Eutrophication alters Si cycling and litter decomposition in wetlands

Original research article

Eutrophication alters Si cycling and litter decomposition in wetlands

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Abstract

Anthropogenic eutrophication of wetlands may have a significant impact on the global biogeochemical silicon (Si) cycle, as Si filtering by wetland vegetation codetermines fluxes of Si towards the oceans. We experimentally investigated how macronutrient (NPK) enrichment alters total Si storage and Si stoichiometry in litter from six wetland species of *Carex*, which we related to other parameters of litter quality and litter decomposition rates. Nutrient enrichment stimulated primary biomass production, which resulted in an increased total Si storage in plants. However, this eutrophication-induced stimulatory effect on Si fixation in plant biomass was counterbalanced by consistently lower (up to 50% reduction) litter Si concentrations in all species, suggesting a plant-physiological response following the relief of nutrient stress. Moreover, competitive species (typical for eutrophic conditions) tended to accumulate less Si (per g DM) than slow-growing species (typical for nutrient-poor conditions). Finally, a negative correlation between litter Si concentrations and litter decomposition rates in nutrient-poor environments suggested an inhibiting effect of Si on decomposition. However, negative correlations between litter Si concentrations and litter macronutrient concentrations as well as positive correlations between litter Si concentrations and C:N and lignin:N ratios indicated a strong interdependence of Si with other litter quality parameters that determine decomposition. We conclude that stimulatory effects of eutrophication on total Si storage in wetland vegetation (following an increase in biomass production) need to be balanced with the plant-physiological response of lower tissue Si concentrations. We argue that rates of Si cycling are likely to be altered through shifts in litter quality and decomposition rates.

Introduction

Wetlands have a significant impact on the global biogeochemical silicon (Si) cycle (Struyf & Conley 2009). Wetland plants take up dissolved silicon (DSi) from the soil solution, which accumulates as biogenic silica (BSi) in the plants' tissue in concentrations that often exceed > 1% dry weight (Schoelynck et al. 2010). Once fixed in plant material (mainly as phytoliths), Si is only gradually released back into the abiotic environment after vegetation dieback and subsequent litter decay or herbivory (Struyf & Conley 2009; Vandevenne et al. 2013). In wetland areas, such Si filtering by the vegetation affects the rate of Si export towards open water and eventually oceans (Borrelli et al. 2012; Struyf & Conley 2012). For example, more than 40 % of DSi export from a tidal marsh has been attributed to the decomposition of reed (*Phragmites australis*) (Struyf et al. 2007).

The Si cycle is largely governed by natural biogeochemical processes, but anthropogenic activities can significantly alter this cycle. For instance, deforestation (Conley et al. 2008), agricultural harvest (Vandevenne et al. 2012), or dam building (Maavara et al. 2014) have all been shown to affect rates of terrestrial Si export towards oceans. Currently, one of the main global anthropogenic shifts in wetlands is NPK-eutrophication due to an intensified use of fertilizers (Conley et al. 2009; Khan & Ansari 2005). The impact of eutrophication on Si cycling has remained uninvestigated, although it may interact with Si cycling through at least three nonexclusive mechanisms. First, eutrophication induces a successional shift towards competitive fast-growing plant species (Hautier et al. 2009), and primary plant production increases as growth is no longer limited by macronutrient shortages (Smith et al. 1999). Such shift in total biomass production could potentially lead to a concomitant increase in total Si fixation by the vegetation (Schoelynck et al. 2014). In other words, a larger biomass stock can theoretically take up and store more Si (*ceteris paribus*). Second, the elimination of an important plant stressor, i.e. nutrient limitation, may affect internal plant stoichiometry. Evidence is emerging that the magnitude of Si accumulation in individual plants is adaptable depending on local abiotic conditions (Carey & Fulweiler 2014; Schoelynck & Struyf 2016). Although plants grown under stressful conditions have been shown to store relatively more BSi (per g dry mass) in their tissue (Schoelynck et al. 2015; Schoelynck & Struyf 2016), which enhances their physical strength and recalcitrance (Ma & Yamaji 2006; Meharg & Meharg 2015), opposite patterns have been observed as well (Cooke & Leishman 2016). Finally, if eutrophication indeed triggers shifts in Si fixation by plants as suggested, then this may affect litter decomposition rates and rates of nutrient cycling as well.

To date, the few studies in which BSi concentrations in plants have been linked to litter decomposition rates report contrasting results (Cornelissen & Thompson 1997; Schaller & Struyf 2013). This may be due to inconclusive interactions between litter BSi concentrations and other parameters of litter quality (lignin, phenols, C:N ratio) (Cooke & Leishman 2012; Schaller et al. 2012; Schoelynck et al. 2010). The effects of litter BSi concentrations on decomposition rates thus remain unclear.

In the present study, we address the previously mentioned knowledge gaps by investigating how macronutrient (NPK) enrichment alters total Si storage and nutrient stoichiometry in wetland plants, and we relate litter BSi concentrations to other parameters of litter quality. Additionally, we link litter BSi concentrations to rates of litter decomposition. We show that ongoing wetland eutrophication will alter mechanisms of Si uptake and retention.

