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Abstract

Silicic acid uptake kinetics for a field population of benthic diatoms were examined using a radioisotope tracer over a concentration range of 0-300 μmol L^{-1}. The microphytobenthos half saturation constant (54 μmol L^{-1}) and specific uptake rates (0.096 h^{-1}) for silicic acid were well above those usually found in the pelagic environment. Silicic acid kinetics were hyperbolic only at low concentrations (< 60 μmol L^{-1}). At higher concentrations, a second mechanism that did not suggest saturation was activated. Many benthic diatoms are motile and migrate vertically in the upper few centimeters of sediment where there are strong gradients of nutrient concentrations. The multiphasic uptake may allow them to take maximum advantage of the high silicic acid concentrations found at depth.

Introduction

Benthic diatoms constitute the major taxa of the microphytobenthic biomass in most marine sedimentary environments (Cahoon 1999). They colonize intertidal and subtidal areas in all latitudes and can contribute significantly to primary production in coastal zones (Mac Intyre et al. 1996; Cahoon 1999). By forming dense microbial mats at the sediment-water interface, benthic diatoms also alter the properties of sediments (Ziervogel and Forster 2006), and play a key role in controlling nutrient fluxes across the sediment-water interface (Sigmon and Cahoon 1997) with important implications for the functioning of the pelagic ecosystem. For example, due to their direct uptake of dissolved silica (DSi) at the sediment-water interface, benthic diatoms can greatly influence DSi released to the water column (Sigmon and Cahoon 1997; Srithongouthai et al. 2003) and play a important role in the dominance of specific pelagic phytoplankton groups (Conley 1993). Indeed, when DSi becomes limiting, a shift from diatoms to non-siliceous algae can occur, which may increase the likelihood of harmful algal blooms and have dramatic consequences for the transfer of carbon to higher trophic levels.

To our knowledge, the dynamic of DSi uptake by these benthic microalgal communities remains unquantified. The purpose of this study was thus to examine the silicic acid uptake kinetics for a field population of benthic diatoms.

Methods

Extraction of microphytobenthos

Sediment cores were collected by scuba divers during September 2005, at the Saint Anne site (48° 21’ 610’’N, 4° 33’ 000’’W), in the Bay of Brest (France). At the site, the tidally controlled water depth varies between ca. 4 and 11 meters, and the median grain size of

the muddy sediments is 100 μm. Seawater temperature on the day of sampling was 16.4 (± 0.1) °C.

To obtain enough material to perform the experiment in triplicate and concentrate the microphytobenthos, the surface (1 cm) of 6 undisturbed cores (9 cm diameter) were sectioned and transferred to a beaker containing filtered sea water. Because diatoms are less dense than most mineral grains (Klein and Hurlbut 1985), they may be separated from the sediment by differential settling. Accordingly, the sample suspension was thus left aside to allow particles to settle. After a short period of time (5-10 min), the supernatant with suspended diatoms was transferred to a carboy containing 8 L of filtered seawater at low DSi concentration, suitable for DSi uptake kinetic experiments.

Determination of silicic acid uptake kinetics

In order to prevent nitrate and phosphate depletion during the incubation, concentrations in the carboy were increased by adding $NaNO_3$ and Na_2HPO_4 to 50 and 1.5 μmol L^{-1}, respectively. Aliquots of the sample suspension were then dispensed into 3 series of 10 polycarbonate bottles (150 mL). Additional subsamples were set aside for triplicate analysis of the major inorganic nutrients (phosphate, nitrate, and ammonium), biogenic silica (BSi), particulate organic carbon (POC) and nitrogen (PON), chlorophyll *a* (Chl *a*), phaeopigments, cell counts, and species determinations. Increasing amounts of DSi, as sodium metasilicate, were then added to the bottles resulting in 3 series of flasks with DSi concentrations ranging from 0 to 300 μmol L^{-1}.

