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Silicon isotopes in juvenile and mature *Cyperus papyrus* from the Okavango Delta, Botswana

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Abstract. The three most abundant stable isotopes of Silicon (Si), ^{28}Si, ^{29}Si, and ^{30}Si, all occur in plants. Isotope studies are a potential tool to explore uptake and function of plant Si, and it is a developing field. However, there is a lack of studies from natural environments, and species from the African continent, and all plant parts including reproductive structures. In this study, naturally grown papyrus plants were sampled from the Okavango Delta and divided into five organs, i.e. umbel, culm, scales, rhizome, and roots. Samples were analysed for TN, TOC, BSi, TP concentrations, and for Si isotopes. Each organ of papyrus is represented by two samples, one from juvenile tissue and one mature (apart from the roots where age is difficult to determine). The study confirms that papyrus is a high Si-accumulating species, with BSi ranging from 0.88% in rhizomes to 6.61% in roots. High Si precipitation in the roots leads to an enrichment in heavy Si isotopes in the residual mobile Si pool, as light Si isotopes precipitate in phytoliths in the roots, even though in this study phytoliths were identified for all organs except for roots. In papyrus, shoot organs gradually become enriched in heavy Si isotopes along the transpiration stream, with an increase in heavy isotopes from rhizomes to scales, culm, and umbel, same pattern that has been observed for other plants in literature.

1 Introduction

Silicon (Si) is the second most abundant element in the Earth's crust, and an important element in global biogeochemical cycles in which plants are a key driver. Silicon is known to be beneficial for plant growth, predominantly through the alleviation of the impacts of a range of both abiotic and biotic stresses (Cooke et al., 2016; Frew et al., 2018). Si is taken up by plants as silicic acid and deposited as amorphous silica (subsequently termed biogenic silica, BSi) in phytoliths and other smaller deposits. Plant Si accumulation varies enormously among species with clear phylogenic patterns (Epstein, 1999). Much of the knowledge about Si accumulation comes from agricultural studies, and crop species have been classified as active, passive, or restricted in Si uptake, depending on their level of Si accumulation (Epstein, 1999). This variation in Si uptake implies that the benefits associated with Si will also vary at the species level or even at the individual plant level

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providing some plants with competitive advantages (Cooke and Leishman, 2011). Though there is comprehensive evidence for the beneficial importance of Si accumulation in plants, the accumulation costs and links between fitness and environment are not yet fully understood (de Tombeur et al., 2023). The mechanisms of uptake, allocation and drivers for variation amongst species remain subject to debate also (Frick et al., 2020).

The three most abundant stable isotopes of Silicon, ^{28}Si, ^{29}Si, and ^{30}Si, all occur in plants. Most plants, across the spectrum of low to high Si-accumulating, preferentially uptake light Si isotopes from the surrounding environment (Frick et al., 2020). Fractionation during the uptake of Si by plant roots had been previously noted (L. Sun et al., 2008). The successive precipitation of light isotopes into phytoliths has been used to explain Si isotope fractionation in shoots (Dupuis et al., 2015). The result is an enrichment of heavy isotopes that are then transported along the transpiration stream and eventual deposition of the heaviest isotopes at transpiration termini. This leads to a Rayleigh fractionation (Y. Sun, Wu, Li, et al., 2016), a process that, in general, describes isotopic partitioning between two reservoirs where one reservoir decreases in size (Kendall et al., 2014).

Isotope studies have been identified as a potential tool to explore uptake and function of plant Si, and it is a developing field. Studies on Si isotopes in plants have been carried out on crop species grown in the laboratory, including tomato, mustard, wheat, rice, cucumber, and banana, and in bamboo (Ding et al., 2005, 2008; Opfergelt et al., 2006; L. Sun et al., 2008; Y. Sun, Wu, and Li, 2016; Y. Sun, Wu, Li, et al., 2016). In previous studies, plant seeds were germinated and grown under controlled conditions, with a constant supply of nutrient solutions (Ding et al., 2008; Opfergelt et al., 2006; L. Sun et al., 2008). There is a lack of studies from natural environments, and species from the African continent, and all plant parts, including reproductive structures. More studies are needed to understand if Si isotopes are indeed a promising tool to resolve outstanding questions around plant Si use, and biogeochemical studies.

The Poales are an order characterised by high Si accumulation (Hodson et al., 2005) with rice arguably the best studied species in relation to Si. The largest families are Poaceae, Cyperaceae, Bromeliaceae and Eriocaulaceae. *Cyperus* is one of the major genera in the Cyperaceae and contains more than 600 tropical and subtropical species (Denny, 1985). *Cyperus papyrus*, or papyrus, is perhaps the most ecologically important aquatic emergent in the genus. The productivity of *C. papyrus* ranges between 48 and 143 t/ha/year (Thompson et al., 1979). At the top of the culm, there is a spherical and large reproductive umbel which acts as the main photosynthetic surface (Jones and Humphries, 2002). Papyrus is native to Africa (Thompson, 1976), but it can also be found in Madagascar, Sicily (Italy), and in the Levant countries, and it is invasive in the USA. It is also one of the biggest sedges worldwide, reaching up to 5 metres in height (Jones and Muthuri, 1985). Papyrus swamps are typically found in stable hydrological regimes, but they can also survive with varying water levels. In the Okavango Delta (Botswana), papyrus swamps make up ~20% of the total reed swamp (Denny, 1985). The most important plant family in permanently herbaceous swamps is Cyperaceae.