Materials and methods

Species selection and classification

We selected six sedge species of the *Carex* genus. Carices form a diverse and abundant plant group in wetlands, and most species store large quantities of Si in their tissue (Opdekamp et al. 2012; Struyf et al. 2010). The selected species are typically found in wetlands of the Northern hemisphere and naturally occupy different parts of a fertility gradient. Based on the species' revised Ellenberg values for nitrogen (Hill et al. 1999), we order *Carex paniculata* L. (6) > *C. appropinquata* Schumacher (4) > *C. diandra* Schrank (3) > *C. lepidocarpa* Tausch (2) = *C. nigra* (L.) Reichard (2) = *C. echinata* Murray (2) (from eutrophic to oligotrophic conditions). The low-competitive (= low Ellenberg-N) sedges have their natural optimum in nutrient-poor environments (Kotowski et al. 2006), whereas competitive (= high Ellenberg-N) species thrive in more eutrophic environments.

Germination and transplantation

Seeds were germinated on moist filter paper (24-15°C day-night regime, 12/12h photoperiod) in spring 2013. Next, seedlings were temporarily grown on a mixture of universal potting soil and white sand to allow an optimal initial growth (to an approximate height of 10 cm). After seven weeks, 180 seedlings (30 individuals of each species) were removed from the pots, rinsed, and transplanted into experimental mesocosms.

Experimental design

30 seedlings of each of the 6 species were divided (in monocultures) over 60 experimental mesocosms (V = 5 L), with 3 seedlings per replicate mesocosm. Half of the mesocosms received a nutrient-rich treatment while the other half was kept nutrient-poor (setup = 2 nutrient levels x 6 species (3 individuals per mesocosm) x 5 replicate mesocosms). Mesocosms were installed in an unheated greenhouse in full daylight and had a 2 cm layer of river gravel at the bottom, covered by clean white sand. 5 multi-channel peristaltic pumps (Masterflex 7521-57, each with 12 connections attached to light-sealed water reservoirs containing tap water) were connected to the mesocosms. Water from the reservoirs was pumped in at the bottom of each mesocosm at a rate of 0.18 L d^{-1} to guarantee a constant supply of essential base cations, trace elements, and minimal amounts of nutrients (tap water characteristics in Table S1). This setup allowed for a continuous and equal inflow of DSi into each mesocosm of 0.6 mg d^{-1}. A water outlet was placed at the top of the mesocosms to allow runoff of excess water.

Extra macro-nutrients (NPK) were injected biweekly directly into the mesocosm inlets, using a 2 mL syringe, as dissolved KNO_3 (N and K) and KH_2PO_4 (P and K) from stock solutions so that total amounts of N, P and K allocated to each mesocosm at the end of the growing season (n = 119 days) equaled 363.1, 21.1 and 959.8 mg respectively for the nutrient-rich treatment and 58.8, 3.8 and 88.6 mg respectively for the nutrient-poor treatment. For N and P, this corresponds with an approximate 6x increase in input (from nutrient-poor to nutrient-rich), whereas input of K (11x increase) was the cumulative result of using both KNO_3 and KH_2PO_4 as fertilizer. In our study,

"nutrient-poor" implies conditions of severe macro-nutrient limitation whereas "nutrient-rich" implies a non-limiting macro-nutrient availability (for the selected species). In other words, the nutrient-poor treatment more closely resembles the natural range of the low Ellenberg-N species (e.g. *C. echinata* or *C. lepidocarpa*), whereas the nutrient-rich treatment more closely resembles the natural range of the higher Ellenberg-N species (e.g. *C. paniculata* or *C. appropinquata*). Every two weeks, the mesocosms were spatially randomized. Pore water DSi concentrations averaged 1.6 ± 0.14 (= SE) mg L^{-1} and did not differ between nutrient treatments (Wilcoxon rank sum test, W = 7, p = 0.31).

SLA measurements and plant harvest

Besides a species' Ellenberg-N value, Specific Leaf Area (SLA) can also be used as a proxy for plant-growth strategy: fast-growing, competitive species (typical for eutrophic systems) tend to have higher SLA-indices than slow-growing, stress-tolerant species (typical for nutrient-limited systems) (Lambers & Poorter 1992; Westoby 1998). To determine SLA, we randomly collected one fresh, fully expanded and illuminated leaf per plant in the middle of the day and cut out a 5-6 cm fragment from the middle of the leaf. We immediately calculated the surface area by combining digital photography with image-processing software ImageJ (Schneider et al. 2012), and fragments were oven-dried (70°C) for 24h and weighed. SLA was calculated as leaf area divided by dry mass ($cm^2\ g^{-1}$). We used mesocosm averages of SLA (= 3 sub-replicates) for further data analysis. From October onwards, senesced leaves (= litter) were collected every week, rinsed, air-dried, and stored. At the end of the growing season (November 2013, n = 119 days), mesocosms were placed in a dark cooling room (~4°C) to simulate winter conditions and to initiate rapid leaf senescing. Mid-January 2014, all sedges were removed from the cooling room and aboveground (senesced) biomass was harvested and rinsed. Per mesocosm, litter from the final harvest was mixed with the senesced leaves that had been collected in the weeks before. Next, all biomass was dried at 45°C and weighed (g). We used this 45°C-dried rather than 70°C-dried material in the decomposition experiments as this more closely resembles natural material for decomposition. Subsamples (1.4-1.9 g) of all 45°C-dried homogenized samples were then oven-dried at 70°C to calculate a moisture conversion factor (to estimate total 70°C-dried biomass production). Finally, these subsamples were ground using a rotary mill (Retsch zm 200) and used for chemical analysis.

