General comments to the reviewer and editor:

First of all we would like to thank the reviewer for the very helpful comments on our manuscript. In general, we tried to consider all comments of the reviewer to improve the quality of our manuscript (please see our answers/comments below).

Answers (highlighted) to the comments of Reviewer #2:

The authors present an interesting and detailed examination of the various constituents of the biogenic silicon pools within a constructed watershed in Germany. There is a detailed methodology outlined in this paper with some interesting results-both through time and through space.

My biggest concern is that this paper starts with a claim to uncover truths about biogenic Si pools other than phytogenic pools with in systems, but the work is mainly focused on a highly-disturbed, constructed watershed. Which is incredibly important to study, but I am not sure much of the introduction fits into what the study actually is. There is question of the broad, applicability of these findings which I only point out given the sweeping nature of much of the introduction. A recasting of this could solve this issue. There needs to be more focus on the disturbance aspect-that is one way to take this work. In Lines 66-70 you bring up the idea of disturbance and disequilibrium and how that affects Si cycling, but don't really explore that. Further in the work, results are presented in a manner that there is explicit testing of various regions of the watershed and it is a bit confusing as to what is being tested as there are "initial values" that seem to be for the whole watershed that are considered to homogenous (which the authors allude to) and then each section is independently tested against these initial values. Part of my confusion here stems I think from my misunderstanding of the closing section of the introduction as there is some obfuscation about what is being hypothesized.

"Thanks a lot for your critical comments and helpful suggestions. In general, we are still convinced of our study design and the corresponding presentation of results. At t_0 there are some slightly differences in abiotic soil conditions (which is also stated in the manuscript), but we assume no differences in the distribution of biogenic siliceous structures (phytoliths, testate amoeba shells etc.) shortly after construction of the study site (a fact that has already been shown in Puppe et al. 2016). Thus, a differentiation

between different sections already at t_0 would produce redundant results for biogenic Si, i.e., BSi for t_0 (east) ≈ BSi t_0 (west) ≈ BSi t_0 (south). This is why we only show one value for BSi at t_0 (which holds true for all sections). However, to accurately describe changes of abiotic soil conditions in every section we differentiated between the different sections already at t_0 (as these slight differences have been known since the end of construction work of Chicken Creek). However, we reworked especially the introduction and the material & methods section of our manuscript to make these points clearer for the reader."

At it's core, this manuscript is a good survey of the biogenic Si pools in the Chicken Creek watershed. But currently, I feel the introduction and discussion read as if they are from two separate papers. I believe the introduction needs to be reshaped to fit the paper that is here. The methods section is excellent and there is a thorough write-up of the procedures presented with adequate documentation. This should be lauded as many papers are often lacking in such detail for those who would like to replicate experiments. Some of the background for this paper that is necessary is in the Puppe et al. 2016 paper in Geoderma-but this manuscript submitted here reads as a good companion piece to the Geoderma one.
There is an appeal here to many readers of Biogeosciences mainly in that Si cycling is not well understood broadly in the biogeochemical community and this work has the potential to make inroads towards expanding the understanding of Si cycling and its relevance. There is a lot of potential here with a need to make the paper more uniform and clear. Presently, there it is too disjointed.

"Thanks a lot for your detailed critical comments and encouraging words. We tried to follow your very helpful suggestions (please see our answers and comments below)."

INTRODUCTION
50 – I would consider changing the line "pro- and eukaryotic organisms…" to "prokaryotes and eukaryotes."
"Done."

59 – The phrase "big scale" reads to colloquially and I would suggest changing that.
"Done."

60-61 – I would clarify this sentence a bit and tone it done the claim. Though this is a substantial amount, this one facet will not regulate all of the climate.
"You are right. We rephrased this sentence."

62 – Change "since" to "for" for clarity.
"Done."

70-72 – I am interested here in how these are unbalance. Could you expand more here? In what direction and magnitude, please.
"We added some information on this topic here."

73 – "…allow to analyze…" is not grammatically correct.
"We corrected this sentence."

75-84 – I really like this summary of pervious work and major findings here.
"Thank you for your encouraging words."

83-84 – Shorten "…as well as uptake into 83 biological systems" to just "biological uptake"
"Done."

85-92 – There is to much effort need to suss out what the hypotheses motivating the work are. They are in there, but need to be clarified.
"You are right. We reworked this paragraph and hope it is clearer now."

METHODS
107 – What do you mean by "serving as aquifer" here? This is worded strangely and could be interpreted in different ways.
"We added some more information here and hope it is reasonable now."

112-115 – I think this area could be improved by considering the area of each portion of the watershed, and maybe even something like an upslope accumulated area calculation. There is some work here that depends heavily on hydrology, but there is not so much hydrology in here. Some GIS work could help.

“You are right; we added some information on the area of the different parts of Chicken Creek. However, we did not include GIS work in our manuscript as we generally focus on biogenic Si pools and not on hydrology, but we will consider this aspect (and corresponding GIS work) in a forthcoming paper (Sommer et al., in preparation).”

116 – Wait, what is skeleton content?
“We added a definition of skeleton content.”

*Also, it is not necessary to put Chicken Creek in quotations each time.
“Okay, we changed this throughout the manuscript.”

157 – “weighted” should be “weighed”
“Done.”

160 – Change “was not used” to “avoided”
“Done.”

183-184 – This sentence is awkwardly constructed and could be clarified.
“You are right. We rephrased this sentence.”

195-203 – The verb tense vacillates a bit and should standardized throughout.
“Done.”

254 – Why were there two replicates before, and now three?
“Different laboratory analyses were done in different replicates (two or three replicates), which is why we give the information on replicates for every subsection independently. However, we added the corresponding method in each sentence to make it clearer.”

Overall, good methods section.
“Thank you for your kind words.”

RESULTS

290-295 – I generally like this section, but you could present some percent change too as a normalized difference. This usually a good way to focus what you want the reader to notice.

"We followed your recommendation."

295-297 – This decrease in pH, this is interesting here.

"You are right."

301 – Usually you see it written 7.4 x 10^{-3} g kg^{-1}

"You are right, we changed it."

323 -328 – This part gets really confusing when you say "increase to" and you present a range. I am not really sure where to follow with this. Could means with a standard deviation or error or some measure of uncertainty be more clear?

"We followed your recommendation and added means here."

* In general there is the presentation of results by different section of the watershed, though this is not something presented as a hypothesis. If this is the manner you want to present, maybe consider making this a research question and present a mechanism that could potentially describe the patterns.

"You are right. We reworked the introduction and our hypotheses/aims."

365-367 – I am of the opinion that you cannot refer to a figure as self-evident of your results. The results section is the to describe the overview of these different pools. Our you could just cut this line.

"We followed your recommendation and changed the heading of this subsection and deleted its first sentence."

372 – Do you have a sense for the total above-ground storage of Si in raw numbers?

"This is an interesting question. Unfortunately, until now we only have the data presented in our manuscript. However, we will consider this aspect in a forthcoming paper (Wehrhan et al., in preparation)."

DISCUSSION

378 – This reads more like a topic sentence than a sub-heading

"You are right. We reworked it."

* The discussion leads of with the origin of where the Si in the system is coming from. Obviously there is an importance imparted to this point, perhaps make this something you are testing then rather than just throwing out initially in the discussion.

"We reworked our manuscript, especially the introduction and material & methods sections and hope it is more consistent now."

393-397 – Great, here is the stuff about the sections being different. Maybe bring some of this up in the methods section where you describe the site.

"This information already can be found in the material & methods section."

* Also, what is skeleton content?

"We added a definition of the skeleton content in the material & methods section."

428 – Larger, instead of bigger.

"Corrected."

FIGURES

Fig 3- What are the error bars here? Without knowing it is difficult to believe that the South plot is statistically significantly different unless these are SD as much of the other paper, but with ANOVA wouldn't confidence intervals or standard error be a good alternative?

"We added information on error bars in the figure caption."

Fig 4 – You highlight the different axes, but the differences are between A +B and C+D. Again, error bars. This really highlights the internal variance at the south site. What is going on there? You could really dig in there more in the future maybe.

"We corrected the hint on different axes and added information on boxes as well as whiskers."

Fig 5 – A couple of notes here, technically this graph is pretty good. But given the large differences in total Si pool size, I don't think normalizing the scales is the best way to present this as it obscures relationships among the sites. It makes the t_0 sites look much larger when we know they aren't. Also, thatching is often distracting when you could go full color for this journal. Again though, interesting stuff going on in the south section.

"You are right. We added a hint on different BSi pool sizes in the figure caption and reworked the layout of the diagram."

Fig 6 – This is a really interesting way to present this as you have combined a table with a conceptual diagram similar to that of a textbook. I really like this. The font color differences are a bit distracting, but well done.

"Thanks a lot for your kind words. We reworked the figure and adjusted font colors."

Answers (in bold type) to the comments of Reviewer #3:

Silicon is one of the most important elements in terrestrial and marine ecosystems. Its flow and fate within these systems help to understand biogeochemistrial function and influences in between. Because of biological performance, the Si cycling is motivated via synthesized hydrated amorphous Silica. The authors selected an unique artificial catchment to observe the change of bio-Si driving by different biological functions, especially phytogenic pathway. The research findings are rather interesting.

First of all we would like to thank you for the very helpful comments on our manuscript. In general, we considered all your comments to improve the quality of our manuscript (please see our detailed answers/comments below).

However, there are a few points needed to be addressed in the MS prior to the acceptance for publication.

1) Total Si analysis. If total Si also significantly changed during this 9 years because of other factors than biological, portion of total SI, i.e., BSi may also change. Unfortunately, I did not see the analysis of total Si changes in tables or figures.

In our study we only measured total Si contents of plants but not of soils because the expected changes in total Si during such a small time period are too small - given the high background Si contents (quartz is the dominating mineral). Any changes during 10 years are lower than the precision of any lab analysis (either XRF or HF extractions), i.e., not detectable. However, we quantified the so-called 'amorphous' Si fraction in soils (by Tiron method) and discussed the corresponding results (please see ll. 398-415).

2) are other sources of Si outside this catchment significant? this applied to wet and dry air deposition. I would like to see the data on this.

This is an important comment. Actually, dust depositions (dry deposition) at Chicken Creek are very low (73-230 mg m^{-2} d^{-1} during storm events) and only slightly above the annual average (70-90 mg m^{-2} d^{-1}) measured in the state of Brandenburg (*Wanner, M., Elmer, M., Sommer, M., Funk, R. & Puppe, D., 2015. Testate amoebae colonizing a newly exposed land surface are of airborne origin. Ecological Indicators, 48, 55-62*). As Si is a lithogenic element the total Si input by precipitation (wet deposition) is negligible as well (<1 kg Si ha^{-1} yr^{-1}, *Sommer et al. 2013*). Nevertheless, we will add this information to the revised version of our manuscript to improve its quality.

3) are erosion/runoff significant for the temporary and spatial change of BSi? The data of land slope and erosion/runoff will help to address this issue.