After mixing and prior to the addition of the radioisotope ^{32}Si, 10 mL of the sample were taken and filtered for DSi determination. The same amount of ^{32}Si labelled- $Si(OH)_4$ (in Na_2SiO_3 solution, Los Alamos National Laboratory), was added to each bottle (4.17 Becquerels mL^{-1}), corresponding to an increase in DSi concentration of 0.02 μmol L^{-1} at the most. Bottles were then hung in situ on a mooring, just above the sea floor. The

photosynthetically available radiation (PAR) at that depth was estimated indirectly from surface measurements (MAREL Buoy, Observatoire du Domaine Côtier, IUEM) and light extinction coefficients measured at the site (J. Grall unpubl. data). PAR varied from 100 to 150 μmol quanta m^{-2} sec^{-1} during the incubation. After a 4 hour period, the bottles were returned to the laboratory and cells were collected immediately by filtration through 0.6 μm Nuclepore membranes. Each filter was then rinsed with filtered seawater, and placed in a 20-mL plastic scintillation vial. Hydrofluoric acid (2 mL of 2.9 N HF) was added to each sample to dissolve the BSi. One hour later, 10 mL of the scintillation cocktail Ultima Gold XR was added. ^{32}Si incorporation was measured using a scintillation counter, as described by Leynaert et al. (1996). The DSi uptake rate was determined for each sample and normalized to the BSi concentration present at the beginning of the incubation, to calculate the specific uptake rate (V).

Particulate matter analysis

BSi, POC, PON, Chl *a*, and phaeopigments were determined in triplicate on particulate matter collected by filtration of 100 mL of sample out of the carboy. BSi analysis was performed using the alkaline digestion method (Ragueneau and Tréguer 1994). Concentrations of Chl *a* and phaeopigments were estimated using the method of Lorenzen (1966) and a KONTRON fluorometer (Kontron Instruments AG). Material for POC and PON measurements was analyzed in a Thermoelectron elemental analyzer (Flash EA). Samples for phytoplankton cell counts and determination were fixed with Lugol's solution and counted using the method of Utermöhl (1958).

Determination of silicic acid concentration in the sediment

Sediment cores were taken in triplicate in May and October. Immediately subsequent to sampling, the water at the surface of the sediment was collected for the determination of the

DSi concentration at the water-sediment interface. The sediment was then vertically sectioned into 0.5 cm intervals (0-1 cm), and 1 cm intervals (1-3 cm), down to 3 cm. The sectioned mud was then centrifuged (15 minutes - 3500 rpm, in a refrigerated centrifuge), and the extracted water was filtered through a 0.2 μm Nuclepore filter and analyzed for DSi, using standard colorimetric techniques (Tréguer and Le Corre 1975).

Results

Characteristics of the sample biomass

The microscopic examination of the microphytobenthos revealed that when the number of cells per liter is considered, *Bacillaria paxilifer* was the dominant species (600 cells L^{-1}) along with *Amphora* sp. and *Cylindrotheca closterium*. *Nitzschia sigma*, *Pleurosigma* cf. *angulata* and *Pleurosigma* cf. *strigosum* were also present at concentrations higher than 100 cells L^{-1} (Fig. 1). However, an estimate of the cells biovolume, from microscopically measured linear dimensions of each species, revealed that *Gyrosigma* cf. *balticum* and *Pleurosigma* cf *strigosum*, because of their large size (1.8 x 10^5 μm^3 and 4.3 x 10^4 μm^3, respectively), greatly dominated the overall biomass (Fig. 2). As these two species are raphid diatoms, they are principally epipelic, i.e., they live freely and are capable of moving vertically within the upper layers of the sediment, in synchronization with day–night and tidal cycles (Round 1971). Only benthic diatom species were observed in these samples.

Biomass characteristics are reported in Table 1. Detritus and empty frustules were abundant in the sample. However, we found a low phaeopigment content as compared to Chl *a*, and the C:Chl *a* ratio (52) is in the range of ratios reported for microphytobenthos (de Jonge 1980). Together, this information indicates that a great proportion of the material was alive.