Chemical analyses

BSi was extracted from 30 mg plant material by incubation in a 0.5 M NaOH mixture at 80°C for 4 h (DeMaster 1981): Si was analyzed on a colorimetric segmented flow analyzer (Skalar, Breda, The Netherlands). Total litter N, P, K, Ca and Mg concentrations were determined following Walinga et al. (1989), in which ground samples were digested with H_2SO_4, salicylic acid and H_2O_2. N and P were analyzed on a segmented flow analyzer (Skalar, Breda, the Netherlands); K, Ca and Mg were analyzed on ICP (Thermo Fisher, Franklin, MA, USA). Litter C concentrations were analyzed though combustion using a CNS-analyzer (Flash 2000). We used the van Soest method to determine alpha-cellulose ("cellulose" hereinafter) and ADF-lignin content ("lignin"

hereinafter) (Van Soest 1963). In short, cetyltrimethylammonium-bromide (CTAB) is added to 0.5-1 g of ground plant material and heated, which dissolves proteins. Samples were then rinsed, dried at 105°C and weighed. Next, 72% sulfuric acid (H_2SO_4) was added to dissolve cellulose, after which samples were again rinsed, dried and weighed. Mass loss, corrected for initial mass of the sample, was used to calculate cellulose content. Finally, lignin was removed from the samples by ashing the remaining material at 550°C for 4 hours. Again, mass loss was used to calculate lignin content. Due to unforeseen procedural errors during the heating phase and sulfuric-acid phase respectively we lost two cellulose- and three lignin samples (out of the sixty samples). Concentrations are reported in mg g^{-1} dry mass.

Decomposition experiments

Decomposition experiments were run in 10 decomposition beds (57 x 39 x 28 cm (l x b x h)). Each bed was filled with 25 kg of limed (25 g $CaCO_3$) clean white sand, and inoculated with 1 L fresh peat soil from a rich fen (collected in the Weerribben, the Netherlands (N52° 47' 02.4" E5° 58' 58.8")) to attain a relevant decomposer community. Beds were placed in a basement in full darkness with temperatures between 15.2 and 19.9 °C, six months prior to initiation of the decomposition experiment. Water levels were manually kept at surface level with a mixture of 90% demineralized water and 10% tap water, the latter to provide a minimal supply of essential trace and base elements (Table S1). Soil pH in the decomposition beds equaled 7.2 ± 0.04 (measured with a HI 99121 portable pH meter (Hanna Instruments, USA)). After five months (= one month prior to actual litter incubation), half of the beds (= 5) were heavily eutrofied by mixing 200 g slow-release NPK-fertilizer (17-9-11 Substral Osmocote) with the soil. The other half of the decomposition beds was kept nutrient-poor: soil was also "mixed" to guarantee an equal treatment.

Plant litter (0.82 g (SE = 0.01)) was cut into 5 cm fragments and put in litter bags. We only incubated litter from plants that had grown under the nutrient-rich treatment as plants from the nutrient-poor treatment had produced insufficient biomass. This however is unlikely to significantly impact our results, as the use of different species guaranteed an equally large variation in litter Si concentrations and nutrient stoichiometry.10 replicate litterbags of each of the 6 species (two litter samples were collected from each of the 30 nutrient-rich mesocosms) were divided pairwise over the nutrient-poor and nutrient-rich decomposition beds.

The litter bags (8 x 5.5 cm) were made from polyester netting with mesh size 325 µm (TopZeven, Haarlem, the Netherlands). Litter bags were placed horizontally on the soil surface and were pushed 2-3 mm into the top soil to guarantee contact of the plant litter with surrounding soil and pore water. Litterbags were retrieved after 116 days, and samples were rinsed with demineralized water, dried (45°C), and remaining mass (g) was determined. Litter decomposition was calculated based on ash-free dry mass, i.e. after subtraction of litter ash content as determined by loss-on-ignition (4h 550°C) before (subsample) and after litter incubation.

To correlate (ash-free) litter decomposition rates with measures of litter quality, we calculated decomposition constants "k" for each litter replicate from a single-exponential decay model (Wider & Lang 1982):

$\ln(M_t/M_0) = -k*t$

where M_t is the (ash-free) mass at time t, M_0 is the initial (ash-free) mass, k is the decay constant and t is time (year).