Good point. Erosion/deposition processes were clearly evident in the Chicken Creek catchment during the first years without plant cover. Substantial surface changes resulted from rill erosion as can be seen in aerial photographs (rill network) and from a comparison of DEMs over time (*Schneider, A., Gerke, H. H., Maurer, T., Nenov, R., 2013. Initial hydro-geomorphic development and rill network evolution in an artificial catchment. Earth Surface Processes and Landforms 38, 1496-1512*). Interrill erosion did not lead to surface changes larger than 20 cm during the first 5 years. Afterwards the establishment of an area-wide plant cover substantially reduces interrill erosion. Because all soil data of t_0 refer to a depth increment of 30 cm we can reasonably assume the same soil conditions after the first years of

more intense erosion. Furthermore, we carefully selected sampling points (2015) to be not influenced by erosion, i.e., at places with low surface roughness and outside rills, of course. We will add this information to the revised version of our manuscript to improve its quality.

4) Root, a substantial portion of plant biomass, actually are more important in the activating or demobilizing the Si from the soil or earth case, because of the interaction between root biomass and root exudate like acid, and mineral Si. Please add the analysis on this.

You are right, the release of organic acids in the rhizosphere of plants can lead to increased weathering rates in general. However, we assumed this effect to be negligible at Chicken Creek due to the quartz dominance in combination with the large proportion of the sand fraction (western section 83 %, eastern section 82 %, southern section 83%). We will add a new table including information on sand, silt, and clay fractions at Chicken Creek to the revised version of our manuscript to corroborate our assumption.

Other specific points:

- please report soil texture in table and MS, a important indicator of soil erosion/runoff.

You are right. As we stated above we will add a new Table including information on sand, silt, and clay fractions at Chicken Creek to the revised version of our manuscript.

- line 141-143: From October 2007 to 2016 (I assume 2016 because the authors did not tell the sampling year), and plant sampling year is 2015, it is only 9 years instead of 10 years. This is 10% difference of duration!

You are right; this sentence is some kind of misleading and we will delete it from the revised version of our manuscript as we do not present results of soil solution analyses in the current manuscript (we will do this in a forthcoming paper, which is currently in preparation). Soil samples were taken in 2005 (t_0) as well as 2015 (t_{10}) (please see ll. 141-142), while plant samples were only taken in 2015 (please see ll. 258-259), but contemporaneously with soil sampling. We will add the corresponding years of soil sampling to ll. 141-142 in the revised version of our manuscript to enhance transparency of our study.

- line 169-170: are MgCO3 contents not significant in the soil? please provide the data and if not negligible, MgCO3 should also be analyzed.

At Chicken Creek the primary mineral component in all particle size fractions at t_0 was quartz (only small amounts of K-feldspar, plagioclase). Calcite comprises 0.5-4.5 % of the initial sediment; dolomite was only detectable in 2 out of 11 samples showing 0.5 %. Magnesite ($MgCO_3$) was not detected in the mineralogical analysis (XRD). Consequently, we assumed $MgCO_3$ contents to be negligible at Chicken Creek. However, we will add the information above to the revised version of our manuscript to improve its quality.

- line 258: why not including root?

In general, monomeric silicic acid (H_4SiO_4) enters the plant via its roots and is carried in the transpiration stream towards transpiration termini (e.g., leaves) in the aerial plant parts. When water evaporates, silicic acid becomes supersaturated and is precipitated as hydrated silica in the form of phytoliths. The vast majority of Si in plants is located at these

transpiration termini, while considerably less Si can be found in other plant portions like stems, roots, and rhizomes. Sangster (1983) (*Sangster, A. G., 1983. Anatomical features and silica depositional patterns in the rhizomes of the grasses Sorghastrum nutans and Phragmites australis. Canadian Journal of Botany 61, 752-761*), for example, found no significant Si depositions in rhizomes of *Phragmites australis*. Consequently, we only analyzed the aboveground vegetation (including transpiration termini and stems). We will add this information to the material and methods-section in the revised version of our manuscript.

- line 389-391: for each year or 9 years?

The reported values apply to samples taken at t_{10}. We will add this information to the revised version of our manuscript.

How big is the influence of biogenic silicon pools on short-term changes of water soluble silicon in soils? Implications from a study of a ten-year-old plant-soil-system

Daniel Puppe[a,*], Axel Höhn[a], Danuta Kaczorek[b], Manfred Wanner[c], Marc Wehrhan[a], Michael Sommer[a,d]

[a] Leibniz-Centre for Agricultural Landscape Research (ZALF) e.V., Institute of Soil Landscape Research, 15374 Müncheberg, Germany

[b] Department of Soil Environment Sciences, Warsaw University of Life Science (SGGW), Nowoursynowska 159, 02-776 Warsaw, Poland

[c] Brandenburg University of Technology Cottbus-Senftenberg, Department Ecology, 03013 Cottbus, Germany

[d] University of Potsdam, Institute of Earth and Environmental Sciences, 14476 Potsdam, Germany

* Corresponding author. E-mail address: daniel.puppe@zalf.de

Abstract

The significance of biogenic silicon (BSi) pools as a key factor for the control of Si fluxes from terrestrial to aquatic ecosystems has been recognized since decades. However, while most research has been focused on phytogenic Si pools, knowledge on other BSi pools is still limited. We hypothesized different BSi pools to influence short-term changes of the water soluble Si fraction in soils to different extents. To test our hypothesis we took plant (*Calamagrostis epigejos*, *Phragmites australis*) and soil samples in an artificial catchment in a post-mining landscape in the state of Brandenburg, Germany. We quantified phytogenic

(phytoliths), protistic (diatom frustules and testate amoeba shells) and zoogenic (sponge spicules) Si pools as well as Tiron extractable and water soluble Si fractions in soils at the beginning (t_0) and after ten years (t_{10}) of ecosystem development. As expected the results of Tiron extraction showed, that there are no consistent changes of the amorphous Si pool at 'Chicken Creek' as early as after ten years. In contrast, compared to t_0 we found increased water soluble Si and BSi pools at t_{10}, thus we concluded BSi pools to be the main driver of short-term changes of water soluble Si. However, because total BSi represents only small proportions of water soluble Si at t_0 (<2 %) and t_{10} (2.8-4.3 %) we further concluded smaller (<5 µm) and/or fragile phytogenic Si structures to have the biggest impact on short-term changes of water soluble Si. In this context, extracted phytoliths (>5 µm) only amounted to about 16 % of total Si contents of plant materials of *C. epigejos* and *P. australis* at t_{10}, thus about 84 % of small-scale and/or fragile phytogenic Si are not quantified by the used phytolith extraction method. Analyses of small-scale and fragile phytogenic Si structures are urgently needed in future work as they seem to represent the biggest and most reactive Si pool in soils, thus the most important driver of Si cycling in terrestrial biogeosystems.

Keywords

biosilicification, initial biogeosystem, phytogenic Si, protistic Si, zoogenic Si

1. Introduction

Various prokaryotes and eukaryotes ~~and eukaryotic organisms~~ are able to synthesize hydrated amorphous silica ($SiO_2 \cdot nH_2O$) structures from monomeric silicic acid (H_4SiO_4), a process called biosilicification (Ehrlich et al. 2010). In terrestrial biogeosystems biogenic silicon (BSi) synthesized by bacteria and fungi, plants, diatoms, testate amoebae and sponges can be found forming corresponding microbial, phytogenic, protophytic, protozoic and zoogenic BSi pools, respectively (Puppe et al. 2015, Sommer et al. 2006). BSi has been recognized as a key factor for the control of Si fluxes from terrestrial to aquatic ecosystems as it is in general more soluble compared to silicate minerals (e.g., Fraysse et al. 2006, 2009). These fluxes influence marine diatom production on a global scale (Dürr et al. 2011, Sommer et al. 2006, Struyf & Conley 2012). Marine diatoms in turn can fix large quantities of carbon dioxide via photosynthesis because ~~on a big scale (~~up to 54 % of the biomass in the oceans is represented by diatoms~~),~~ thus diatoms have an important influence on ~~regulate~~ climate change (Tréguer & De La Rocha 2013, Tréguer & Pondaven 2000).

While the importance of phytogenic Si pools for global Si fluxes has been recognized ~~since~~for three decades (e.g., Bartoli 1983, Meunier et al. 1999, Street-Perrott & Barker 2008), information on the other BSi pools is comparably rare (Clarke 2003). However, in recent publications the potential importance of diatoms, testate amoebae and sponge spicules in soils for Si cycling has been highlighted (Aoki et al. 2007, Creevy et al. 2016, Puppe et al. 2014, 2015, 2016). Furthermore, evidence arises that BSi pools are in disequilibrium at decadal time scales due to disturbances and perturbations by humans, e.g., by changes in forest management or farming practices (Barão et al. 2014, Keller et al. 2012, Vandevenne et al. 2015). In consequence, BSi accumulation and BSi dissolution are not balanced, which influences Si cycling in terrestrial biogeosystems not only on decadal but also on millennial

scales (Clymans et al. 2011, Frings et al. 2014, Sommer et al. 2013, Struyf et al. 2010). Sommer et al. (2013), for example, found the successive dissolving of a relict phytogenic Si pool to be the main source of dissolved Si in soils of a forested biogeosystem. Due to the fact that the continuous decomposition of this relict phytogenic Si pool is not compensated by an equivalent buildup by recent vegetation the authors concluded a BSi disequilibrium on a decadal scale. On a millennial scale Clymans et al. (2011) estimated the total amorphous Si storage in temperate soils to be decreased by approximately 10 % since the onset of agricultural development about 5,000 years ago. This decrease not only has consequences for land-ocean Si fluxes but also influences agricultural used landscapes because Si is a beneficial element for many crops (e.g., Epstein 2009, Ma & Yamaji 2008).

For a better understanding of BSi dynamics, chronosequence studies are well suited, because they allow us to analyze time-related changes of BSi pools during biogeosystem development. In the present study we analyzed various BSi pools in differently aged soils of an initial artificial catchment ('Chicken Creek') in a post-mining landscape in NE Germany. Chicken Creek represents a study site with defined initial conditions and offers the rare opportunity to monitor BSi dynamics from the very beginning. Former studies at this site revealed i) a formation of protophytic (diatom frustules), protozoic (testate amoeba shells) and zoogenic (sponge spicules) Si pools within a short time (<10 years) and ii) a strong relation of spatiotemporal changes of protistic (diatoms and testate amoebae) BSi pools to the vegetation, because plants provide, e.g., rhizospheric micro-habitats including enhanced food supply (Puppe et al. 2014, 2016). From these results it can be concluded that especially vegetated spots at initial biogeosystem sites represent hot spots of BSi accumulation of various origin (compare Wanner & Elmer 2009). Furthermore, construction work with large machines resulted in differently structured sections of Chicken Creek with slight differences

in abiotic conditions (for details see subsection 2.1.) (Gerwin et al. 2010). These differences in turn lead to section-specific vegetation dynamics at Chicken Creek (Zaplata et al. 2010). Knowledge about BSi accumulation dynamics is crucial for the understanding of Si cycling in terrestrial biogeosystems. We regard water extractable Si as an useful proxy for desilication and biological uptake (plants, testate amoebae etc.). In addition, we used an alkaline extractant (Tiron) to detect eventual short-term changes of the amorphous Si fraction. ~~and hypothesized different BSi pools to influence short-term changes of water soluble Si in initial soils.~~ We hypothesized i) BSi pools to influence short-term changes of water soluble Si in initial soils, but no short-term changes in amorphous Si fractions, ii) the phytogenic Si pool to be the most prominent one in size, thus the biggest driver of short-term changes of water soluble Si, and iii) BSi pool changes to be section-specific, i.e., related to vegetation ~~Further we assumed the phytogenic Si pool to be the most prominent in size (compare Sommer et al. 2013), thus the biggest driver of short-term changes of water soluble Si.~~dynamics. The aims of the present study were i) to quantify various BSi pools, i.e., protophytic, protozoic, zoogenic and phytogenic Si pools, during initial soil and ecosystem development, (ii) to analyze potential section-specific short-term changes of these BSi pools after a decade of ecosystem development, and iii) to evaluate the influence of different BSi pools on water soluble Si in these soils.