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The Okavango Delta is historically important as a source of water during drought and dry seasons for both wildlife and livestock. It is also economically important as a source of tourism and of water for northern and central Botswana. Moreover, it is ecologically important as it supports a significant amount of Botswana's wildlife and because of its biodiversity, its association of abundant water and the arid environment in which the Okavango Delta resides, and because of the impressive paleoclimatic record contained in its sediments (Shaw, 1988). The Panhandle is a permanently flooded landscape in the Okavango Delta characterised by the flowing of the Okavango River, one of the largest rivers in southern Africa. This river forms in the Angola highlands and then flows southwards entering Namibia and finally reaching Botswana. Here, the Okavango River creates the inland Okavango Delta (actually an alluvial fan) in the Kalahari Desert (West et al., 2015). The δ^{30}Si in dissolved silica (DSi, hereafter) in the Delta's surface water does not show big variations over time, ranging from 0.36 to 1.19‰. This results from a balance in the processes adding and removing DSi from the water. One big control on DSi is the aquatic vegetation, which uptakes DSi from the water and produces BSi (Frings et al., 2014).

Current understanding of Si and other nutrient cycles in the Okavango Delta is still moderate, as well as the key drivers of these cycles and which processes take place (Mendelsohn, 2010). This study aims to provide new knowledge on the plant communities living in the area, focusing on *C. papyrus*, one of the most abundant sedges in the Delta. The few studies on nutrient concentrations are particularly focused on Nitrogen (N). Due to the abundance of *C. papyrus* in this system, to understand more about Si cycling in this system and uptake by this species, we sought to (1) Quantify the amounts of macronutrients, including Si, in papyrus plant parts, and (2) Explore Si isotope distribution/fractionation in papyrus plants.

2 Material and Methods

2.1 Sampling site

Naturally-grown papyrus plants were sampled in 2017 from Sepopa, a site located in the Panhandle region of the Okavango Delta, Botswana (Fig. 1). Ten samples were collected at a 60-m distance from the river channel (called the backswamp, hereafter). Papyrus plants were harvested and separated into the different organs with respect to the age of the plants in the field. The organs of papyrus are umbels, culms, scales, roots, and rhizomes, and all of them were collected both as juvenile and mature tissues except for roots (Fig. 2). Harvesting of plants was executed during flood recession or low flood (August-November 2017). The samples were rinsed, dried, and ground. Later, the samples were analysed for Total Nitrogen (TN), Total Organic Carbon (TOC), BSi, Total Phosphorus (TP) in % by dry weight (hereafter %), and for Si isotopes, with high-temperature combustion and wet chemical analyses.

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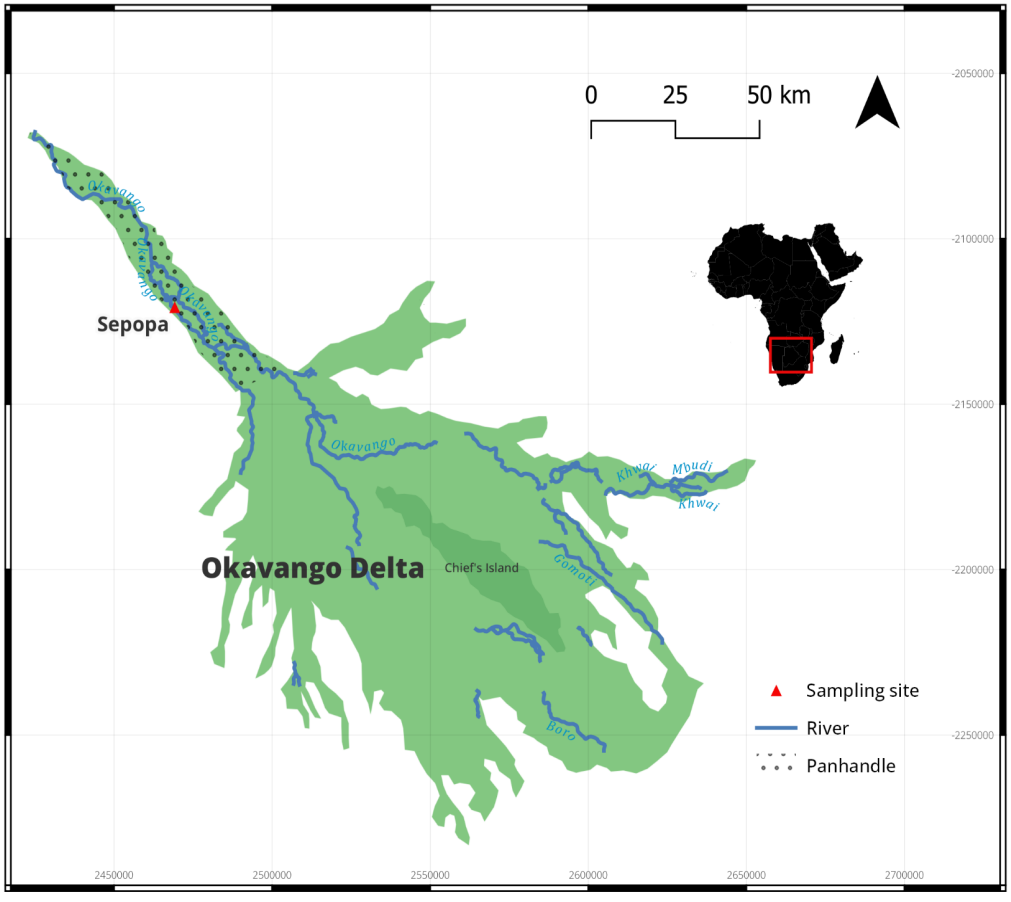

Figure 1: Overview of the Sample Locality in the Okavango Delta, Botswana.