Data analysis

We calculated total BSi storage (= BSi_{tot} (in mg)) in each individual plant by multiplying tissue BSi concentrations (= BSi_{con} (in mg g^{-1})) with total biomass produced by the same plant (in g). To test if nutrient addition affects total BSi storage as well as BSi concentrations, we used Linear Mixed Effect (LME) modelling with REML in SPSS 22 (IBM Corp. 2013) in which we treated "Species" (1 to 6) as well as "Nutrient level" (rich or poor) as fixed-effect predictors. Prior to analysis, leaf litter BSi concentration was log10-transformed to attain normality of the model residuals. In all models, we tested for significance of the interaction terms of the two predictors. Next, we ran Spearman's correlation tests on the original data (not pooled per species) to correlate untransformed litter BSi concentrations with chemical litter parameters (N, P, K, but also C:N, C:P, lignin, cellulose, Lignin:N, Lignin:P, Ca, and Mg), as well as Ellenberg-N values and SLA-indices. Correlation tests were run separately for plants that were grown in the nutrient-poor and the nutrient-rich treatments. Finally, we used Spearman's correlation tests to see if litter BSi concentration correlates with litter decomposition constants "k". We ran two separate tests: one for litter that was incubated in nutrient-poor decomposition beds and a second for litter in the nutrient-rich beds. For all tests, significance was accepted at $p < 0.05$.

Results

Litter chemistry

Under the nutrient-poor treatment, all *Carex* species had higher concentrations of BSi (in mg g^{-1}) in their leaf litter (Figure 1a). The interaction effect between species and nutrient treatment was non-significant ($F_{5,48} = 1.93$, $p = 0.108$, Table 1), indicating that the magnitude of BSi accumulation under nutrient-poor conditions was equal for all species. When we omitted the non-significant interaction effect from the model, both main effects (nutrient level and species) retained their significant influence on litter BSi concentration ($p < 0.001$). However, as total plant biomass production was 2 - 5 times lower in the nutrient-poor treatment, total BSi storage per plant (in mg) was also lower in some species (Figure 1b), as indicated by the significant interaction term ($F_{5,48} = 45.24$; $p = 0.003$, Table 1). Overall, nutrient addition resulted in a tissue quality shift: plants from the nutrient-poor treatment were characterized by relatively high BSi and low N(P)K concentrations, whereas plants from the nutrient-rich treatment were characterized by relatively low BSi and high N(P)K concentrations (Figure 2). For plant litter from the nutrient-rich and nutrient-poor treatment separately, tissue BSi concentrations correlated negatively with tissue N and K concentrations (Figure 2a,c; Table 2). The negative correlation between tissue BSi concentration and P concentration was non-significant (Figure 2b; Table 2).

Correspondingly, litter BSi concentrations correlated positively with C:N and lignin:N ratios (Table 2). Litter Ca- and Mg concentrations correlated positively with BSi concentrations in litter from the nutrient-poor treatment (Table 2), but positive correlations were non-significant in litter from the nutrient-rich treatment. Furthermore, negative correlations were found between litter BSi concentrations and SLA in both nutrient treatments, and there was a tendency for high Ellenberg-N species to store relatively less BSi (Table 2), but the latter pattern was only significant in litter from the nutrient-rich treatment (Table 2).