2. Material and methods

2.1. Study site

The study site ~~'~~Chicken ~~Creek'~~Creek (51°36'18" N, 14°15'58" E) represents an artificial catchment in a post-mining landscape located in the active mining area 'Welzow-South' (lignite open-cast mining, 150 km south-east of Berlin) in the state of Brandenburg, Germany

(Kendzia et al. 2008, Russell et al. 2010). Climate at ~~'Chicken Creek'~~Chicken Creek is characterized by an average air temperature of 9.6°C and an annual precipitation of 568 mm comprising data from 1981 to 2010 (Meteorological Station Cottbus, German Weather Service).

For construction of the about 6 ha sized catchment an 1-3 m thick base layer (aquiclude) of Tertiary clay was covered by a 2-3 m thick sandy, lignite- and pyrite-free Quaternary sediment serving as water storage layer (aquifer) (Gerwin et al. 2010, Kendzia et al. 2008). Quaternary material was taken from a depth of 20-30 m during lignite mining process and its texture is classified as sand to loamy sand (Table 1) with low contents of carbonate (Gerwin et al. 2009, 2010, Russell et al. 2010). Dumping of material and construction work with large machines (e.g., stackers and bulldozers) resulted in differently structured sections of ~~'Chicken Creek'~~Chicken Creek. Generally, the catchment area can be divided into four sections: i) an eastern part (ca. 1.8 ha), ii) a western part (ca. 1.6 ha), iii) a central trench (ca. 0.9 ha) separating the eastern from the western part and iv) a southern part (ca. 1.5 ha) with a pond at the lowest point (Fig. 1). Construction work was completed in September 2005 (time zero, t_0). Analyses subsequent to catchment completion indicated slight differences in abiotic conditions (soil pH, conductivity, skeleton content (soil particle diameter >2 mm), proportions of sand, silt and clay, concentration of organic and inorganic carbon) between the eastern and the western part (Gerwin et al. 2010). The primary mineral component in all particle size fractions at t_0 was quartz (only small amounts of K-feldspar, plagioclase). Calcite comprised 0.5-4.5 % of the initial sediment, dolomite was only detectable in few samples with contents of 0.5 % and magnesite ($MgCO_3$) was not detectable by mineralogical analysis (W. Schaaf, pers. comm., 2011). ~~In October 2007 four soil pits in combination with suction cups were installed at 'Chicken Creek' Creek for soil solution analyses.~~ For detailed

information on site construction and ~~soil pit installation~~initial ecosystem development see Gerwin et al. (2010) and Schaaf et al. (2010), respectively.

2.2. Soil sampling

We took samples shortly after construction of ~~'Chicken Creek'~~Chicken Creek (2005, t_0) and after an ecosystem development period of about ten years (2015, t_{10}). For t_0 (no vegetation detectable) we assumed only very few biogenic siliceous structures homogenously distributed on the whole area of ~~'Chicken Creek'~~Chicken Creek, i.e., no section-specific distribution of BSi (BSi t_0 east ≈ BSi t_0 west ≈ BSi t_0 south) at the beginning of ecosystem development (~~compare~~ Puppe et al. 2016). This is why we did not sample all different sections of the catchment, but took soil samples in six field replicates to quantify BSi pools at t_0. However, for t_{10} we hypothesized section-specific differences~~changes~~ in BSi pool quantities related to section-specific vegetation dynamics~~ (Puppe et al. 2016).~~. To evaluate these differences~~changes~~ after a decade of ecosystem development and to cover the biggest possible BSi accumulation in soil we focused on spots where Si accumulating plant species, i.e., *Calamagrostis epigejos* and *Phragmites australis* became dominant (Zaplata et al. 2010). Thus we took samples in the eastern (*C. epigejos* dominant) and western (mainly *C. epigejos* dominant, one spot with *P. australis*) and southern section (*P. australis* dominant) of ~~'Chicken Creek'~~Chicken Creek.

For an accurate description of changes of abiotic soil conditions and related phytogenic Si in every section we took soil and plant samples in eastern, western and southern sections at t_0 as well as t_{10}. Erosion and deposition processes were clearly evident in the Chicken Creek catchment during the first years without plant cover. Substantial surface changes resulted from rill erosion as aerial photographs (rill network) and a comparison of photogrammetry-

based digital elevation models showed (Schneider et al. 2013). Interrill erosion did not lead to surface changes larger than about 20 cm during the first five years. Afterwards the establishment of an area-wide plant cover substantially reduces interrill erosion. Because all soil data at t_0 referred to a depth increment of 30 cm we reasonably assumed the same soil conditions for the sampled t_0-spots during the first years. Furthermore, we carefully selected sampling points at t_{10} to be not influenced by erosion, i.e., at spots with low surface roughness and outside rills. Soil samples for the determination of soil properties and plant samples were taken in five (western and southern section) and six (eastern section) field replicates at t_0 and t_{10} (Fig. 1). At every sampling point three undisturbed soil cores where taken with a core cutter (diameter = 3.4 cm, depth = 5 cm) and transferred into plastic bags. Bulk densities were calculated from dividing weight of dried (105°C) soil samples by corresponding volume.

2.3. Determination of basic soil properties

Soil samples were air dried and sieved and the fine earth fraction (<2 mm) was used for laboratory analyses. Soil pH was measured based on the DIN ISO Method 10390 (1997) in 0.01 M $CaCl_2$ suspensions at a soil to solution ratio of 1:5 (*w/v*) after a 60 minute equilibration period using a glass electrode. The total carbon content was analyzed by dry combustion using an elemental analyzer (Vario EL, Elementar Analysensysteme, Hanau, Germany). Carbonate ($CaCO_3$) was determined conductometrically using the Scheibler apparatus (Schlichting et al. 1995). Organic carbon (C_{org}) was computed as the difference between total carbon and carbonate carbon. ~~All analyses~~Analyses of basic soil properties were performed in two lab replicates per sample.

193 *2.3.1. Water Extractable Si (Si_{H2O})*

194 Water extractable Si was determined based on a method developed by Schachtschabel &
195 Heinemann (1967). Ten grams of dry soil (<2 mm) were ~~weighted~~weighed into 80 ~~ml~~mL
196 centrifuge tubes and 50 mL distilled water added together with three drops of a 0.1% NaN_3-
197 solution to prevent microbial activity. Total extraction time was seven days in which tubes
198 were shaken by hand twice a day for twenty seconds. Mechanical (constant) shaking by
199 using, e.g., a roll mixer, was avoided ~~not used~~ to prevent~~avoid~~ abrasion of mineral particles
200 colliding during shaking (McKeague & Cline 1963). The solutions were centrifuged (4000
201 rpm, 20 min), filtrated (0.45 µm polyamide membrane filters) and Si was measured by ICP-
202 OES, (ICP-iCAP 6300 DUO, Thermo Fisher SCIENTIFIC GmbH). Analyses of water extractable Si
203 were performed in two lab replicates per sample.
204
205 *2.3.2. Tiron extractable Si (Si_{Tiron}), aluminum (Al_{Tiron}) and iron (Fe_{Tiron})*
206 The Tiron ($C_6H_4Na_2O_8S_2 \cdot H_2O$) extraction ~~follows~~followed the method developed by Biermans
207 & Baert (1977), modified by Kodama & Ross (1991). It has been used to quantify amorphous
208 biogenic and pedogenic Si (Kendrick & Graham 2004), although a partial dissolution of
209 primary minerals is well known (Kodama & Ross 1991, Sauer et al. 2006). The extraction
210 solution was~~is~~ produced by dilution of 31.42 g Tiron with 800 mL of distilled water, followed
211 by addition of 100 mL sodium carbonate solution (5.3 g Na_2CO_3 + 100 mL distilled water)
212 under constant stirring. The final pH of 10.5 was~~is~~ reached by adding small volumes of a 4M
213 NaOH-solution. For the extraction 30 mg of dry soil were~~is weighted~~ weighed into 80 mL
214 centrifuge tubes and a 30 mL aliquot of the Tiron solution was added. The tubes were~~are~~
215 then heated at 80°C in a water bath for 1h. The extracted solutions were centrifuged at 4000
216 rpm for 30 min, filtrated (0.45 µm polyamide membrane filters, Whatman NL 17) and Si, Al

and Fe measured by ICP-OES. Analyses of Tiron extractable Si, Al and Fe were performed in three lab replicates per sample.

2.4. Microscopical analyses of diatoms, sponge spicules and testate amoebae

~~Field fresh, i.e., not air-dried,~~ Fresh soil samples were homogenized by gentle turning of the plastic bags before air drying. ~~and afterwards~~Afterwards 2 g of fresh soil were taken per sample and stored in 8 ~~ml~~mL of formalin (4 %). Subsequently, biogenic siliceous structures, i.e., diatom frustules, testate amoeba shells and sponge spicules (Fig. 2A-D), were enumerated in soil suspensions (125 mg fresh mass (FM)) received from serial dilution (1000-125 mg soil in 8 ~~ml~~mL of water each) using an inverted microscope (OPTIKA XDS-2, objectives 20:1 and 40:1, equipped with a digital camera OPTIKAM B9).