Silicic acid concentrations in the sediment

Pore water profiles of DSi are presented in Fig. 3. Both profiles, performed at two different seasons (in spring and at the end of the summer) are very similar. They suggest a steep increase in concentrations until a nutrient maximum is reached, around 150 μmol L^{-1}, between 1 and 2 cm below the interface. DSi concentrations of interstitial waters from the 0-0.5 cm layer of the sediment (91 and 103 μmol L^{-1}) were more than one order of magnitude higher than in the overlying water (2.7 and 8.2 μmol L^{-1}) in the two cores sampled.

We note that overall DSi pore water concentrations in October were lower than in May. . However, due to the paucity of data and the high variance in concentration, a seasonal trend can not be determined. Although pore water profiles are from May and October, and the experiments were conducted in September, we feel the range of DSi concentrations in the upper 1 cm of the sediment column is representative of the concentrations that benthic diatoms encounter during vertical migration.

Kinetics of silicic acid uptake

Specific uptake rates (V, h^{-1}) are plotted against the ambient DSi concentrations (Fig. 4A). At low concentrations, the data seem to follow a rectangular hyperbola, similar to the Michaelis-Menten equation for enzyme kinetics. However, at high concentrations, the data do not suggest uptake saturation, but rather a linear dependence on the DSi concentrations.

As the kinetic profiles suggest a discontinuity, we calculated a half-saturation constant (K_s) and a specific uptake rate (V_{max}) for DSi uptake at low concentrations only. The best fit to the Michaelis-Menten function is obtained when considering the range of data from 0 to 60 μmol L^{-1}, which gives a K_s of 54 μmol L^{-1} and a V_{max} of 0.096 h^{-1}. As the second part of the curve does not show any sign of saturation at higher concentrations, it has been analysed as a linear component, excluding the Michaelis-Menten portion. Thus, for DSi concentrations above 60 μmol L^{-1}:

$$V\ (h^{-1}) = 0.0049 * S\ (\mu mol\ L^{-1}) - 0.2806 \quad (r^2 = 0.88) \qquad (1)$$

Closer examination of the curve (Fig. 4B) shows that the linear term does not overlap with the lower range of data, and thus does not affect the estimate of the Michaelis-Menten kinetics at low concentrations.

Potential experimental artifacts

Kinetic experiments for DSi uptake are seldom performed in triplicate because of the high cost of the ^{32}Si isotope. The good fit and very low scatter in the data provide great confidence in the results.

We recognize that our experimental conditions are designed to exclude particles. However, the method of microphytobenthos extraction results in the presence of empty frustules as well as some clay contamination of the sample and could lead to experimental artifacts due to passive adsorption of the label, particularly when exposed to enriched DSi concentrations. Nonetheless, previous studies in the Danube estuary have shown, using $HgCl_2$ killed controls, that there is little likelihood of an artefact related to ^{32}Si adsorption in turbid waters (Ragueneau et al. 2002). Moreover, studies on DSi adsorption processes carried out on sediment of similar characteristics (Gehlen and Van Raaphorst 2002) reported a maximal rate of 1250 μmol Si adsorbed per kg of dry sediment at DSi concentrations of 250 $\mu mol\ L^{-1}$. Considering a maximum of 100 mg of sediment per liter in our sample, we calculate that 0.125 $\mu mol\ Si\ L^{-1}$ could be adsorbed, i.e., two orders of magnitude lower than the uptake rates measured in the kinetic experiment for the same range of DSi concentrations. Thus, it is very unlikely that the adsorption process, if it occurred, affected the measured uptake rate.

DISCUSSION

Silicic acid uptake by microphytobenthos: a multiphasic mechanism

It has long been recognized that DSi uptake by diatoms in the pelagic environment is carrier mediated and tends to conform to Michaelis-Menten hyperbolic saturation function (Paasche 1973). Our results are the first data reporting a half saturation constant and maximum specific uptake rate of DSi for a natural benthic diatom community. The data show that the DSi uptake kinetics follows a hyperbolic pattern at low concentrations only (< 60 μmol L^{-1}). At higher silicate concentrations, the influence of another mechanism becomes apparent (Fig. 4A).