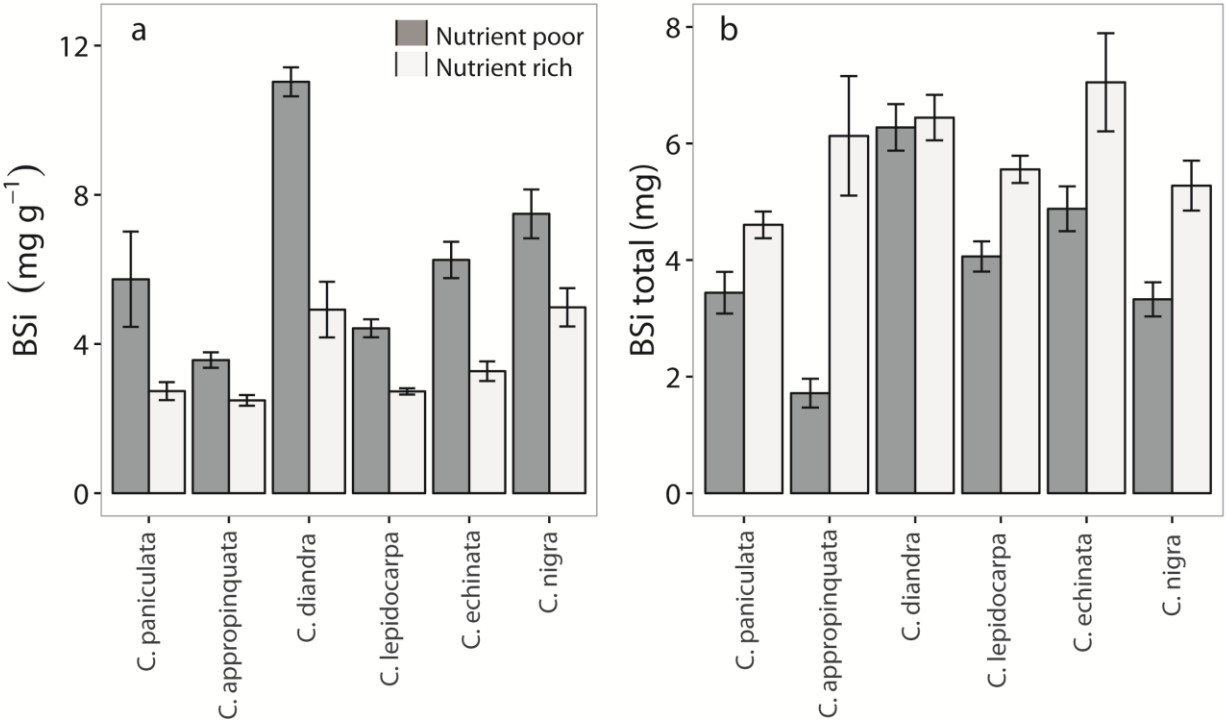

Figure 1: Leaf litter (a) BSi concentrations (mg g^{-1}) and (b) total BSi storage per plant (mg) in 6 species of *Carex* grown under nutrient-poor and nutrient-rich conditions. Bars represent averages ± SE (n=5).

Table 1: Output of the fixed-effect model on the interactive effects of "Species" and "Nutrient level" on leaf litter BSi concentrations (BSi_{con} ; mg g^{-1}) and total Si storage per plant (BSi_{tot} ; mg) for 6 species of *Carex*. Values in bold indicate significance ($p < 0.05$)

Effect	Dependent variable	d.f.	F-value	p-value
Species		5,48	25.11	**<0.001**
Nutrient level	BSi_{con} [log10(x)]	1,48	107.03	**<0.001**
Species * Nutrient level		5,48	1.93	.108
Species		5,48	9.02	**<0.001**
Nutrient level	BSi_{tot}	1,48	45.24	**<0.001**
Species * Nutrient level		5,48	4.25	**.003**

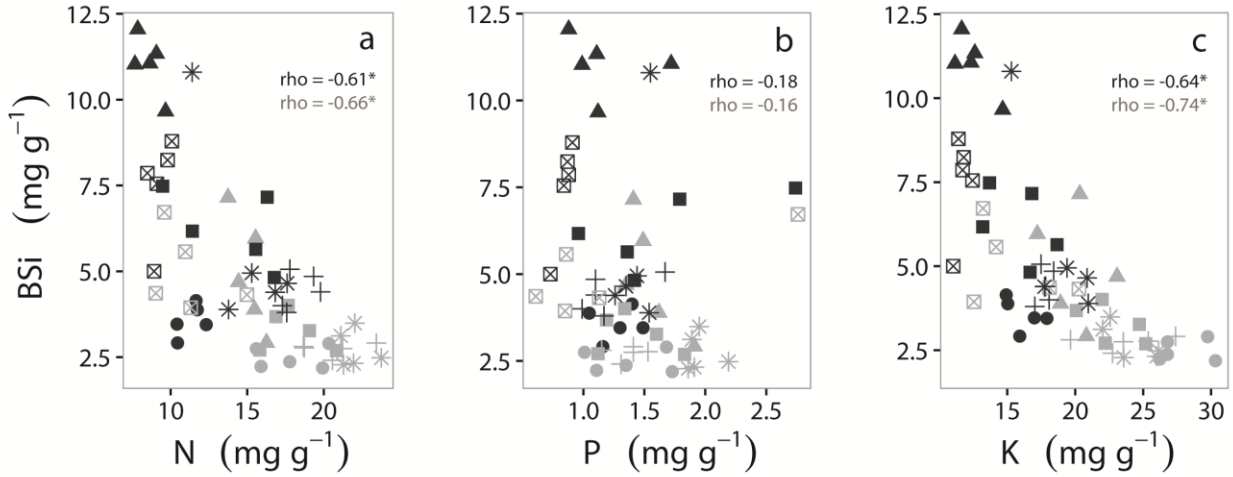

Figure 2: Correlations between leaf litter BSi concentration and (a) N concentration, (b) P concentration and (c) K concentration (mg g^{-1}) from 6 species of *Carex* grown under nutrient-poor (black markers) and nutrient-rich (grey markers) conditions. ● = *C. appropinquata*, ▲ = *C. diandra*, ■ = *C. echinata*, + = *C. lepidocarpa*, ⊠ = *C. nigra*, ✳ = *C. paniculata)*. * indicates significance ($p < 0.05$).

Table 2: Nonparametric correlations between tissue quality parameters and BSi concentrations of *Carex* leaf litter (from 6 species) of individuals grown under nutrient-poor conditions (left) and nutrient-rich conditions (right). Values in bold indicate significance ($p < 0.05$).