2.5. Determination of phytoliths in soil samples

10 g of dry soil material (<2 mm) were processed in four steps (adapted from Alexandre et al. 1997). First organic matter was oxidized using H_2O_2 (30 Vol. %), HNO_3 (65 Vol. ~~%),~~%) and $HClO_4$ (70 Vol. %) at 80°C until reaction subsides. Secondly, carbonates and Fe oxides were dissolved by boiling the sample in HCl (10 Vol. %) for 30 min. Thirdly, the <2 µm granulometric fraction was removed by dispersion of the remaining solid phase of step 2 with 2 Vol. % sodium hexametaphosphate solution (6–12 h), centrifugation at 1000 rpm for 2–3 min, and subsequent decantation. Finally, the phytoliths were separated by shaking the remaining solid phase of step 3 with 30 mL of sodium polytungstate ($Na_6(H_2W_{12}O_{40})\cdot H_2O$) with a density of 2.3 g cm^{-3}~~,~~ and subsequent centrifugation at 3000 rpm for 10 min~~,~~. Afterwards, ~~carefully pipetting~~ the supernatant was carefully pipetted ~~,~~ and ~~filtering~~

240 ~~by~~filtered using 5 µm teflon filters. This step was repeated three times. The filter residue was
241 washed with water, bulked, dried at 105°C, and weighted.
242
243 *2.6. Quantification of biogenic Si pools*
244 In general, biogenic siliceous structures consist of hydrated amorphous silica ($SiO_2 \cdot nH_2O$).
245 We assumed an average water content of about 10 % for these structures to avoid an
246 overestimation of BSi pools (Mortlock & Froelich 1989).
247 Protophytic Si pools (represented by diatom frustules) were quantified by multiplication of Si
248 contents per frustule with corresponding individual numbers (see Puppe et al. 2016).
249 Protozoic Si pools (represented by testate amoebae) were quantified by multiplication of
250 silica contents of diverse testate amoeba taxa (Aoki et al. 2007) with corresponding
251 individual numbers (living plus dead individuals, for details see Puppe et al. 2014, 2015).
252 Zoogenic Si pools (represented by sponge spicule fragments) were calculated by multiplying
253 volumes (μm^3) of the found spicule fragments with the density of biogenic Si (2.35 g cm^{-3})
254 and summing up the results. Volume measurements were conducted using a laser scanning
255 microscope (Keyence VK-X110, magnification 200-2.000x) (details in Puppe et al. 2016). For
256 laser scanning microscopy spicule fragments were taken from soil suspensions by
257 micromanipulation, washed in dist. H_2O and placed on clean object slides. After air drying
258 images of spicule fragments were acquired (software Keyence VK-H1XVD) and analyzed
259 (software Keyence VK-H1XAD).
260 Phytogenic Si pools were estimated by multiplying the numbers of found phytoliths with
261 corresponding mean volumes (μm^3) of phytoliths, multiplying these results with the density
262 of biogenic Si (2.35 g cm^{-3}) and summing up the results. Volume measurements with the
263 laser scanning microscope of 30 typical elongate (Fig. 2E) and 30 typical bilobate phytoliths

(Fig. 2F) resulted in mean volumes of 3765 µm^3 and 707 µm^3, respectively. For laser scanning microscopy extracted phytoliths were placed on clean object slides and images were acquired and analyzed analogous to sponge spicules. For bilobate phytoliths we measured the upper half per phytolith and doubled the result to obtain the corresponding total volume, thus we assumed bilobate phytoliths to be symmetric. We assumed phytoliths to consist of 95 % SiO_2 and 5 % other elements, i.e., carbon (Song et al. 2012) and other elements like iron, aluminum or calcium (Buján 2013).

BSi pools (mg m^{-2}) were calculated considering bulk density (g cm^{-3}), thickness (5 cm) and – for protistic and zoogenic Si pools – water content (% of fresh mass) per soil sample. Silica (M = 60.08 g mol^{-1}) pools were converted to Si (M = 28.085 g mol^{-1}) pools by multiplication with 28/60 (details in Puppe et al. 2014, 2015, 2016).

2.7. Plant analyses

Plant ~~(aboveground plant material only)~~ and litter samples of *C. epigejos* and *P. australis* were collected in the summer of 2015. In general, monomeric silicic acid (H_4SiO_4) enters the plant via its roots and is carried in the transpiration stream towards transpiration termini. When water evaporates, silicic acid becomes supersaturated and is precipitated as hydrated silica in the form of phytoliths. The vast majority of Si in plants is located at the transpiration termini (e.g., leaves) in the aerial plant parts, while considerably less Si can be found in other plant portions like stems, roots and rhizomes. Sangster (1983), for example, found no significant Si depositions in rhizomes of *P. australis*. Consequently, we only analyzed the aboveground vegetation (including transpiration termini and stems). The collected plant material was washed with distilled water to remove adhering soil minerals and oven-dried at 45°C for 48 hours.

288 *2.7.1. Total Si content in plant materials*

289 Plant samples were milled using a knife mill (Grindomix GM 200, Retsch) in two steps: 4.000
290 rpm for 1 min and then 10.000 rpm for 3 min. Sample aliquots of approximately 100 mg
291 were digested under pressure in PFA digestion vessels using a mixture of 4 ~~ml~~mL distilled
292 water, 5 ~~ml~~mL nitric acid (65 %), and 1 ~~ml~~mL hydrofluoric acid (40 %) at 190°C using a
293 microwave digestion system (Mars 6, CEM). A second digestion step was used to neutralize
294 the hydrofluoric acid with 10 ~~ml~~mL of a 4 %-boric acid solution at 150°C. Silicon was
295 measured by ICP-OES (ICP-iCAP 6300 Duo, Thermo Fisher Scientific GmbH) with an internal
296 standard. To avoid contamination, plastic equipment was used during the complete
297 procedure. Analyses of total Si content were performed in three lab replicates per sample.
298

299 *2.7.2. Determination of phytoliths in plants and litter*

300 Plant material was washed with distilled water and oven-dried at 45°C for 48 hours. Removal
301 of organic matter was conducted by burning the samples in a muffle furnace at 450°C for 12
302 hours. Next, the material was subject to additional oxidation using 30 % H_2O_2 for 12 hours.
303 The obtained material was filtered through a teflon filter with a mesh size of 5 µm. The
304 isolated phytoliths and siliceous cast (>5 µm) were subject to analysis via polarized light
305 microscopy (Nikon ECLIPSE LV100 microscope) for full characteristics. We used laser
306 scanning microscopy for measurements of the surface-area (μm^2) of the 30 typical bilobate
307 and 30 typical elongated phytoliths used for volume measurements (see 2.6) and calculated
308 corresponding surface-area-to-volume ratios (A/V ratios) as an indicator for the resistibility
309 of these siliceous structures against dissolution. Higher A/V ratios indicate a bigger surface-
310 area available for dissolution processes.
311

312 *2.8. Statistical analyses*

313 Correlations were analyzed using Spearman's rank correlation (r_s). Significances in two-
314 sample (n = 2) cases were verified with the Mann-Whitney U-test. For *k*-sample (n > 2) cases
315 the Kruskal-Wallis analysis of variance (ANOVA) was used followed by pairwise multiple
316 comparisons (Dunn's post hoc test). Statistical analyses were performed using software
317 package SPSS Statistics (version 19.0.0.1, IBM Corp.).

318

319 **3. Results**

320 *3.1. Basic soil parameters*

321 Soils at the initial state (t_0) showed in the upper 5 cm organic carbon contents (C_{org}) between
322 1.1 g kg^{-1} and 4.4 g kg^{-1} in the western section, 0.8 g kg^{-1} and 1.8 g kg^{-1} in the eastern section
323 and 0.2 g kg^{-1} and 3.3 g kg^{-1} in the southern section. This corresponded to mean carbon
324 stocks of 237 g m^{-2} (west), 123 g m^{-2} (east) and 160 g m^{-2} (south, Table 2~~1~~). After 10 years
325 (t_{10}) of ecosystem development the C_{org} stocks increased up to a factor of 3 (396-556 g m^{-2} in
326 the upper 5 cm) compared to corresponding values at t_0. This resulted in a surprisingly high
327 mean annual CO_2-C sequestration rate of 27-32 g m^{-2} (upper 5 cm). Hereby the largest C_{org}
328 stock changes were found in the western section of the area followed by the eastern section
329 and the southern section (Table 2~~1~~).
330 The carbonate contents ($CaCO_3$) at t_0 varied between means of 1.0 g kg^{-1} (west), 0.9 g kg^{-1}
331 (east) and 1.8 g kg^{-1} (south). The corresponding stocks were 88 g m^{-2} (west), 91 g m^{-2} (east)
332 and 174 g m^{-2} (south, Table 2~~1~~). The carbonate pools in the western and eastern section
333 were very similar, while the high carbonate values in the southern section were due to the
334 original soil properties. At t_{10} the distribution of carbonate was as follows: in the western
335 section there was an increase of about 17 % (from 88 g m^{-2} to 101 g m^{-2}), in the eastern part

a distinct decrease of about 67 %~~one third~~ (from 91 g m^{-2} to 30 g m^{-2}) was detected and in the southern section again a decrease of about 28 % (from 174 g m^{-2} to 126 g m^{-2}) was identified.

At t_0 the pH values of the soils showed a range between 7.9 and 8.3 (Table 2~~1~~) with relatively low variation between the different sections. After 10 years the pH values decreased to 7.1-7.4 in all sections.

3.2. Water and Tiron extractions

The mean water soluble Si (Si_{H2O}) contents in the upper 5 cm showed low variation between the different sections at t_0: 7.3 mg kg^{-1} ~~x 10^{-3}~~ (west), 7.2 mg kg^{-1} ~~x 10^{-3}~~ (east) and 8.6 mg kg^{-1} ~~x 10^{-3}~~ (south). The corresponding stock values were 0.7 g m^{-2} (west), 0.87 g m^{-2} (east) and 0.84 g m^{-2} (south) for all sections at t_0 (Table 2~~1~~). After 10 years (t_{10}) an overall significant increase of Si_{H2O} in each of the different sections compared to t_0 was found. The corresponding stock values were 1.7 g m^{-2} (west), 1.5 g m^{-2} (east) and 2.2 g m^{-2} (south, Table 2~~1~~).

At t_0 the mean Tiron extractable Si contents in the upper 5 cm varied between 5.5 g kg^{-1} (west), 5.2 g kg^{-1} (east) and 4.1 g kg^{-1} (south). The related stock values were 524 g m^{-2} (west), 503 g m^{-2} (east) and 399 g m^{-2} (south, Table 2~~1~~). After 10 years (t_{10}) the Tiron extractable Si content showed a slight increase in the western section to 6.5 g kg^{-1} (552 g m^{-2}), while the concentration in the eastern section decreased significantly to 2.6 g kg^{-1} (196 g m^{-2}, Table 2~~1~~). In the southern section only a slight decrease to 3.8 g kg^{-1} (317 g m^{-2}) was found. The Al and Fe extractable Tiron contents followed the distribution of the Si concentrations with one exception in the western section, where contrary to Si the Al and the Fe contents slightly increased at t_{10} (Table 2~~1~~). Si/Al ratios ranged between 1.6 and 2.2 at ~~'Chicken Creek'~~Chicken

Creek. Tiron extractable Si and Al fractions as well as Tiron extractable Al and Fe fractions were strongly correlated (Table 3~~2~~).

3.3. Biogenic Si pools in soils

In general, total biogenic Si pools increased in every section after ten years of ecosystem development with statistically significant differences between t_0 (11.6 ± 6.5 mg Si m^{-2}) and the southern section at t_{10} (96.0 ± 87.2 mg Si m^{-2}) (Fig. 3). Total BSi showed strong positive and statistically significant correlations to water soluble Si (Table 3~~2~~). Phytogenic (phytoliths >5 µm) Si pools ranged from 0-18 mg m^{-2} (mean: 6.6 mg m^{-2}) at t_o and significantly increased to means of 20.7 mg m^{-2} (range: 7-52 mg m^{-2} ~~(eastern section~~) and 12.9 mg m^{-2} (range: 14-15 mg m^{-2} ~~(southern section~~) at the eastern and southern section during 10 years, respectively (Fig. 4A). Protophytic Si pools (diatom frustules) ranged from 0-7 mg m^{-2} (mean: 2.6 mg m^{-2}) at t_0 and increased up to a mean of 47.4 mg m^{-2} (range: 0.1-162 mg m^{-2}) at t_{10} (southern section) (Fig. 4B). At t_0 no sponge spicules were found with one exception representing an extreme value (12.7 mg m^{-2}). After one decade of ecosystem development zoogenic Si pools increased to a maximum of 46 mg m^{-2} at the southern section (t_{10}) (Fig. 4C). Protozoic Si pools were zero at t_0 with one exception representing an extreme value (1.8 mg m^{-2}) and significantly increased to 4.6 mg m^{-2} (range: 1-11 mg m^{-2}) and 11.5 mg m^{-2} (range: 2-36 mg m^{-2}) in the eastern and the southern section at t_{10}, respectively (Fig. 4D).