Although these are the result of a single experiment, they suggest the possibility of multi-phase DSi uptake by benthic diatoms. Indeed, despite extensive studies, there is still no consensus about the number and nature of uptake mechanisms in plants (Nissen 1989). For phytoplankton, Michaelis-Menten type saturable kinetics explain the movement of molecules across the cell membranes. However, in higher plants, two types of transfer have been described: the first (carrier mediated transport plus simple diffusion) involves the contribution of a hyperbolic saturation transfer (with a carrier) plus simple linear diffusion (non-carrier-mediated). In the second, two different carriers, with different kinetic constants are involved (Chrispeels et al. 1999). Among these different types of nutrient transfer, the transport of DSi for benthic diatoms could be explained by the sequential induction of two uptake systems: a carrier mediated transport following a hyperbolic saturation function at "low" concentrations (< 60 μmol L^{-1}), and a linear term at higher concentrations, whose nature remains to be defined.

- A hyperbolic saturation transfer at "low" concentrations (< 60 μmol L^{-1}) suggests exceptionally high K_s and V_{max}

In this study, in the range of concentrations considered to fit reasonably well with Michaelis-Menten saturation functions (0 - 60 μmol L^{-1}), the calculated K_s is 54 (± 6) μmol L^{-1}

and V_{max} is 0.096 (± 0.020) h^{-1}. As no data are available for benthic diatoms, we can compare our results to values obtained for pelagic diatom assemblages from different environments. Such data were summarized by Claquin et al. (2006) where overall means for K_s and V_{max} were 2.48 (± 1.93) μmol L^{-1} and 0.026 (± 0.60) h^{-1}, respectively. Our calculated values are thus in the higher range of kinetic data reported for pelagic diatoms.

Particularly, the value of K_s is at least one order of magnitude above those found in pelagic environments suggesting a very low affinity of the benthic diatom for DSi. In some exceptional situations, extremely high K_s values also have been reported for natural pelagic diatom communities (Brzezinski et al. 1998; Nelson et al. 2001). However, and as stated by the authors, these values were marked with large uncertainties because the relationship between the specific uptake rate and DSi only poorly fitted the Michaelis-Menten hyperbola.

The V_{max} we calculated for benthic diatoms also stands above those typically reported for pelagic communities. It is 3 to 4 times higher than the average reported by Claquin et al. (2006), despite the presence of detrital biogenic silica (empty frustules, shell fragments, dead cells, etc.). This is a recurring problem when studying natural diatom communities (whether pelagic or benthic), because viability is not easily determined. Thus, it is possible that the V_{max} for the living diatoms could be even higher than the calculated one.

- The linear component

The linear term is usually considered to represent passive diffusion in higher plants (Nissen 1989). However, diatoms maintain supersaturated DSi concentrations inside the cell (ranging between 19 to 340 mmol L^{-1}) (Binder and Chisholm 1980). This means that if the intracellular free pool of DSi can reach millimolar concentrations, passive diffusion would occur against a concentration gradient. However, the soluble form of Si present in the free pool is still ambiguous. Several studies have suggested the existence of organosilicon

compounds which form a water-insoluble Si pool and would allow for an inward concentration gradient (Sullivan 1979). This is consistent with Thamatrakoln and Hildebrand's (2008) model for uptake in which they suggest that at high DSi, uptake would not be carrier mediated but would rather occur by diffusion.