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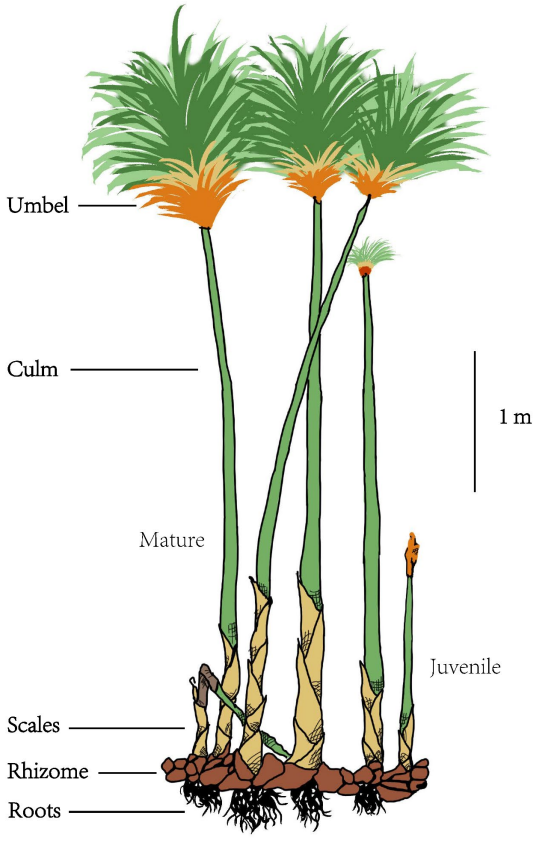

Figure 2. Sketch of *Cyperus papyrus*. The different organs and growth stages of papyrus are presented.

2.2 TN and TOC Determination

TN and TOC concentrations were determined via high-temperature combustion using a Costech ECS 4010 elemental analyser. Subsamples of ~2-3 mg were packed in tin capsules and combusted at temperatures of 1700-1800 °C. The measurements were calibrated with a 5-point calibration curve with acetanilide as a standard (10.34% N, 71.10% C). Precision and accuracy were checked with reference soil samples with known carbon and nitrogen contents (0.06% N, 0.73% C), barley flour standards (1.9% N, 43.51% C), and high organic sediment standards (0.52% N, 7.45% C).

2.3 Wet Chemical Digestions

2.3.1 BSi Determination

Subsamples of ~28-31 mg were placed into polypropylene round bottles. To digest the papyrus, 40 mL of 1% Na_2CO_3 solution was added to the subsamples. The bottles were put in a covered shaking water bath (Julabo) at 85 °C for 3 hours, with caps slightly loosened to vent gases. Then, the bottles were removed from the shaking bath and placed into a room

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temperature water bath and cooled for 3-5 minutes to slow down further dissolution. Subsequently, 1 mL of the sample was pipetted into plastic bottles containing 9 mL of 0.017M HCl to neutralise the Na2CO3 digestion solution. The subsamples were then analysed for dissolved silicate using the Smartchem 200 (AMS System) wet chemistry analyzer. For this study, an automated molybdate-blue method was used (Grasshoff, 1999). The principle behind this method is the reaction of monomeric silicic acid with ammonium molybdate, that results in a molybdosilicic acid complex. This complex is then reduced to heteropoly-molybdenum by ascorbic acid and measured at 660 nm by a spectrophotometer.

2.3.2 TP Determination

Subsamples of ~0.1 g were weighed and placed into glass vials. The glass vials were acid-washed beforehand with 10% HCl for 24 hours. 0.5-1.0 mL of 50% $Mg(NO_3)_2$ were added to the subsamples. The subsamples were ashed for 2 hours at 550 °C in a muffle furnace (Nabertherm B180). When the samples had cooled down, 10 mL of 1M HCl were added to the subsamples and vortexed to get all the sample into the solution. Then, the subsamples were shaken for 16 hours and analysed for total phosphate using the Smartchem 200 (AMS System) wet chemistry analyser.