At t_0 most BSi (>50 %) is represented by phytoliths >5 µm followed by diatom frustules, sponge spicules and testate amoeba shells (Fig. 5). After ten years of ecosystem development the proportion of the different BSi pools to total BSi changed. While the proportion of protozoic Si pools increased in all sections at t_{10}, the other BSi pools showed more variable changes over time. The proportion of phytogenic Si pools either increased

383 (western section) or decreased (eastern and southern sections). In contrast, the proportion
384 of protophytic Si pools decreased at the western section and increased in the eastern and
385 southern sections. The proportion of zoogenic Si pools decreased in the western and eastern
386 sections, but increased slightly in the southern section at t_{10}.
387
388 *3.4. Phytoliths and total Si content in plant materials*
389 The total content of Si was determined for two Si accumulating plant species *Calamagrostis*
390 *epigejos* and *Phragmites australis* dominating distinct catchment sections. For *C. epigejos*
391 the mean total content of Si was 2.25 % (range: 1.8-3.1 %), whereas for *P. australis* a mean
392 total Si content of 2.70 % (range: 2.0-3.2 %) was determined (Fig. 6A, B). For litter we found
393 mean total Si contents of 3.1 % (range: 2.8-3.3 %) and 2.9 % (range: 1.7-3.2 %) for *C. epigejos*
394 and *P. australis*, respectively.
395 Phytoliths >5 µm were also isolated from both plants; for *C. epigejos* the mean extracted
396 phytolith content was 0.37 % (range: 0.31-0.46 %), whereas for *P. australis* a mean phytolith
397 content of 0.43 % (range: 0.37-0.50 %) was determined (Fig. 6A, B), i.e., related to the total
398 Si content of plants 16.4 % and 15.9 % of phytogenic Si were represented by extracted
399 phytoliths >5 µm in *C. epigejos* and *P. australis*, respectively. Thus, small-scale (<5 µm)
400 and/or fragile (siliceous structures mostly thinner than 5 µm, but up to several hundred
401 micrometers long, Fig. 7) phytogenic Si represented 83.6 % and 84.1 % of total phytogenic Si
402 in *C. epigejos* and *P. australis*, respectively. Mean extracted phytolith contents in plant litter
403 were 0.47 % (range: 0.35-0.70 %) and 0.51 % (range: 0.41-0.59 %) for *C. epigejos* and *P.*
404 *australis*, respectively.
405 Surface-areas of 30 typical bilobate and 30 typical elongate phytoliths were in a range of 216
406 µm^2 to 3,730 µm^2 and 2,302 µm^2 to 22,203 µm^2, respectively (Table 4~~3~~). The corresponding

volumes of bilobate and elongate phytoliths were in a range of 36 µm^3 to 2,046 µm^3 and 390 µm^3 to 14,649 µm^3, respectively. Surface-to-volume ratios of bilobate and elongate phytoliths were in a range of 0.7 to 9.8 and 0.6 to 5.9 with means of 2.8 and 2.6, respectively.

3.5. BSi and Si fractions under Calamagrostis epigejos *and* Phragmites australis ~~*Synopsis of ecosystem development in respect to Si*~~

~~In Figure 6 a comprehensive overview of the different pools and their changes over time is given. Our main findings are: water~~Water soluble Si fractions increased by 99 % and 163 %, total BSi by 281 % and 660 % after ten years of ecosystem development in soils under *C. epigejos* and *P. australis*, respectively (Fig. 6A, B). In contrast, Si_{Tiron} decreased by 42 % and 1.4 % from t_0 to t_{10} in soils under *C. epigejos* and *P. australis*, respectively. If we assume mean dry biomasses of 115 g m^{-2} and 186 g m^{-2} for *C. epigejos* and *P. australis* (M. Wehrhan, pers. comm., 2017) about 2.6 g Si m^{-2} and 5.0 g Si m^{-2} are stored in the aboveground biomass at ~~'Chicken Creek'~~Chicken Creek at t_{10}, respectively. For litter of *C. epigejos* and *P. australis* (mean dry biomasses of 59 g m^{-2} and 94 g m^{-2} at t_{10}, M. Wehrhan, pers. comm., 2017) we calculated corresponding pools of about 1.8 g Si m^{-2} and 2.7 g Si m^{-2} at t_{10}, respectively.

4. Discussion

4.1. Drivers of short-term changes of water soluble Si at ~~'Chicken Creek'~~Chicken Creek

In general, weathering of silicates represents the ultimate source of $Si(OH)_4$ in terrestrial biogeosystems in the long term (Berner 2003). In this context, the long-term accumulation of BSi can influence the total amorphous (Tiron extractable) Si as it is known from forested catchments or old chronosequence soils (Conley et al. 2008, Kendrick & Graham 2004,

Saccone et al. 2008). Contrary, short-term changes of BSi pools likely do not influence Tiron extractable Si in initial soils (total BSi represents only 0.002-0.03 % of Tiron extractable Si at ~~'Chicken Creek'~~Chicken Creek). Thus, the major proportion of Tiron extractable Si at ~~'Chicken Creek'~~Chicken Creek seems to be of pedogenic origin (e.g., Si included in Al/Fe oxides/hydroxides). This is supported by relatively low Si/Al ratios (<5) indicating a minerogenic origin of Tiron extractable Si instead of BSi as a source of Si_{Tiron} (Bartoli & Wilding 1980). We further exclude changes of Tiron extractable Si as the main driver of water soluble Si at ~~'Chicken Creek'~~Chicken Creek in the short term, because i) Si_{Tiron} and Si_{H2O} showed no statistical relationship at all and ii) a significant change of the Tiron extractable Si fraction ~~only~~ occurred only in the eastern section, whereas in the western and southern section Si_{Tiron} did not change significantly over time. We assume that these changes of Si_{Tiron} in the eastern section are related to abiotic conditions (soil pH, conductivity, skeleton content, proportions of sand, silt and clay, concentration of organic and inorganic carbon), which were slightly different to the conditions of the western section already at t_0 (Gerwin et al. 2010). Furthermore, we excluded atmospheric inputs as potential drivers of short-term changes of water soluble Si at Chicken Creek. On the one hand, dust depositions (dry deposition) at Chicken Creek are very low (73-230 mg m^{-2} d^{-1}) and only slightly above the annual average (70-90 mg m^{-2} d^{-1}) measured in the state of Brandenburg (Wanner et al. 2015). On the other hand, the total input of Si (as a lithogenic element) by precipitation (wet deposition) is negligible as well (<1 kg Si ha^{-1} yr^{-1}, Sommer et al. 2013).

Our results indicate a strong relationship between water soluble Si and total BSi. In this context, two different causal chains can be discussed: Either SiO_2-synthesizing organisms are drivers of the amount of $Si(OH)_4$ in the soil or – *vice versa* – the amount of water soluble Si in the soils is the main driver of SiO_2-synthesizing organisms as biosilicification is limited by

455 $Si(OH)_4$. Laboratory studies, for example, revealed that SiO_2-synthesizing organisms, i.e.,
456 testate amoebae, can deplete the amount of $Si(OH)_4$ in culture media due to biosilicification
457 (Aoki et al. 2007, Wanner et al. 2016). However, Wanner et al. (2016) also showed that
458 culture growth of SiO_2-synthesizing testate amoebae was dependent on Si concentration in
459 the culture media. Furthermore, *in situ* analyses showed that marine diatom blooms can
460 deplete $Si(OH)_4$ concentrations in the oceans (Hildebrand 2008). In forested biogeosystems
461 Puppe et al. (2015) found high individual numbers of SiO_2-synthesizing testate amoebae at
462 study sites with low amounts of $Si(OH)_4$ and *vice versa*. However, it is unlikely that testate
463 amoebae depleted amounts of $Si(OH)_4$ at these sites, because corresponding protozoic Si
464 pools are relatively small compared to phytogenic ones (Puppe et al. 2015, Sommer et al.
465 2013). Regarding vegetation and corresponding phytogenic Si pools their influence on the
466 amount of $Si(OH)_4$ in soils has been shown in several studies (e.g., Bartoli 1983, Farmer et al.
467 2005, Sommer et al. 2013). On the other hand, phytolith production is probably more
468 influenced by the phylogenetic position of a plant than by environmental factors like
469 temperature or Si availability (Hodson et al. 2005, Cooke & Leishman 2012).
470 From our results and the discussion above we conclude short-term changes of water soluble
471 Si to be mainly driven by BSi. However, total BSi represents only small proportions of water
472 soluble Si at t_0 (<2 %) and t_{10} (<4.5 %). From this result the question arises, where does the
473 major part of the increase in water soluble Si at ~~'Chicken Creek'~~Chicken Creek come from?
474 We will discuss this question in the subsection (4.2.) below.
475
476
477

4.2. ~~Small-scale and fragile phytogenic Si has the biggest~~ Sources of ~~impact on~~ water soluble Si at ~~'Chicken Creek'~~ Chicken Creek

From ~~further~~form~~urth~~er results of BSi analyses in forested biogeosystems, we assumed the phytogenic Si pool to be the most prominent in size. In this context, results of Sommer et al. (2013) and Puppe et al. (2015) showed that phytogenic Si pools in soils of forested biogeosystems were up to several hundred times ~~bigger~~larger than protozoic Si pools. However, phytogenic Si pools in soils are surprisingly small compared to other BSi pools at ~~'Chicken Creek'~~Chicken Creek. Our findings can be attributed to at least two reasons. Firstly, phytogenic Si is stored in a developing organic litter layer where it is temporarily protected against dissolution and secondly, the used methods were not able to accurately quantify the total phytogenic Si pool, but only the larger (>5 µm) and stable part ~~(>5 µm)~~.

Total Si and phytolith contents of litter samples at ~~'Chicken Creek'~~Chicken Creek did not differentiate from total Si and phytolith contents of plants. This fact indicates that litter decomposition and related Si release into the subjacent soil are relatively slow processes and we interpret our findings as a hint for a developing compartment of dead plant tissue above the mineral soil surface. Esperschütz et al. (2013) showed in a field experiment in initial soils near ~~'Chicken Creek'~~Chicken Creek that after 30 weeks only 50 % of the litter of *C. epigejos* were degraded, whereby degradation rates were highest in the first four weeks. Estimations of biomasses of *C. epigejos* and *P. australis* at ~~'Chicken Creek'~~Chicken Creek via remote sensing with an unmanned aerial system showed that the relation between phytogenic Si pools plant biomass and litter biomass is almost the same for both plant species (factor about 1.5, based on the total area of ~~'Chicken Creek'~~Chicken Creek), i.e., Si in the plants was about one third higher than in litter (M. Wehrhan, pers. comm., 2017, manuscript in preparation). At the sampling points about 1.8 g Si m^{-2} and 2.7 g Si m^{-2} were

stored in the litter of *C. epigejos* and *P. australis* at t_{10}, respectively, which is in the range of published data for annual Si input through litterfall in a short grass steppe (2.2-2.6 g Si m^{-2} per year, Blecker et al. 2006).