Another explanation is active carrier-mediated transport of DSi at high concentrations. Based on the likelihood that any carrier-mediated transport should eventually saturate, what we observed as linear uptake would be only the linear portion of a saturating kinetic curve for a very high K_s (low affinity) transporter. This is consistent with the fact that different Si-transporters (SIT) may co-exist within individual cells (Hildebrand et al. 1998). Since each transporter has a different affinity or capacity for transport, one mode of regulating the uptake of DSi by the diatoms would be by controlling the amount or location of these different types of SITs. This is also in agreement with results from chemostat studies which indicate that 3 distinct modes of DSi uptake can be distinguished in some diatom species (Conway et al. 1976), depending on the intra and extra cellular Si levels. Del Amo and Brzezinski (1999), in reference to the unusual DSi uptake kinetic of the pelagic diatom *P. tricornutum*, also suggested the possibility of two different transport systems. Recently, Thamatrakoln and Hildebrand (2007) suggested that SIT activity could be internally controlled by the rate of silica incorporation. This calls into question the relationship between uptake and silica deposition. Further studies should be design to examine the evolution of the efflux to influx ratio as a function of DSi concentrations.

The shape of the kinetic curve and the potential advantages it conveys

Many benthic diatoms are motile and migrate vertically in the sediment in a close relationship with the diel cycle in subtidal areas (Ní Longphuirt et al. 2006), and the daytime and emersion periods in intertidal zones (Serôdio et al. 1997). In muddy sediments, the range of vertical migrations can reach centimeters (Saburova and Polikarpov 2003). It is in these

first centimeters of sediment that the gradient concentration of DSi is the steepest. What is crucial is that over a migration cycle, benthic diatoms may experience changes in DSi concentrations from a few micromolar at the sediment-water interface up to more than 100 μmol L^{-1} in the first cm of sediment (Fig. 3). Other studies have reported concentrations even higher, exceeding 300 μmol L^{-1} in the first cm of sediment (Sundbäck 1991).

This environment differs from the situation encountered by pelagic diatoms. For example, DSi concentrations in the water column overlying the site of sampling in the Bay of Brest range from depletion in spring, to a maximum of about 15 μmol L^{-1} in winter (data from Somlit-Brest, Coastal time-series station). Thus whereas pelagic diatoms experience an order of magnitude difference in the DSi concentration throughout the year, benthic diatoms will undergo variations in DSi concentrations sometimes greater than two order of magnitude, over a 24-hour period, through the process of migration from the sediment surface down to a depth of one cm. Therefore, for benthic diatoms, the variation in concentration is more pronounced, and the frequency at which these variations occur is also much higher.

In such an environment, the multiphasic mechanism for uptake gives benthic diatoms the possibility to take maximum advantage of the high DSi concentration found deeper in the sediment. Consequently, this suggests that microphytobenthos would reliably take up silicic acid from sediment, rather than from the water column because they are much less effective in their ability to collect and transport substrate at low surface silicic acid concentrations. However, we must acknowledge that the uptake experiment was conducted in light and thus did not simulate the conditions where the linear uptake with higher concentrations would be occurring in the field (in the dark and often anoxic subsurface sediment). The direct availability of light is not necessary for DSi uptake as silicon metabolism is not directly connected to photosynthesis (Brzezinski 1992). But the availability of oxygen might be of a concern as energy for silicification is provided by aerobic respiration (Lewin 1955).

Saburova and Polikarpov (2003) also suggested that diatoms were more likely to assimilate DSi in subsurface sediments. They found a higher percentage of cells in G2 and mitosis stages of the cell cycle below the surface, which are the stages of the cell cycle that are more specifically coupled to the DSi assimilation by diatom species (Claquin and Martin-Jézéquel 2005). Conversely, others (Sundback 1991; Sigmond and Cahoon, 1997) have shown benthic DSi fluxes to decrease, and even to reverse, during periods of illumination, suggesting the influence of microphytobenthos on DSi is strongest at midday, i.e., when cells are concentrated at the surface of the sediment. Thus, whether diatoms utilize DSi when they are concentrated at the surface during day light, or from sediment pore water, or both, is still unclear but is an interesting area for future research.