	Nutrient-poor BSi (mg g^{-1}), n = 30		**Nutrient-rich** BSi (mg g^{-1}), n = 30	
Parameter	**Spearman's rho**	**P value**	**Spearman's rho**	**P value**
N (mg g^{-1})	**-0.61**	**<0.001**	**-0.66**	**<0.001**
P (mg g^{-1})	-0.18	.352	-0.16	0.396
K (mg g^{-1})	**-0.64**	**<0.001**	**-0.74**	**<0.001**
C:N ratio (g g^{-1})	**0.59**	**<0.001**	**0.61**	**<0.001**
C:P ratio (g g^{-1})	0.17	.380	0.16	0.397
Lignin (mg g^{-1})	0.12	.526	0.25	0.206
Lignin:N ratio (g g^{-1})	**0.43**	**.019**	**0.50**	**0.008**
Lignin:P ratio (g g^{-1})	0.12	.543	0.15	0.470
Cellulose (mg g^{-1})	0.23	.214	0.07	0.742
Ca (mg g^{-1})	**0.62**	**<0.001**	0.31	0.099
Mg (mg g^{-1})	**0.44**	**.015**	0.06	0.771
SLA (cm^2 g^{-1})	**-0.60**	**<0.001**	**-0.51**	**0.004**
Ellenberg-N ranking	-0.24	.209	**-0.38**	**0.041**

BSi and litter decomposition

In the nutrient-poor decomposition beds, litter decomposition constants "k" correlated negatively with litter BSi concentrations, indicating a relatively slow decay of BSi-rich litter in nutrient-poor environments (Spearman's rho = -0.44, df = 28, p = 0.014, Figure 3a). This pattern however did not hold in the nutrient-rich decomposition beds, where no relation was found between litter BSi concentrations and decomposition constants (Spearman's rho = 0.05, df = 28, p = 0.78, Figure 3b).

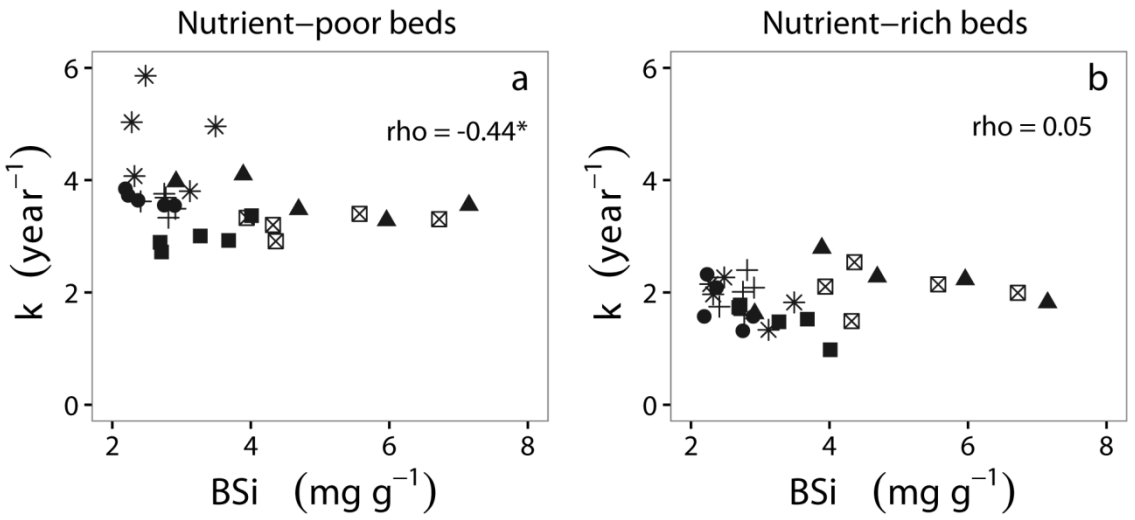

Figure 3: Correlations between leaf litter BSi concentrations and decomposition constants "k" in (a) nutrient-poor decomposition beds and (b) nutrient-rich decomposition beds. ● = *C. appropinquata,* ▲ = *C. diandra,* ■ = *C. echinata,* ┼ = *C. lepidocarpa,* ⊠ = *C. nigra,* ✳ = *C. paniculata).* * indicates significance ($p < 0.05$).

Discussion

We investigated how eutrophication affects Si fixation in wetland plants. An increase in nutrient levels led to lower BSi concentrations in all species. However, this inhibiting effect of eutrophication on Si concentration in the vegetation was counterbalanced by a higher total BSi storage due to increased primary production, which led to an increase in total Si fixation. A negative correlation between litter BSi concentrations and litter decomposition rates in nutrient-poor environments suggests an inhibitory effect of BSi on decomposition processes.

Eutrophication and intraspecific stoichiometric shifts

An increase in macronutrient (NPK) availability directly affected BSi stoichiometry of wetland plants: high ambient nutrient levels corresponded with low plant BSi concentrations (up to 50 % reduction in *C. diandra*). Moreover, this pattern of lower BSi concentrations upon macronutrient addition was consistent in all six study species, and the magnitude of the effect did not differ between the slow-growing (low Ellenberg-N) and more competitive (high Ellenberg-N) species. Such stoichiometric response to stress has been shown for hydrodynamic stress (Schoelynck et al. 2012) and herbivory (Massey & Hartley 2006), but our study is the first to experimentally relate high BSi concentrations to ambient nutrient stress. We hypothesize that the observed increase in BSi concentration under nutrient-poor conditions increases chances of plant survival. Overall, Si accumulation has beneficial effects on plants, especially when grown under stressful conditions (Ma et al. 2001). For instance, high tissue BSi concentrations make plants (1) less attractive for