Altogether, these results clearly underline our interpretation of a developing organic layer where litter accumulates and phytogenic Si is temporarily stored and protected against dissolution, thus Si release is delayed biologically controlled as it can be observed at forested biogeosystems (Sommer et al. 2013). The Si pools in the aboveground biomass of *C. epigejos* (2.6 g Si m^{-2}) and *P. australis* (5.0 g Si m^{-2}) at ~~'Chicken Creek'~~Chicken Creek at t_{10} are comparable to reported values of Great Plains grasslands (2.2-6.7 g Si m^{-2} in the aboveground biomass) (Blecker et al. 2006) and reach about 30 % (*C. epigejos*) or 59 % (*P. australis*) of published data for a beech forest (8.5 g Si m^{-2} in the aboveground biomass of *Fagus sylvatica* trees) in northern Brandenburg, Germany (Sommer et al. 2013), after (only) ten years of ecosystem development.

Regarding methodological shortcomings of the used phytolith extraction procedure there are several aspects to be discussed. Wilding & Drees (1971), for example, showed that about 72 % of leaf phytoliths of American beech (*Fagus grandifolia*) are smaller than 5 µm. This is in accordance with our findings. Phytoliths >5 µm only amounted to about 16 % of total Si contents of plant materials of *C. epigejos* and *P. australis*, thus about 84 % of phytogenic Si (<5 µm and /or fragile phytogenic Si structures) are not quantified by the used phytolith extraction method. Watteau & Villemin (2001) found even smaller (5-80 nm) spherical grains of pure silica in leaf residues in topsoil samples of a forested biogeosystem. In addition, silica depositions can be found in intercellular spaces or in an extracellular (cuticular) layer (Sangster et al. 2001), whereat no recognizable phytoliths are formed. These structures might be too fragile for preservation in soils and are likely lost to a great extent in the used

phytolith extraction procedure due to dissolution. Meunier et al. (2017) analyzed different phytolith morphotypes, e.g, silica bodies originating from cells of the upper epidermis, silica casts of trichomes or parenchyma/collenchyma cells, of durum wheat plant shoots. They found fragile sub-cuticular silica plates (2-4 µm thick, up to several hundred micrometers long and wide) to be the second most common phytolith morphotype. This is corroborated by our own findings as the biggest part (about 84 %) of total plant Si is represented by small-scale (<5 µm) and/or fragile phytogenic Si in *C. epigejos* and *P. australis*. If we assume that total Si contents of plants at ~~'Chicken Creek'~~Chicken Creek are one-to-one reflected by phytogenic Si pools in soils we can easily calculate these small-scale and fragile pools resulting in about 130 mg m^{-2} and 100 mg m^{-2} (84 % of total, i.e., 156 mg m^{-2} and 119 mg m^{-2}, phytogenic Si each) under *C. epigejos* and *P. australis*, respectively. These calculated phytogenic Si pools are about 13 (diatom frustules), 38 (testate amoeba shells) and 45 (sponge spicules) or 3 (diatom frustules) and 10 (testate amoeba shells, sponge spicules) times bigger than the other BSi pools at *C. epigejos* and *P. australis* sampling points, respectively. If we further assume an input of this phytogenic Si for at least seven years (Zaplata et al. 2010) phytogenic Si might be the main driver of short-term changes of water soluble Si at ~~'Chicken Creek'~~Chicken Creek. This is supported by relatively high surface-to-volume ratios of bilobate and elongate phytoliths. These ratios are about three times higher compared to ratios of other biogenic siliceous structures, i.e., testate amoeba shells, diatom frustules and sponge spicules.

In addition, Si pools represented by single siliceous platelets of testate amoeba shells have to be considered as well as these platelets can be frequently found in freshwater sediments, for example (Douglas & Smol 1987, Pienitz et al. 1995). Unfortunately, there is no information on the quantity of such platelet pools in soils available, but it can be assumed

550 that these platelets can be frequently found in soils as they are used by some testate
551 amoeba genera (e.g., *Schoenbornia*, *Heleopera*) for shell construction (Meisterfeld 2002,
552 Schönborn et al. 1987). In general, it can be assumed that phytogenic Si structures <5 µm
553 and single testate amoeba platelets (about 3-12 µm in diameter, Douglas & Smol 1987) are
554 highly reactive due to their relatively high surface/volume ratios. However, to the best of our
555 knowledge there is no publication available dealing with corresponding physicochemical
556 analyses or dissolution kinetics of these siliceous structures. In general, experiments with
557 phytoliths (>5 µm) showed that surface-areas and related dissolution susceptibilities are, for
558 example, age-related due to changes in specific surface areas and the presence of organic
559 matter bound to the surface of phytoliths (Fraysse et al. 2006, 2009).
560
561 **5. Conclusions**
562 Decadal changes of water soluble Si at ~~'Chicken Creek'~~Chicken Creek are mainly driven by
563 BSi, thus Si cycling is biologically controlled already at the very beginning of ecosystem
564 development. In this context, especially phytogenic Si plays a prominent role. However, a
565 developing organic layer (L horizon) at the soil surface temporarily protects phytogenic Si
566 against dissolution, because phytogenic Si is still incorporated in plant structural elements
567 (tissues). In consequence a delaying biogenic Si pool is built up and Si release into the soil is
568 retarded. Furthermore, established phytolith extraction methods alone are not suitable to
569 quantify total phytogenic Si pools as phytoliths >5 µm seem to be only a minor part of this
570 pool (about 16 % in the current study). In general, information on small-scale (<5 µm) and/or
571 fragile phytogenic Si structures are urgently needed as they seem to represent the biggest
572 and most reactive Si pool in soils, thus the most important driver of Si cycling in terrestrial
573 biogeosystems. Future work should focus on i) the quantification of this pool, ii)

physicochemical analyses of its components, and (iii) their dissolution kinetics in lab experiments. The combination of modern microscopical (SEM-EDX, laser scanning microscopy) (this study, Puppe et al. 2016, Sommer et al. 2013) and spectroscopical (FTIR and micro-FTIR spectroscopy) (Liu et al. 2013, Loucaides et al. 2010, Rosén et al. 2010) methods might introduce new insights in this field.

Acknowledgements

This study has been financed by the DFG project 'Spatiotemporal dynamics of biogenic Si pools in initial soils and their relevance for desilication' (SO 302/7-1). Many thanks to Christian Buhtz and Reneé Ende for their excellent laboratory support. We would like to thank the members of the 'Chicken Creek project' at BTU Cottbus-Senftenberg for providing soil samples from 2005 and organizational support. Vattenfall Europe Mining AG provided the research site. This study is a contribution to the Transregional Collaborative Research Centre 38 (SFB/TRR 38) financially supported by the German Research Council (DFG, Bonn) and the Brandenburg Ministry of Science, Research and Culture (MWFK, Potsdam). Last but not least we would like to thank the anonymous reviewers for their very helpful comments on our manuscript.

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Figures and Figure captions

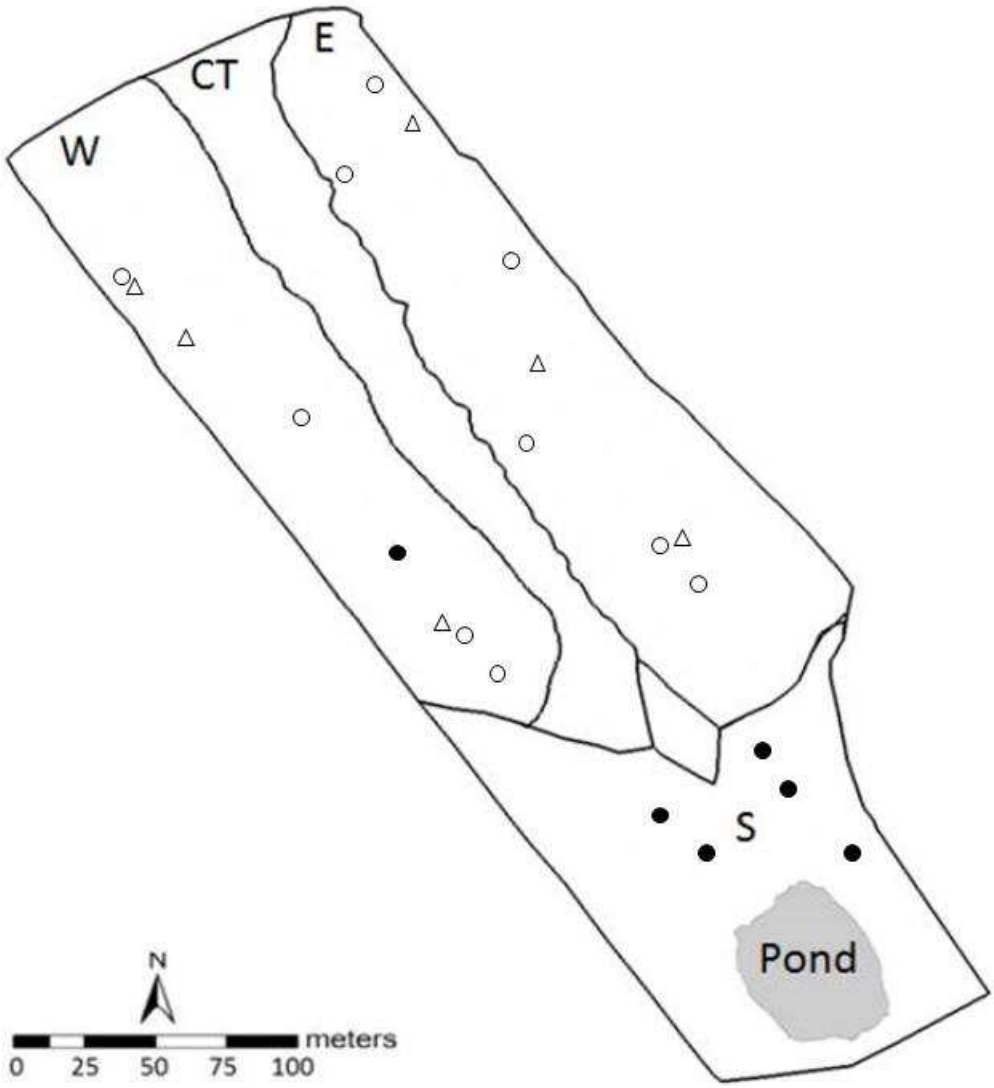


Fig. 1. Map of ~~'Chicken Creek'~~Chicken Creek (W = western section, CT = central trench, E = eastern section, S = southern section with pond). Triangles indicate the sampling points used for BSi analyses at t_0 (n = 6). Circles indicate the sampling points used for measurements of soil parameters (at t_0 and t_{10}) and plant analyses (only at t_{10}) (W, n = 5; E, n = 6; S, n = 5). Empty and filled circles represent sampling points where *Calamagrostis epigejos* and *Phragmites australis* became dominant. Note that the size of sampling points is not to scale.