This study provides first evidence for multiphasic uptake of DSi by benthic diatoms. We can not rule out that pelagic diatoms subjected to high DSi concentrations would not demonstrate the same transport mechanism. It may call into question the concept of nutrient limitation, as the activation of a second uptake system at high concentrations could lead to unsaturable DSi uptake. However, we are aware of the limitations of the data set and that this is the result of a single experiment. Follow up studies are needed to better understand the physiological ecology of benthic diatoms in relation to the strong gradients in physical or chemical microenvironment in surface sediment. These include further work on the evolution of the efflux to influx ratio in diatoms as a function of DSi concentrations, on how multiphasic uptake of silicic acid (and other nutrients) relates to vertical migration by the diatoms, oxygen and light conditions in the subsurface sediments.

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Tables

Table 1: Biomass characteristics of the microphytobenthos sample

	POC (μmol L^{-1})	PON (μmol L^{-1})	BSi (μmol L^{-1})	Chlorophyll *a* (μg L^{-1})	Phaeopigments (μg L^{-1})
concentration	15.14	0.96	2.64	3.49	0.41
SD (n = 3)	0.4	0.10	0.23	0.89	0.60

Figure captions

Figure 1: (A) *Gyrosigma* cf. *balticum* and (B) *Pleurosigma* cf. *strigosum* greatly dominated the overall biomass because of their large size (1.8 x 10^5 μm^3 and 4.3 x 10^4 μm^3, respectively).

Figure 2: Cell abundance (cells L^{-1}) and volume (μm^3 L^{-1}) of the main species present in the microphytobenthos extracted from the sediment.

Figure 3: Pore water profiles of silicic acid.

Figure 4: (A) Specific silicic acid uptake rates (V, h^{-1}) plotted against the ambient silicic acid concentration (μmol L^{-1}). At low concentrations (0 - 60 μmol L^{-1}), the data are fitted to the Michaelis-Menten equation for enzyme kinetics, and to a linear function at higher concentrations. (B) Details of the data analysis showing that the linear part of the curve does not overlap with the lower range of data.

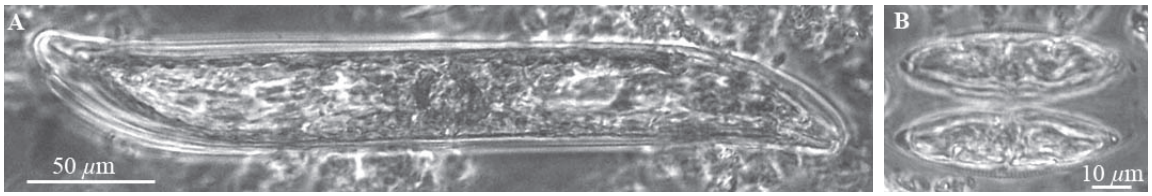

Fig. 1

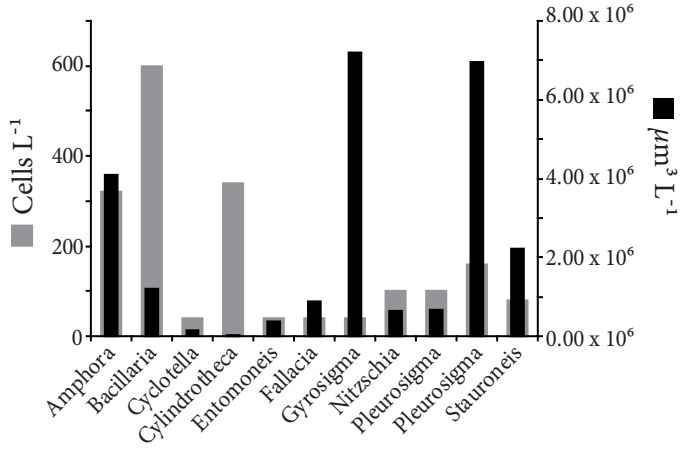
Cells L-1
µm3 L-1
0
200
400
600
0.00 x 10⁶
2.00 x 10⁶
4.00 x 10⁶
6.00 x 10⁶
8.00 x 10⁶
Amphora
Bacillaria
Cyclotella
Cylindrotheca
Entomoneis
Fallacia
Gyrosigma
Nitzschia
Pleurosigma
Pleurosigma
Stauroneis