herbivores (Bonar et al. 1990; Schoelynck & Struyf 2016), (2) more resistant to physical disturbances and abiotic stress by increasing rigidity (Ma & Yamaji 2006; Schoelynck et al. 2012) and (3) less prone to disease (Ma & Yamaji 2006). Under nutrient-poor conditions these are valuable traits as plant growth is slow and costly, and herbivory or damage to living tissue would come at a high cost (Chapin 1991; Grime 1977). When eutrophication lifts nutrient stress on plants, it may result in a lower relative BSi concentration in the vegetation. However, such decrease in BSi concentrations need not be problematic as plants growing in eutrophic environments may be more capable of rapid recovery after disturbances or damage.
The observed response to nutrient stress can be explained by at least two nonexclusive processes. First, differences are possibly linked to different modes of Si accumulation (i.e. active and passive), as shown for *Spartina* (Carey & Fulweiler 2014). Generally, tissue BSi concentrations > 4.6 mg g^{-1} (= 0.46% BSi or 1% SiO_2) are indicative for active Si accumulation (Carey & Fulweiler 2012), with lower values indicating passive (= transpiration-based) accumulation. Based on this threshold, it appears that some of our study species could have switched from passive Si accumulation in nutrient-rich conditions to active Si accumulation in nutrient-poor conditions (Figure 1a). Second, it is possible that plants from the nutrient-poor treatment maximized nutrient uptake by maintaining higher transpiration rates, thereby accelerating mass-flow of pore water (and nutrients) to the roots. Such response has, for example, been shown in *Ehrharta calycina* (Cramer et al. 2008). If so, then (passive) Si accumulation in the plant should increase concomitantly. Although our data do not allow us to disentangle the exact physiological mechanisms, our results do support the hypothesis that the level of BSi accumulation in wetland plants is adaptive (Schoelynck & Struyf 2016), i.e. Si accumulation in the vegetation increases under stressful conditions.

Eutrophication and plant community shifts

If we want to predict how wetland eutrophication affects Si cycling on the ecosystem level, then concomitant shifts in vegetation assembly and primary production need to be taken into account. Eutrophication induces a plant community shift from low-productive communities towards fast-growing, more competitive communities (Ceulemans et al. 2011; Hautier et al. 2009). In our dataset, we see a negative relationship between specific leaf area (SLA) and leaf litter BSi concentrations (Table 2, Figure 4), as well as a negative relationship between Ellenberg-N ranking and leaf litter BSi concentrations in the nutrient-rich treatment (Table 2). As high SLA-indices and high Ellenberg-N values are both indicators for rapid growth (found primarily in competitive plants with high return on investment) (Lambers & Poorter 1992; Westoby 1998), an eutrophication-induced shift towards more productive plant communities will result in decreased concentrations of Si in the vegetation.
Yet, lower vegetation Si concentrations need to be corrected for the eutrophication-induced increase in absolute Si storage due to an increase in primary biomass production. As total Si storage per plant (in mg) was higher in the nutrient-rich treatment, stimulant effects of eutrophication on total primary production and thus total Si storage in the vegetation can outweigh inhibiting effects of eutrophication on BSi concentrations in the vegetation.

We can translate these results to shifts in BSi storage in the vegetation of natural wetlands, using simple calculations. We start from a hypothetical oligotrophic wetland that is mainly dominated by small-stature, low-competitive sedges (Ellenberg-N = 2). If we assume an average BSi concentration of this vegetation type of 6 mg g^{-1} (= average of *C. echinata*, *C. nigra* and *C. lepidocarpa* under nutrient-poor conditions) and a yearly aboveground biomass production between 1–3.5 metric tons ha^{-1} (Ellenberg & Leuschner 1996), then this accumulates to an estimated fixation of 6 – 21 kg BSi ha^{-1} yr^{-1} in the aboveground vegetation. Upon wetland eutrophication however, the small-stature sedges are likely to be replaced by taller sedges (Ellenberg-N > 4). In the new vegetation, we assume an average foliar BSi concentration of 2.6 mg g^{-1} (= average of *C. paniculata* and *C. appropinquata* under nutrient-rich conditions) and a yearly aboveground biomass production between 5.5–8 metric tons ha^{-1} (Ellenberg & Leuschner 1996). This corresponds with an estimated 14 –21 kg BSi ha^{-1} yr^{-1} in the eutrophic vegetation. Hence, despite an average 2-5 fold increase in biomass production after eutrophication, total Si fixation in the vegetation does not necessarily increase.

We should note that the above calculations only apply for sedge-dominated wetlands. For example, dense hypertrophic *Phragmites* stands have a different Si stoichiometry (Struyf et al. 2007), and may reach a much higher yearly production of > 20 metric tons ha^{-1} (Ellenberg & Leuschner 1996), resulting in more Si accumulation in the vegetation.