2.3.3 Si Isotope Digestions

Subsamples of the bulk material were pre-cleaned prior to digestion. To do this cleaning step, 1 g subsamples were weighed into 50-mL centrifuge tubes and were successively treated with HCl, HNO_3, and H_2O_2 to remove carbonates and organic matter following robust BSi cleaning protocols (Hendry et al., 2010; Hendry and Robinson, 2012). The first step was a HCl leach. 10-15 mL of 10% HCl was added and the samples were left overnight in a water bath at 80 °C. Subsequently, the samples were centrifuged at 3500 rpm for 7 minutes and the acid was decanted. New 10% HCl was added and the samples were centrifuged again. The sample material was rinsed with Milli-Q® (18.2 MΩ * cm) water and centrifuged at 3500 rpm for 5 minutes. This rinsing step was repeated 3 times. The second step was a nitric digestion. 10-15 mL of 35% HNO_3 was added and the samples were left for 6 hours in the water bath at 80 °C. The samples were centrifuged at 3500 rpm for 7 minutes, the acid was decanted, and sample material was rinsed with Milli-Q® three times as described above. The third step was a peroxide digestion. 10-15 mL of 15% H_2O_2 was added and the samples were left overnight in a 80 °C water bath. Samples were centrifuged and the peroxide was decanted. Fresh 33% H_2O_2 was added, the samples were centrifuged at 3500 rpm for 5 minutes, and the peroxide was decanted. The sample material was then rinsed 3 times with Milli-Q®. In order to check for the presence of any additional siliceous organisms (e.g. diatoms) smear slides were prepared. In samples where diatoms were present, they were dissolved with the following two steps. First, 20 mL of 0.1M Na_2CO_3 was added in a 80 °C water bath for 20 minutes. The samples were centrifuged at 3500 rpm for 5 minutes and the decant liquid was discarded. The sample material was rinsed with Milli-Q® 3 times before moving on. Next, 20 mL of 0.2M NaOH was added to the samples in a 95 °C water bath for 20 minutes. Samples were centrifuged at 3500 rpm for 5 minutes, the decant liquid was discarded, and material was rinsed with Milli-Q® 3 times. Samples were then freeze-dried for 2 days and re-checked via smear slide to confirm the absence of additional siliceous organisms.

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Pre-cleaned subsamples of 4-34 mg (mass was determined by % weight BSi , to ensure there would be >2 ppm of Si in the final sample digestion) were weighed in Teflon tubes and a final digestion was performed adding 3 mL of 0.4M NaOH. Samples were dissolved on a hot plate at 100 °C for 2 days, mixing after 1 day. The samples were then transferred into 15-mL centrifuge tubes, and 0.3 mL of 6M HCl were added to neutralise the samples. The samples were run in Smartchem 200 (AMS System) wet chemistry analyser to check [SiO_2] with the same method used above for SiO_2 content determination (paragraph 2.3.1).

2.4 Measurement of Si Isotopes

Prior to Si isotopic analysis, all samples and standards (NBS28 and Diatomite) were purified by cation exchange chromatography (Bio-Rad AG50W-X12, 200–400 mesh in H+ form resin) following Georg et al. (2006). Briefly, between 0.2-1.0 mL of the digested samples were loaded into pre-charged columns. 4 mL of Milli-Q® were used to elute any sample remaining in the columns, obtaining a final 2-ppm solution in acid-cleaned Teflon bottles. Procedural blanks were treated in the same manner.

For all samples, Si isotopic analyses were performed at Brest, France, on the MC-ICP-MS (Multi-Collector Inductively-Coupled-Plasma Mass-spectrometer, Finnigan Neptune, at the IFREMER PSO facility). Analyses were performed under a dry plasma using an APEX-Q in a medium resolution and analyses were made on the low mass side of the Si peaks to resolve the interferences (e.g., $^{14}N^{12}O$). All samples followed the typical standard-sample bracketing using Pcal Si standard as bracketing standard and Mg for doping from Cardinal et al., 2003. The $\delta^{30}Si$ results are then reported relative to the standard NBS28 following Eq. (1):

$$\delta^{30}Si = 1000 * \frac{\text{Rsample}-\text{Rstd}}{Rstd}, \quad (1)$$

where R is the $^{30}Si/^{29}Si$ ratio of either the sample or the standard, and the standard being the international Si standard Quartz NBS28 (RM8546).

External reproducibility was assessed by repeated measurement of the biogenic opal standard, Diatomite (Reynolds et al., 2007) with a $\delta^{30}Si$ mean value of +1.18 (0.17 2SD, n=16) and $\delta^{29}Si$ = +0.66 (0.16 2SD, n=16) which yield within the errors published in the intercalibration study. For all samples and standards, the three isotopes (^{28}Si, ^{29}Si, ^{30}Si) were measured and results show good agreement with the mass-dependent fraction between $\delta^{29}Si$ and $\delta^{30}Si$ with $\delta^{29}Si = 0.52\ \delta^{30}Si$ (0.16, 2SD).

2.5 Phytolith Imaging with SEM

Following cleanings, samples from each organ group (i.e., umbel, culm, scales, rhizome, roots) were analysed at the Department of Geology, Lund University, using a Tescan Mira 3 High-Resolution Schottky field emission scanning electron microscope (SEM) equipped with an Oxford energy dispersive X-ray spectrometry (EDS). Pre-cleaned samples were

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mounted on carbon tape, and sputter coated with Platinum-Palladium (Cressington sputter coater 108 auto, 20 mA, 20 seconds). The samples were then imaged and mapped for elemental content via SEM-EDS.

3 Results

3.1 TN, TOC, and TP in plant parts by age

TOC, TN, and TP all varied amongst the different tissue types and tissue ages in C. papyrus (Fig. 3). TN concentrations were found to be higher in juvenile organs compared to mature ones. The highest concentrations were found in umbels (1.93% and 1.56% for juvenile and mature, respectively) followed by rhizomes (1.41% and 1.31% for juvenile and mature, respectively). Roots had concentrations of 1.36% and 1.05%. Juvenile and mature culms had concentrations of 0.90% and 0.41% respectively, while juvenile and mature scales 0.73% and 0.49%.

TOC concentrations were rather constant among the different organs, but higher concentrations were found in mature parts than juvenile. Juvenile and mature umbels showed concentrations of 44.23% and 46.57%, respectively; juvenile and mature culms 41.39% and 41.94%; juvenile and mature scales 41.24% and 43.46%; juvenile and mature rhizomes 42.59% and 44.23%; and lastly roots had the lowest TOC concentrations of 40.59% and 35.98%.