Fig. 2

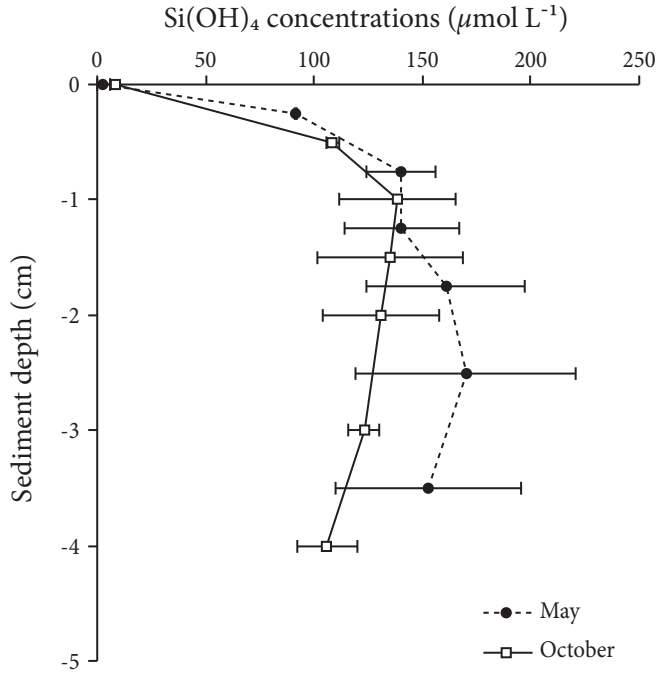
Si(OH)4 concentrations (μmol L-1)
0
50
100
150
200
250
0
-1
-2
-3
-4
-5
Sediment depth (cm)
May
October

Fig. 3

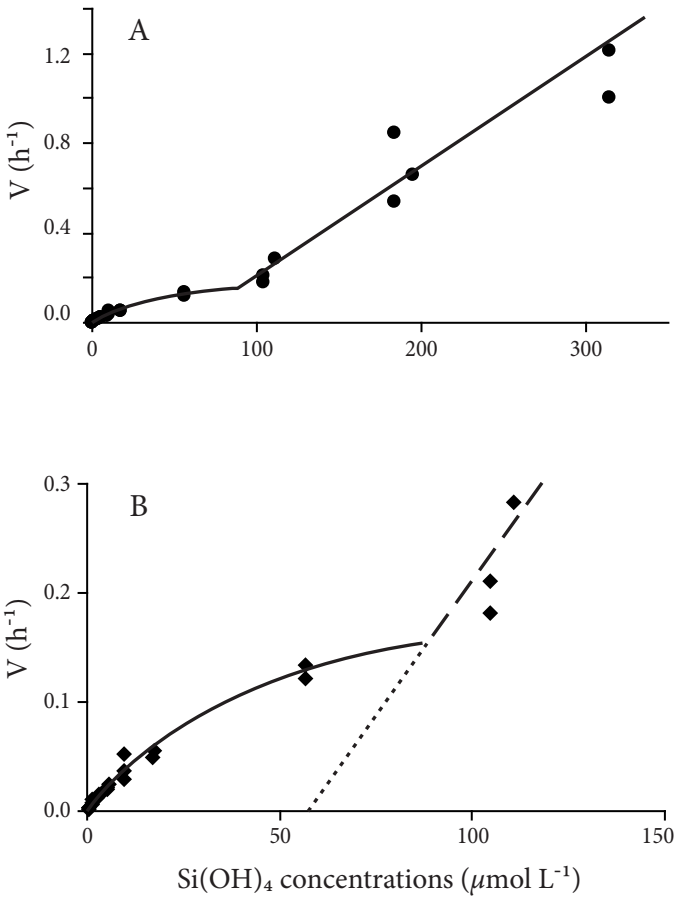
A
1.2
0.8
0.4
0.0
V (h-1)
0
100
200
300
B
0.3
0.2
0.1
0.0
V (h-1)
0
50
100
150
Si(OH)4 concentrations (μmol L-1)

Fig. 4