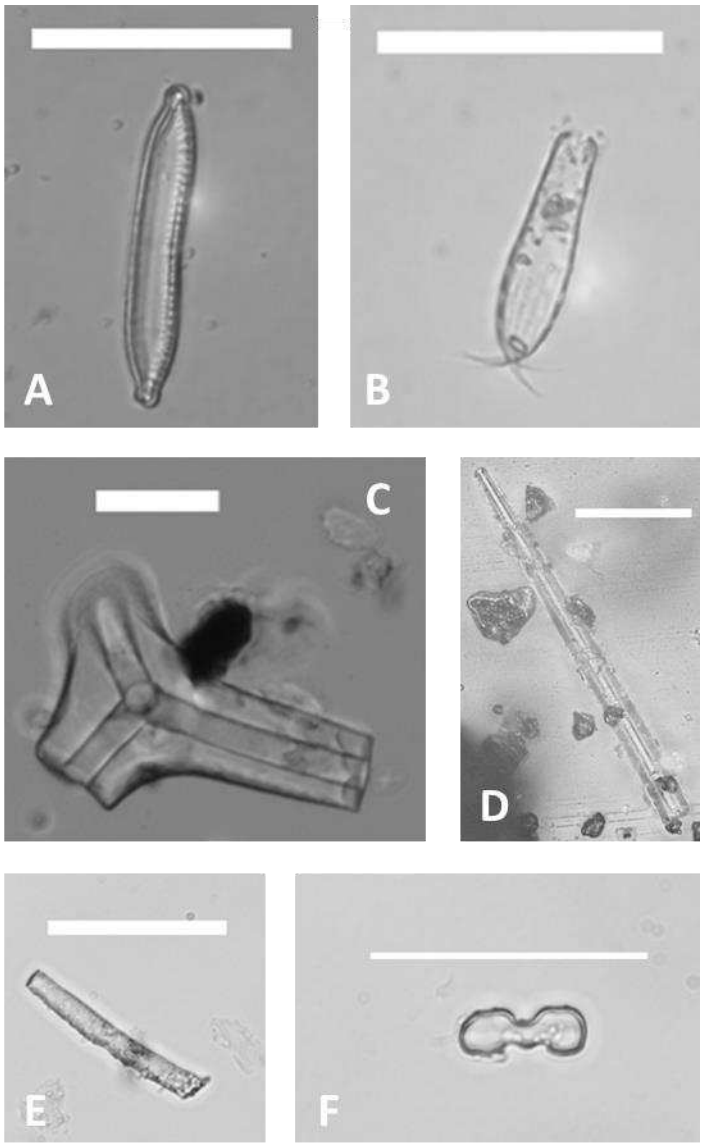
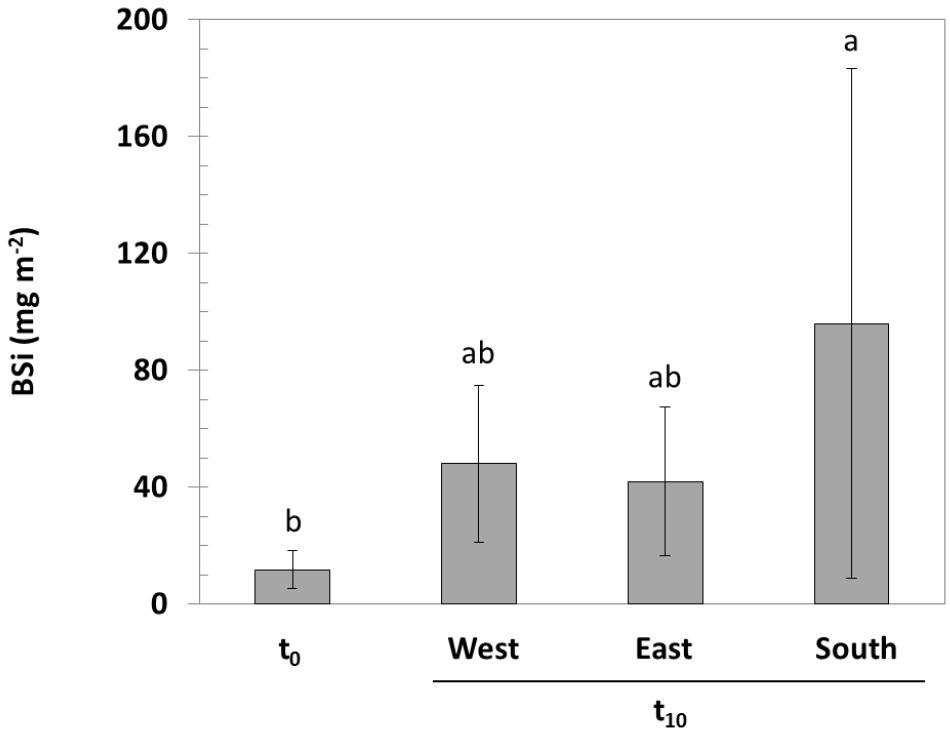


Fig. 2. Micrographs (light microscope) of biogenic silica structures found at ~~'Chicken Creek'~~Chicken Creek. A) pennate diatom (valve view), B) testate amoeba shell (*Euglypha cristata*), C) and D) sponge spicules (fragments), E) elongate phytolith and F) bilobate phytolith. All scale bars: 50 µm.



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810 **Fig. 3.** Total biogenic Si pools in soils (means ± standard deviation, upper 5 cm) at ~~'Chicken~~

811 ~~Creek'~~Chicken Creek at the end of construction work (t_0) and after ten years of ecosystem

812 development (western, eastern and southern sections, t_{10}). Significant differences are

813 indicated by different letters ($p < 0.05$, Kruskal-Wallis ANOVA with Dunn's post hoc test).

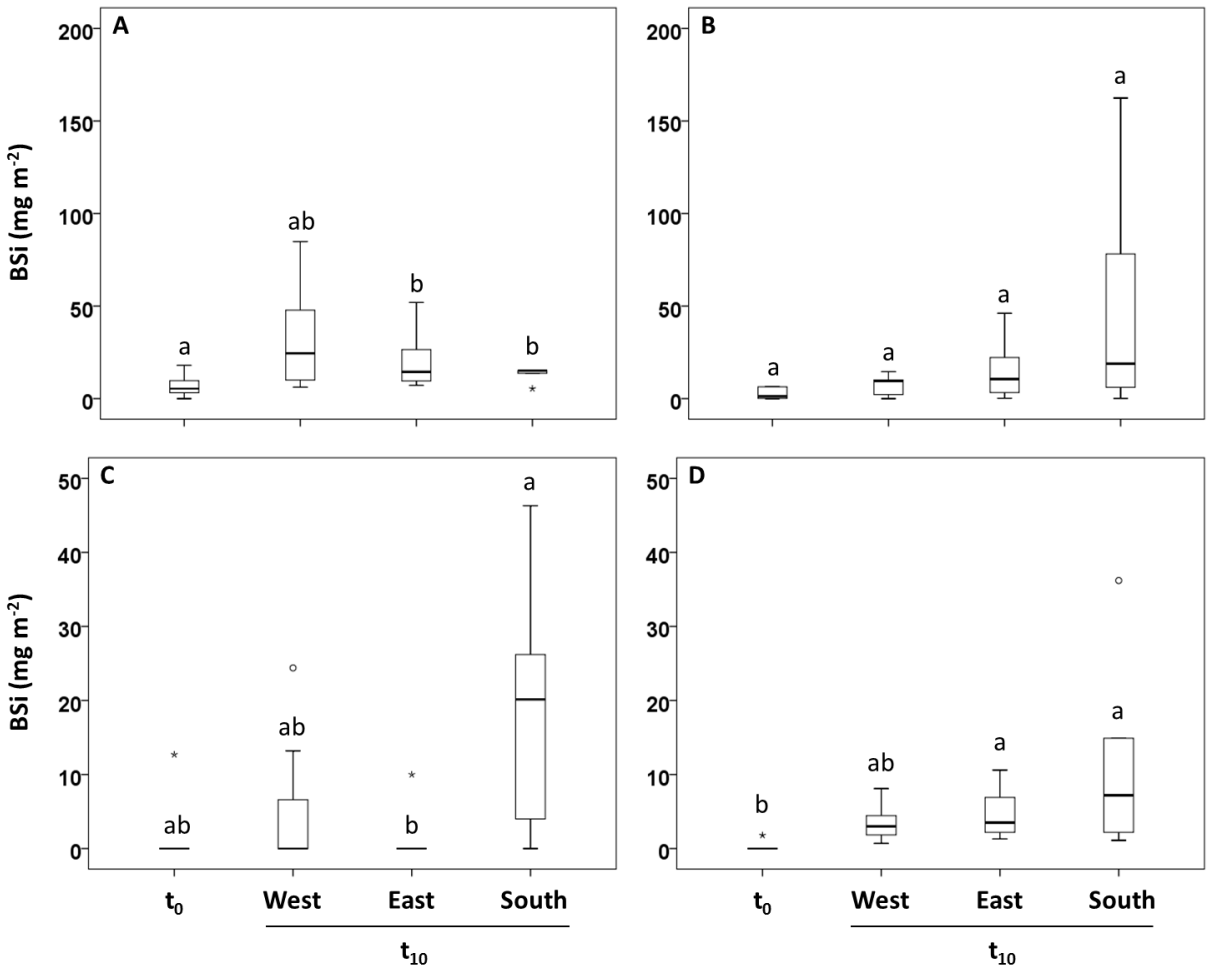


Fig. 4. Boxplots (top, middle and bottom lines of the boxes show the 25th, 50th and 75th percentiles, respectively, and whiskers represent 1.5× the inter-quartile ranges) of biogenic Si pools in soils (upper 5 cm) at ~~'Chicken Creek'~~Chicken Creek at the end of construction work (t_0) and after ten years of ecosystem development (western, eastern and southern sections, t_{10}). A) Phytogenic Si pools (phytoliths), B) protophytic Si pools (diatom frustules), C) zoogenic Si pools (sponge spicules) and D) protozoic Si pools (testate amoeba shells). Significant differences are indicated by different letters ($p < 0.05$, Kruskal-Wallis ANOVA with Dunn's post hoc test). Circles and asterisks indicate outliers and extreme values, respectively. Note different scales for diagrams A+B and C+D~~C and D~~.