TP was found to follow the same trend as TN, i.e., juvenile organs had higher % than mature. The highest concentrations were found in the umbels, with 0.24% and 0.11% respectively. Rhizomes followed with 0.18% and 0.07%. Juvenile and mature culms had concentrations of 0.12% and 0.03%, respectively, and juvenile and mature scales 0.08% and 0.01%. Both roots showed concentrations of 0.07%.

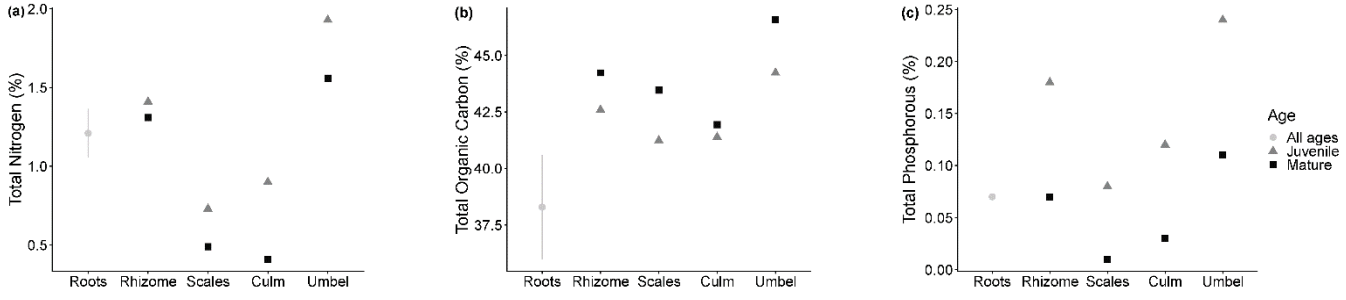

Figure 3: Scatter plots showing (a) TN, (b) TOC , and (c) TP divided by plant part and age.

3.1 BSi and δ^{30}Si in plant parts by age

BSi and δ^{30}Si values are presented in Fig. 4, together with water sample data taken from Frings et al. (2014) as a reference. BSi concentrations were found to be higher in mature organs than in juvenile, apart from the rhizomes. The highest BSi was

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in the roots, 3.88% and 6.61%. Juvenile and mature umbels had concentrations of 1.28% and 2.07%; juvenile and mature culms 1.26% and 2.87%; juvenile and mature scales 1.22% and 1.30%; and lastly juvenile and mature rhizomes 1.12% and 0.88%.

δ^{30}Si in roots were 0.00‰ and -0.06‰; in rhizomes 0.64‰ and -1.14‰ for juvenile and mature; in scales 0.99‰ and -0.18‰ for juvenile and mature; in culms 1.04‰ and 0.74‰ for juvenile and mature; and in umbels 0.84‰ and 1.65‰ for juvenile and mature, respectively. δ^{30}Si in the Panhandle water was 0.56-1.16‰ and [BSi] concentration was 54.74-111.79 µM (Frings et al., 2014), and are presented here as shaded rectangles (Fig. 4b-c).

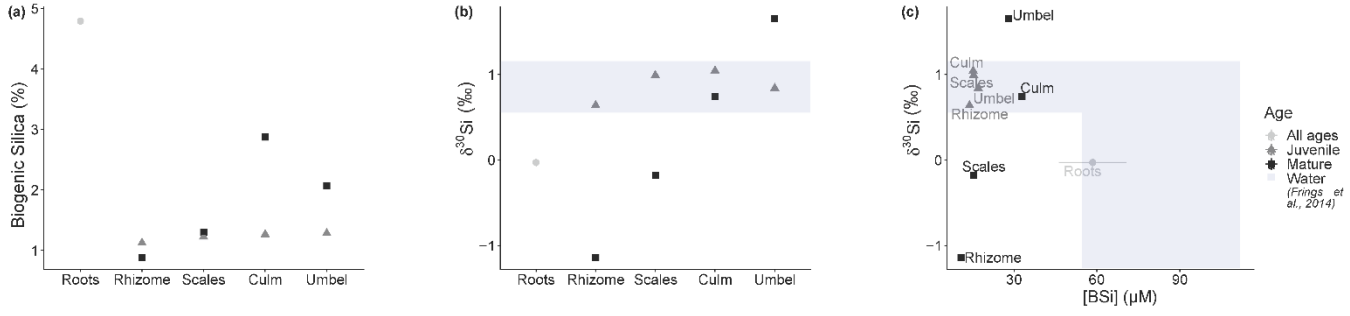

Figure 4: Scatter plots showing (a) BSi divided by plant part and age, (b) δ^{30}Si divided by plant part and age, and [BSi] vs δ^{30}Si divided by plant part and age. δ^{30}Si and [BSi] water data are taken from Frings et al. (2014) as a reference.