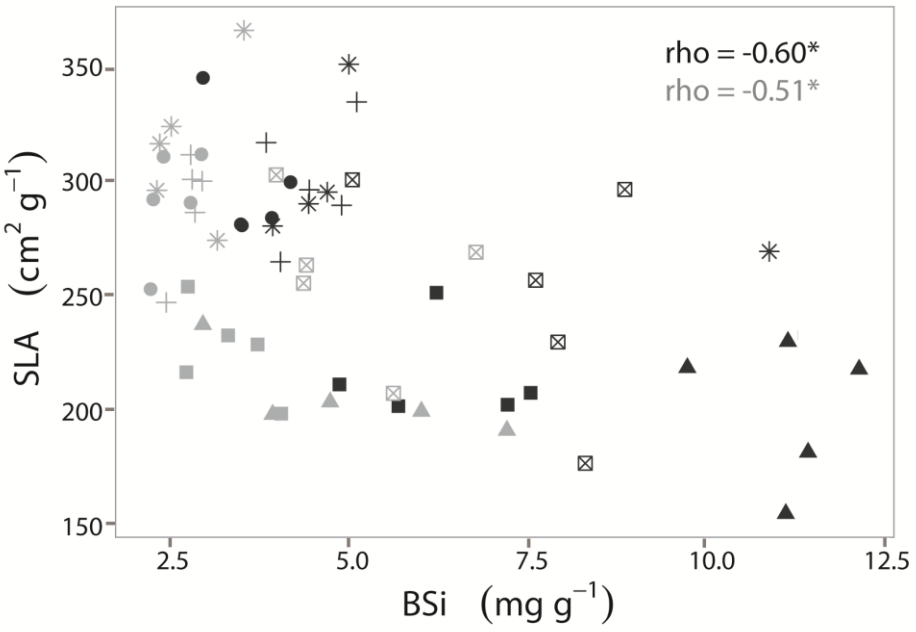

Figure 4: Correlations between specific leaf area (SLA, cm^2 g^{-1}) and leaf litter BSi concentration (mg g^{-1}) from 6 species of *Carex* grown under nutrient-poor (black markers) and nutrient-rich (grey markers) conditions. ✹= *C. appropinquata*, ▲ = *C. diandra*, ■ = *C. echinata*, + = *C. lepidocarpa*, ⊠ = *C. nigra*, ✳ = *C. paniculata*). * indicates significance ($p < 0.05$).

Litter decomposition

Litter decomposition studies are often biased towards well-known tissue quality parameters such as N and lignin content whereas other litter characteristics may be equally important (Hobbie 2015), and our findings suggest that Si concentration is a candidate trait.

To date, the effects of litter Si concentrations on decomposition rates have remained unclear. Some researchers suggest that high litter Si concentrations hamper decomposition (Cornelissen & Thompson 1997), but opposite patterns have been reported as well (Schaller & Struyf 2013). We show that, under nutrient-poor conditions, a high initial litter BSi concentration correlates with lower rates of litter decomposition. This suggests a positive feedback on BSi and carbon retention in litter in nutrient-poor environments, although measurements of BSi dissolution from decaying litter would additionally be required to support this hypothesis. However, we also show that there is a strong interdependence of BSi with other tissue quality parameters (as previously shown by Schaller et al. 2012; Schoelynck et al. 2010): BSi-rich litter was also characterized by low nutrient (NK) contents and high C:N and lignin:N ratios, and these variables hamper decomposition as well (Emsens et al. 2016; Taylor et al. 1989). Therefore, it is impossible to distinguish between causation (= a high foliar BSi concentration directly hampers decomposition) and correlation (= a high foliar BSi concentration correlates with other tissue quality parameters, but only the latter determine rates of decomposition). The correlation between BSi concentration and decomposition did not hold in the nutrient-rich decomposition beds, suggesting that Si-decomposition dynamics may interact with abiotic and biotic factors. Given the potential link between Si incorporation and decomposition, we propose that Si stoichiometry be taken into account in future decomposition studies.

Consequences for the global Si cycle

We show that stimulatory effects of eutrophication on total Si storage in wetland vegetation, i.e. due to an increase in primary production, need to be balanced with the plant-physiological response of lower tissue Si concentrations. In sedge-dominated wetlands, eutrophication is likely to affect the rate at which Si is released back into the environment after vegetation dieback: under nutrient-poor conditions, the dominance of low-competitive species results in the production of recalcitrant BSi-rich litter with a low decomposability. Here, we hypothesize that a large proportion of BSi will eventually be retained in refractory organic matter in the wetland soil. In contrast, plant species in eutrophic environments generally produce easily degradable and (relatively) BSi-poor litter, and most BSi will be released soon after vegetation dieback through fast litter decay. Overall, eutrophication may thus facilitate high rates of net Si export from wetlands to oceans, but this hypothesis requires future testing in natural ecosystems. In conclusion, although the vegetation of eutrophic and nutrient-poor environments may sequester similar total amounts of Si, eutrophication-induced shifts in feedbacks on litter decomposition are likely to increase rates of Si cycling.

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Appendix

Table S1: Chemical characteristics of the tap water (pH = 7.2) used in the mesocosm experiment to grow *Carex* shoots.

Element	Value (mg L^{-1})
DSi	3.35
P	0.03
NO_3^-	11.7
NH_4^+	0.02
K	3.7
Na	33.0
Ca	63.0
Mg	7.1
S	17.4
Cl	46
Fe	0.02
Mn	0.001
Al	0.033
Cu	0.006
B	0.045
Zn	0.009
Ni	0.002
Se	0.002