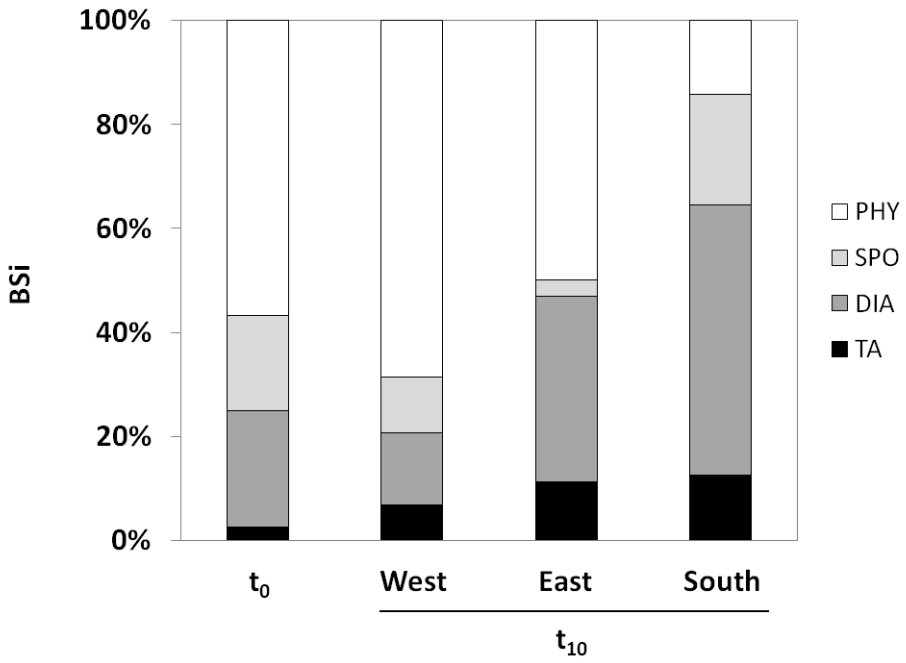
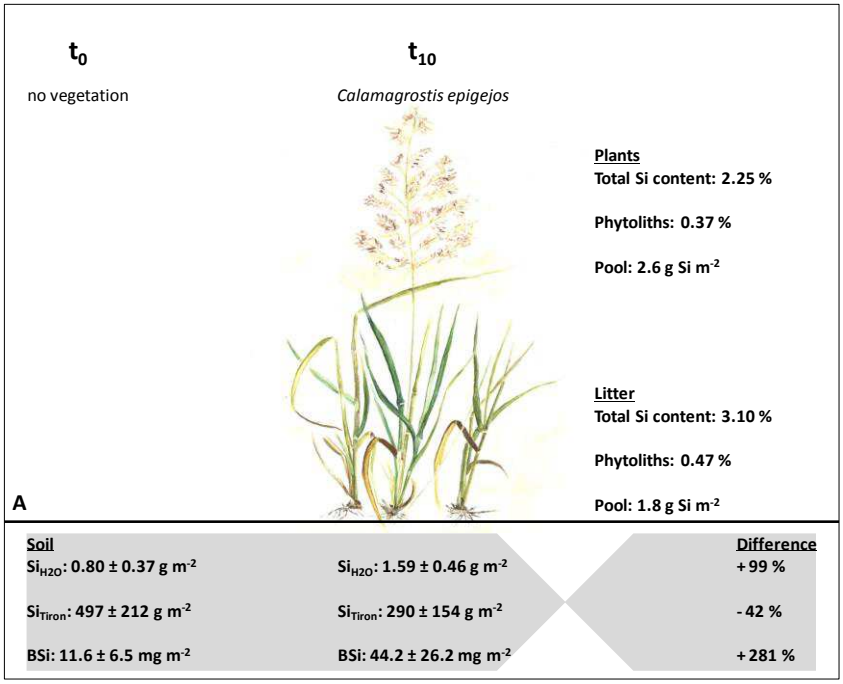


Fig. 5. Proportion of phytoliths (PHY), sponge spicules (SPO), diatom frustules (DIA) and testate amoeba shells (TA) to total BSi in soils (upper 5 cm) at ~~'Chicken Creek'~~Chicken Creek at t_0 and t_{10}. Note that total BSi pools differ in size (see Fig. 3).



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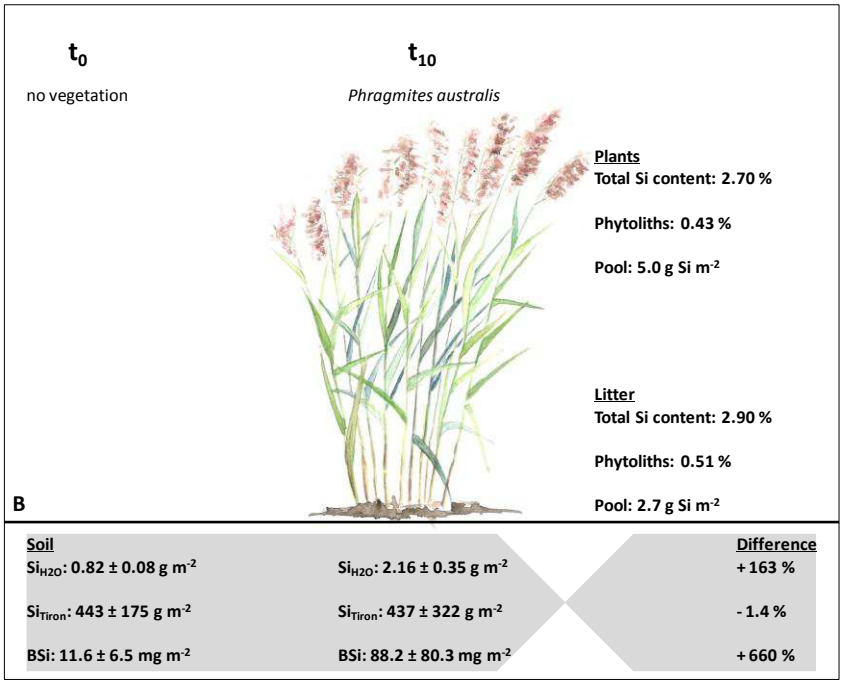

843

844 **Fig. 6.** Comparison of water soluble Si (Si_{H2O}) as well as amorphous Si (Si_{Tiron}) fractions and
845 total BSi in soils (means ± standard deviation, upper 5 cm), where *Calamagrostis epigejos* (A)
846 and *Phragmites australis* (B) became dominant. Data are given for t_0 (no vegetation) and t_{10}
847 (*C. epigejos*, *P. australis*). For t_{10} total plant Si contents, ~~phytolith~~ extracted phytogenic Si
848 (phytoliths) contents and Si pools for *C. epigejos* and *P. australis* (plants and litter) are stated
849 in addition. Paintings from Cornelia Höhn, Müncheberg.

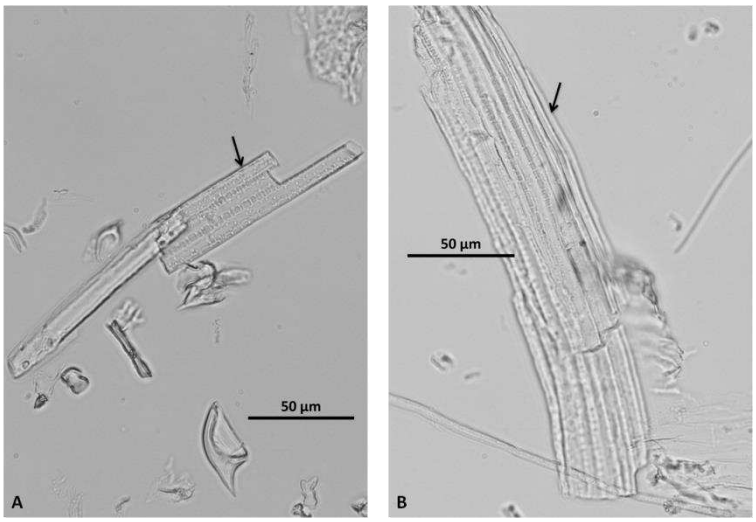

Fig. 7. Micrographs of fragile phytogenic Si structures (arrows) of *C. epigejos* (A) and *P. australis* (B).

Tables and Table headings

Table 1. Contents of skeleton (>2 mm), fine earth (<2 mm), sand, silt and clay fractions (upper 30 cm) at the sampling points in western, eastern and southern sections at Chicken Creek (t_0, calculations based on data of Gerwin et al. 2010). Minimal (Min) as well as maximal (Max) values, means ($\bar{x}$) and standard deviations (SD) are given.

Section		>2 mm	<2 mm	Sand	Silt	Clay
		%			%	
West	Min	9	80	77	7	5
	Max	20	91	88	13	10
	$\bar{x}$	13	87	83	10	7
	SD	5	5	4	2	2
East	Min	2	77	69	6	4
	Max	23	98	91	20	11
	$\bar{x}$	13	87	82	11	7
	SD	7	7	9	6	3
South	Min	0.2	84	78	7	4
	Max	17	99.8	89	17	8
	$\bar{x}$	8	92	83	11	6
	SD	8	8	4	4	2

Table 21. Measured soil parameters (upper 5 cm, means ($\bar{x}$) with standard deviation (SD)) at the different sections of ~~'Chicken Creek'~~Chicken Creek. Significant differences between t_0 and t_{10} are each stated in bold (Mann-Whitney U-test, p <0.05) or marked with asterisks (p <0.1) for the western, eastern and southern section.

Age	Section		Si_{H2O}	Si_{Tiron}	Al_{Tiron}	Fe_{Tiron}	C_{org}	$CaCO_3$	pH
			g m^{-2}						
t_0	West	$\bar{x}$	**0.70**	524	312	249	237*	88	**7.9**
		SD	0.10	95	24	33	156	72	0.1
t_{10}	West	$\bar{x}$	**1.73**	552	254	239	556*	101	**7.4**
		SD	0.22	300	154	104	167	93	0.1
t_0	East	$\bar{x}$	0.87*	**503**	**268**	**261**	**123**	91	**8.1**
		SD	0.48	281	151	130	38	79	0.2
t_{10}	East	$\bar{x}$	1.50*	**196**	**122**	**151**	**396**	30	**7.1**
		SD	0.57	49	27	29	54	18	0.2
t_0	South	$\bar{x}$	**0.84**	399	232	238*	160*	174	**8.3**
		SD	0.06	154	112	65	131	109	0.1
t_{10}	South	$\bar{x}$	**2.24**	317	147	157*	474*	126	**7.4**
		SD	0.33	149	62	57	258	40	0.1

Table 32. Spearman's rank correlations between measured soil parameters and total BSi (upper 5 cm, n = 6) at ~~'Chicken Creek'~~Chicken Creek. Significant correlation coefficients are given in bold (p <0.05).

	Si_{H2O}	Si_{Tiron}	Al_{Tiron}	Fe_{Tiron}	C_{org}	$CaCO_3$	pH	BSi
Si_{H2O}	1.000							
Si_{Tiron}	-0.257	1.000						
Al_{Tiron}	-0.600	**0.829**	1.000					
Fe_{Tiron}	-0.486	0.771	**0.943**	1.000				
C_{org}	0.714	0.086	-0.371	-0.486	1.000			
$CaCO_3$	0.200	0.086	-0.086	-0.029	0.029	1.000		
pH	-0.600	0.200	0.486	0.543	-0.771	0.543	1.000	
BSi	**0.941**	-0.213	-0.577	-0.577	**0.880**	0.152	-0.698	1.000

Table ~~43~~. Surface-areas, volumes and surface-to-volume ratios (A/V) of different biogenic siliceous structures found at ~~'Chicken Creek'~~Chicken Creek.

	Surface-area (µm^2)		Volume (µm^3)		A/V ratio	
	Min.	**Max.**	**Min.**	**Max.**	**Range**	**Mean (SD)**
Bilobate phytoliths	216	3,730	36	2,046	0.7-9.8	2.8 (1.8)
Elongate phytoliths	2,302	22,203	390	14,649	0.6-5.9	2.6 (1.1)
Diatom frustules*	351	9,901	347	28,024	0.3-3.3	0.9 (0.5)
TA shells*	1,229	5,085	900	15,812	0.2-2.7	0.8 (0.7)
Sponge spicules*	305	16,963	291	59,744	0.3-1.6	0.8 (0.4)
Spicule fragments*	2,828	17,268	5,255	34,812	0.5-0.6	0.5 (0.03)

* Data taken from Puppe et al. (2016).