4 Discussion

4.1 Biogenic Silica

This study confirms that *C. papyrus* is a high Si accumulating species, with tissue concentration ranging from 0.88% in mature rhizomes to 6.61% in roots. We noted the big difference in the two BSi concentrations in roots, 3.88% and 6.61%. This could be due to the age of the roots, even though during sampling it was not possible to classify whether the roots were juvenile or mature. Changes in BSi concentration with ontogeny have been shown in many species, with BSi positively correlated with tissue age (Motomura et al., 2004), because once deposited, BSi is not remobilised, and hence BSi accumulates over time. Consistent with this, higher BSi was found in mature than in juvenile tissue for scales, culms and umbels. However, we found higher BSi of juvenile rhizomes compared to mature rhizomes, most likely the result of increased allocation of organic compounds to these organs which are often used for carbohydrate storage, diluting any Si concentration. Most plants accumulate more BSi in leaves than in flowers, though species in the Poales typically accumulate high amounts in their flowers and associated parts (Schoelynck et al., 2023).

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All plants accumulate BSi passively through the transpiration stream and some species additionally take up silicon through specialised transporters (Mitani-Ueno and Ma, 2021). It is not known if *C. papyrus* produces Si-specific channels and transporters that actively take up or move Si around the plant, as Cyperaceae species with genes encoding putative transporters are known (Mandlik et al., 2020). An alternative mechanism for the high BSi accumulation is a high transpiration rate. However, in general, C4 species like *C. papyrus* are characterised by lower transpiration rates compared to C3 species. This is due to higher water use efficiency because of the lack of photorespiration (Hasegawa and Okuda, 1974; Long, 1999). Papyrus swamps can also create a low evapotranspiration microclimate because of their culm height and standing biomass (Jones and Muthuri, 1985). It was observed that evapotranspiration rates in some papyrus swamps were lower than evaporation in open water (Mohamed et al., 2004), and remarkably papyrus stomata can control water loss from swamps (Pacini et al., 2018).

Also of note was the high Si accumulation in roots with a BSi concentration of 6.61% (Fig. 4) with rhizomes also comparatively high. Few other studies report BSi in papyrus, however Struyf et al. (2015) report whole plant concentration as 1.90% and Hodson et al. (2005) report 1.07% for culms. Our findings illustrate the importance of measuring roots and rhizomes to accurately determine plant BSi in general, as the use of the lower values above would lead to underestimations of biomass BSi and related flux calculations.

Following the relatively high BSi concentrations found in *C. papyrus* samples, particularly in the roots, culm and umbels, distinctive phytoliths were found in all organs except for roots (Fig. 5). The phytoliths found can be described as conical morphotypes with satellites (Majumder et al., 2020). The Cyperaceae are known as prolific producers of phytoliths, and in the leaves and/or culms and inflorescences, of multiple species of *Cyperus* spp. (Murungi and Bamford, 2020), including the culms and inflorescences of *C. papyrus* (Albert et al., 2006). To the best of our knowledge, phytoliths have not been isolated from *Cyperus* spp. roots (Murungi and Bamford, 2020).

The function of BSi in flowers still needs further exploration but in general, Si accumulation is lower in floral structures than it is in leaves (Schoelynck et al., 2023). In papyrus, high concentrations of Si were found in the umbels, the reproductive tissue which include the large flower stalks, lack petals and are wind pollinated. Sedges, in the Poales, like *Cyperus*, show a high BSi in inflorescences and it could be related to a protective function to defend against herbivory. Also, flowers being fundamental for reproduction, high BSi could be used to ameliorate potential stresses (Schoelynck et al., 2023) and can be important for seed fertility as Si reduces water loss and protects from infections (Ma, 2004).

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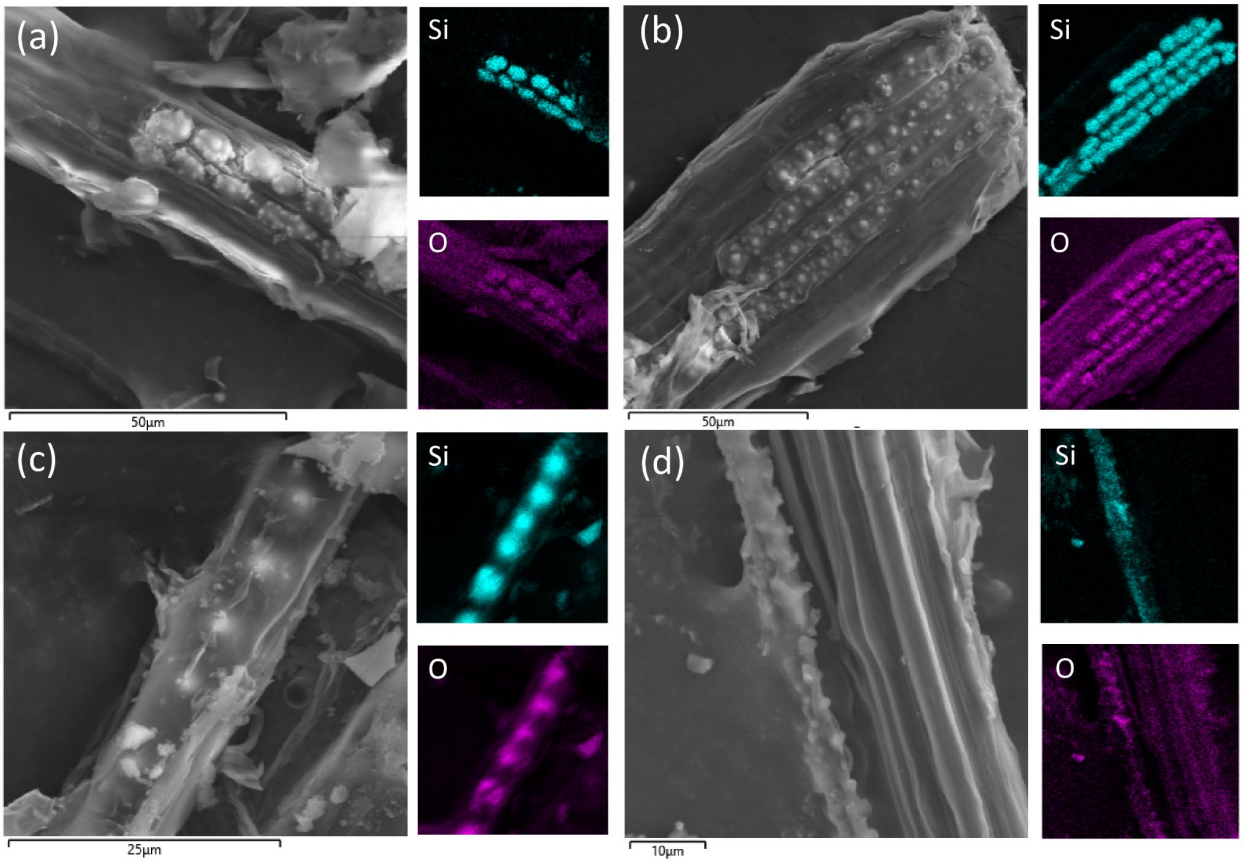

