si) increases antioxidant enzyme activity and reduces lipid peroxidation in roots of salt-stressed barley (Hordeum vulgare L.). J Plant Physiol 160:1157–1164

Liang Y, Sun W, Zhu Y, Christie P (2007) Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: a review. Environ Pollut 147:422–428

Linthurst RA, Seneca ED (1980) The effects of standing water and drainage potential on the *Spartina alterniflora* substrate complex in a North Carolina salt marsh. Estuar Coast Mar Sci 11:41–52

Linthurst RA, Seneca ED (1981) Aeration, nitrogen and salinity as determinants of *Spartina alterniflora* Loisel. growth response. Estuaries 4:53–63

Ma JF (2004) Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. Soil Sci Plant Nutr 50:11–18

Ma JF, Takahashi E (2002) Soil, fertilizer, and plant silicon research in Japan. Elsevier, Amsterdam

Ma JF, Yamaji N (2006) Silicon uptake and accumulation in higher plants. Trends Plant Sci 11:392–397

Matoh T, Kairusmee P, Takahashi E (1986) Salt induced damage to rice plants and alleviation effect of silicate. Soil Sci Plant Nutr 32:295–304

McNaughton SJ, Tarrants JL (1983) Grass leaf silicification: natural selection for an inducible defense against herbivores. Proc Nat Aca Sci USA 80:790–791

McNaughton SJ, Tarrants JL, McNaughton MM, Davis V (1985) Silica as a defense against herbivory and a growth promotor in African grasses. Ecology 66:528–535

Mendelssohn IA, Morris JT (2002) Eco-physiological controls on the productivity of *Spartina alterniflora* Loisel. In: Weinstein MP, Kreeger DA (eds) Concept and controversies in tidal marsh ecology. Kluwer, Dordrecht, pp 59–80

Motomura H, Fujii T, Suzuki M (2004) Silica Deposition in Relation to Ageing of Leaf Tissues in Sasa veitchii (Carriere) Rehder (Poaceae: Bambusoideae). Ann Bot 93:235–248

Norris AR, Hackney CT (1999) Silica content of a mesohaline tidal marsh in North Carolina. Estuar Coast Shelf Sci 49:597–605

Piperno DR (1988) Phytolith analysis: an archaeological and geological perspective. Academic, New York

Piperno DR (2006) Phytoliths: a comprehensive guide for archaeologists and paleoecologists. Altamira, Lanham

Pondaven P, Gallinari M, Chollet S, Bucciarelli E, Sarthou G, Schultes S, Jean F (2007) Grazing-induced changes in cell wall silicification in a marine diatom. Protist 158:21–28

Quéré E, Magnanon S, Annézo N (2010) Vingt ans de suivi et de conservation du *Limmonium humile* Miller en rade de Brest. ERICA 23:101–121

Querné J, Poupart N, Legoff M, Chapalain G, Ragueneau O (in revision) Variations of growth and primary production of invading *Spartina alterniflora* along tidal marshes of a semi-enclosed European bay. Aquat Bot

Raven JA (2003) Cycling silicon: the role of accumulation in plants. New Phytol 158:419–421

Roland RM, Douglass SL (2005) Estimating wave tolerance of *Spartina alterniflora* in coastal alabama. J Coast Res 21:453–463

Saccone L, Conley DJ, Sauer D (2006) Methodologies for amorphous silica analysis. J Geochem Explor 88:235–238

Schoelynck J, Bal K, Backx H, Okruszko T, Meire P, Struyf E (2010) Silica uptake in aquatic and wetland macrophytes: a strategic choice between silica, lignin and cellulose? New Phytol 186:385–391

Sleimi N, Abdelly C (2004) Salt-tolerance strategy of two halophyte species: *Spartina alterniflora* and *Suaeda fruticosa*. In: Lieth H, Mochtchenko M (eds) Cash crop halophytes recent studies – Ten years after Al Ain meeting. Kluwer, Dordrecht, pp 79–85

Smalley AE (1959) The role of two invertebrate populations, Littorina irrorate and Orchelimum fidicinium, in the energy flow of a salt marsh ecosystem. PhD thesis, University of Georgia, Athens

Smetacek V, Assmy P, Henjes J (2004) The role of grazing in structuring Southern Ocean Pelagic ecosystems and biogeochemical cycles. Antar Sci 16:541–558

Sparfel L, Fichaut B, Suanez S (2005) Progression de la Spartine (*Spartina alterniflora* Loisel) en Rade de Brest (Finistère) entre 1952 et 2004: de la mesure à la réponse gestionnaire. Norois 196:109–123

Street-Perrott FA, Barker PA (2008) Biogenic silica: a neglected component of the coupled global continental biogeochemical cycles of carbon and silicon. Earth Surf Proc Land 33:1436–1457

Struyf E, Conley DJ (2009) Silica, an essential nutrient in wetland biogeochemistry. Front Ecol Environ 7:88–94

Struyf E, Van Damme S, Gribsholt B, Middelburg JJ, Meire P (2005) Biogenic silica in tidal freshwater marsh sediments and vegetation (Schelde estuary, Belgium). Mar Ecol Prog Ser 303:51–60

Struyf E, Van Damme S, Gribsholt B, Bal K, Beauchard O, Middelburg JJ, Meire P (2007) *Phragmites australis* and silica cycling in tidal wetlands. Aquat Bot 87:134–140

Sun L, Wu LH, Ding TP, Tian SH (2008) Silicon isotope fractionation in rice plants, an experimental study in rice growth under hydroponic conditions. Plant Soil 304:291–300

Swales A, MacDonald I, Green M (2004) Influence of wave and sediment dynamics on cordgrass (*Spartina anglica*) growth and sediment accumulation on an exposed intertidal flat. Estuar Coast 27:225–243

Tesson Y, Quéré E, Magnanon S (1997) Suivi des populations de Limonium humile en rade de Brest (Rapport final 1997). Rapport Conservatoire Botanique National de Brest pour la Communauté Urbaine de Brest et le Conseil Général du Finistère