Figure 5: SEM images of phytoliths, with Si and O elemental mapping on the side, which are conical morphotypes with satellites, in *Cyperus papyrus*: (a) scales, (b) rhizome, (c) umbel, and (d) culm.

4.2 Silicon isotopes in *C. papyrus*

To the best of our knowledge, this is the first time a non-crop plant species has been analysed for Si isotopes, considering different plant parts.

In rice, shoot organs gradually become enriched in heavy isotopes along the transpiration stream (Ding et al., 2005, 2008; Y. Sun, Wu, and Li, 2016). The same pattern was observed in papyrus, with an increase in heavy isotopes going from rhizomes to scales, stem, and umbel (Fig. 4b). This is especially clear in the mature organs, while juvenile parts seem to be clustered together and not show this trend. This might be due to the young plant organs not being fully differentiated (Fig. 2) and therefore showing a similar behaviour. For the mature parts, they are more specialised and differentiated, and more strongly show the systematic differentiation of Si isotopes up the transpiration stream.

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Si transport in papyrus does not seem to be conservative, in that there is not a linear relationship between δ^{30}Si and plant part as water moves up through the plant (Fig. 4c). In juvenile parts, it appears that Si is transported from the roots to all the other organs. In mature parts, after a first precipitation in the roots, heavy Si seems to follow the transpiration stream moving from rhizome to scales to culm and to umbel. It could also be that there are two different transport pathways (Fig. 4c): one going from the roots up to culm and umbel, and one going from the rhizome to the scales. These two different pathways could be explained by both roots and rhizomes being underwater and therefore directly in contact with water.

The high Si concentration in the roots was expected to reflect phytolith formation, however, we found none. This was hampered by degraded samples, possibly due to the numerous cleaning steps undertaken to remove the numerous diatoms we found on the samples, which are also siliceous. Other studies of phytoliths in Cyperaceae have focused on culm and umbel samples (Majumder et al., 2020; Murungi and Bamford, 2020), hence it is unclear if these tissues do indeed produce phytoliths. However, given the high [BSi] it seems highly likely, and as noted by Novello and colleagues (2012), overlooking this tissue type risks missing valuable information for archaeology and biogeochemical cycle calculations.

Phytolith formation in roots has already been observed in mustard, wheat, and other grasses (Frick et al., 2020; Hodson and Sangster, 1989; Paolicchi et al., 2019). The presence or absence of an efflux transporter (Low Silicon 2, Lsi-2), which is found in roots, influences isotope fractionation along the transpiration stream (Frick et al., 2020). Three possible scenarios have been speculated: Lsi-2 has a preference for light ^{28}Si isotopes as in the case of a passive diffusion; Lsi-2 has a preference for either light ^{28}Si or heavy ^{30}Si, leading to an equilibrium in isotope fractionation; precipitation of H_4SiO_4 in the roots enriches the remaining H_4SiO_4 in heavy ^{30}Si which is then transported up in the shoot (Frick et al., 2020). The big isotopic difference observed in papyrus' roots and umbel (Fig. 4b) could possibly be explained by the third scenario, i.e., Si precipitation in roots. BSi precipitation would then enrich the residual Si pool in heavy ^{30}Si that is transported upstream, as already observed by previous studies in other plants (Ding et al., 2005; Hodson et al., 2005; Y. Sun, Wu, Li, et al., 2016). Moreover, heavy Si isotopes were found to be more mobile than light isotopes in plants, contradicting the belief that the transport of light isotopes is favoured in plant biological processes (Dawson et al., 2002).

4.3 Comparison with other studies

The nutrient concentrations for *C. papyrus* determined in this study are largely comparable to other studies (see Table 1). Overall, TOC values are very consistent with previous literature about the same species, while TN, BSi and TP are more variable. TN and BSi tend to be higher compared to other studies, whereas TP is generally much lower. The variation found amongst datasets could be a reflection of different nutrient availability between the three catchments studied (from Botswana, Kenya and Uganda), dynamic environment of wetlands in general, and, in particular, of the Okavango Delta. The Okavango Delta is characterised by seasonal fluctuations in inundation, so the varying depths of water could potentially result in different nutrient concentrations available to the plant communities and in papyrus.

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Other data for *C. papyrus* BSi concentrations are limited, enabling only some comparisons. As a consequence, Si cycling calculations made with existing literature would lead to an underestimation because, as found in this study, the most BSi-rich organs (i.e., roots, culms and umbels) have not previously been measured for BSi.

Table 1. Table showing the mean TN, TOC, BSi, and TP in every part of papyrus and the comparison with previous studies. The studies of Struyf et al. (2015) and McCarthy et al. (1989) are from the Okavango Delta, Botswana; the studies of Boar et al. (1999) and Muthuri and Jones (1997) are from Lake Naivasha, Kenya; the study from Lind and Visser (1962) is from Lake Victoria, Uganda.

	Lodi et al., 2024 (This Study)	**Struyf et al., 2015**	**Hodson et al., 2005**	**Boar et al., 1999**	**Muthuri and Jones, 1997**	**McCarthy et al., 1989**	**Gaudet, 1977**	**Lind and Visser, 1962**
	TN (%)							
Umbel	1.75			1.98	2.00	0.93	1.74	1.71
Culm	0.66			0.60	0.72	0.63	0.73	0.49
Scales	0.61				0.50			
Rhizome	1.36			0.99	1.40	0.75	1.29	1.11
Roots	1.21			0.94	1.40		1.12	0.75
	TOC (%)							
Umbel	45.40			45.60		41.73		
Culm	41.67			43.60		41.66		
Scales	42.35							
Rhizome	43.41			44.30		39.77		
Roots	38.29			41.60				
	BSi (wt% SiO_2)							
Umbel	1.68							
Culm	2.07		1.07					

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Scales	1.26							
Rhizome	1.00							
Roots	4.79							
Total	2.16	1.90						
	TP (wt% PO_4)							
Umbel	0.18				0.24			
Culm	0.08				0.14			
Scales	0.05				0.11			
Rhizome	0.13				0.31			
Roots	0.07				0.09			

4.4 Silicon and biogeochemical cycling in the Okavango Delta

Given its high Si accumulation, *C. papyrus*, as one of the most abundant plants in Okavango Panhandle and Delta, likely has an important role in the annual Si budget of the system (Struyf et al., 2015). Estimates reveal that the amount of BSi found in sediments in the Okavango Delta is one of the highest reported worldwide (Struyf et al., 2015), and still it is thought as an underestimation because of the abundance of Si accumulating plants like papyrus. It was also noticed as Si tends to decrease with depth in the Okavango sediments, reflecting the efficient recycling provided by papyrus and vegetation in general (Frings et al., 2014). Moreover, along the Okavango Delta, a shift from decreasing to increasing available DSi was recorded, which could be due to the high volume of vegetation (Frings et al., 2014) continuously uptaking Si from the water.

The fact that vegetation, particularly papyrus and reed, tends to discriminate against the heavy isotopes was noticed by Frings et al. (2014) and was again observed in this study. In the lower parts of papyrus, the Si isotopic composition is lower than in water, evidencing the discrimination against heavy isotopes by papyrus. In fact, from our results, it seems that papyrus is preferentially taking up light Si isotopes, depositing them first in the roots as phytoliths, though it remains to be confirmed if this is as phytoliths, and enriching the remaining Si pool inside the plant in heavy isotopes that are then transported up along the transpiration stream.

The abundance of hippos and other grazers in the Okavango Delta is another important point when considering Si cycling. Hippos have been considered “animal Si pumps” because of their large influence on the Si cycle (Schoelynck et al., 2019).

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In the Mara Wetlands (Kenya), hippos are responsible for 32% of BSi flux and more than 76% of the total Si flux (Schoelynck et al., 2019). The role of other grazers in nutrient cycles is not yet known, but the abundance of elephants and antelopes could make them potential Si pumps as well (Schoelynck et al., 2019). Considering the similarities between these two wetlands particularly in terms of vegetation and fauna, we speculate a similar influence on the Si cycle by hippos in the Okavango Delta.

5 Conclusions

Cyperus papyrus, a dominant species in the backswamps of the Okavango River, as well as being an important contributor to C, P and N cycling, is a key regulator of Si as a high Si-accumulating species. Large amounts of Si are accumulated in the roots, rhizomes and umbels - reported for the first time here - and discrete phytoliths were found in all organs, though intriguingly, yet to be confirmed for roots. Isotope analysis suggests that, after an initial preferential uptake of light Si isotopes and deposition of Si in the roots, there is a successive enrichment in heavy Si isotopes along the transpiration stream. Taken together this study highlights that all plant parts should be measured to ensure accurate flux calculations for Si biogeochemical modelling, and it adds support for the use of silicon isotopes to better understand Si uptake and allocation in plants.

Data availability

All original data are presented in this paper and available from the authors upon reasonable request. All raw data are available in the Supplementary Material.

Author contribution

Giulia Lodi: Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing.
Julia Cooke: Conceptualization, Formal analysis, Writing – review & editing.
Rebecca A. Pickering: Methodology, Formal analysis, Investigation, Writing – review & editing.
Lucie Cassarino: Methodology, Formal analysis, Investigation, Writing – review & editing.
Mike Murray-Hudson: Resources, Writing – review & editing, Funding Acquisition.
Keotshephile Mosimane: Resources.
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Competing interests

The authors have no competing interests to declare.

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