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Biochar affects silicification patterns and physical traits of rice leaves cultivated in a desilicated soil (Ferric Lixisol)

Felix de Tombeur^{1*}, Julia Cooke², Laurie Collard¹, Drissa Cisse³, Fatimata Saba³, David Lefebvre⁴, Victor Burgeon¹, Hassan Bismarck Nacro³, Jean-Thomas Cornelis¹

¹TERRA Teaching and Research Centre, Gembloux Agro-Bio Tech, University of Liege, Gembloux, Belgium

²School of Environment, Earth and Ecosystem Sciences, The Open University, Milton Keynes MK7 6AA, UK.

³Institut de développement rural (IDR), Université Nazi Boni (UNB), Burkina Faso

⁴School of Water, Energy and Environment, Cranfield University, College Road, Bedford, MK43 0AL, UK

*Corresponding author; felix.detombeur@uliege.be

Abstract

Aims Increasing the leaf silicification of cereal crops to ameliorate defenses against stresses and improve yield constitutes a major challenge in (sub-)tropical regions with highly desilicated soils. We tested the efficacy of different biochars – as readily available alternatives to commercial fertilizers – to increase leaf silicification and understand subsequent impacts of leaf traits that might benefit crops.

Methods We compared the application of two biochars (rice-derived biochar with 198 g kg⁻¹ of Si and cotton-derived biochar with 4 g kg⁻¹ of Si) and wollastonite (240 g kg⁻¹ of Si) at two application rates on the leaf silicification patterns and leaf traits of rice growing in pots containing highly desilicated soil (Ferric Lixisol) from Burkina Faso.

Results Leaf Si increased from 19.0 to 70.4 g kg⁻¹ with Si addition (control < cotton biochar < wollastonite < rice biochar), resulting in larger epidermal silica deposits. Leaf carbon (C), leaf mass per area (LMA) and leaf arc decreased and were negatively correlated with leaf Si, however, surprisingly, the leaf force to punch and the plant biomass decreased.

Conclusions We demonstrate the effective use of rice biochar in desilicated environments to improve the Si status of cereal crops and their associated leaf traits. In particular, the decrease in LMA with rice biochar application shows a promising capacity of rice biochar to reduce rice leaf

C costs. *In situ* trials are now needed to investigate whether or not these beneficial effects may result in increased crop yields through resilience against environmental stresses.

Keywords silicon; silicon fertilizer; biochar; biosilicification; leaf toughness; biomechanics

Introduction

Vascular plants accumulate silicon (Si) in their tissue, often in concentrations exceeding those of the major macronutrients (Epstein 1994). Silicon is taken up from the soil solution as monosilicic acid (H_4SiO_4), transported in the xylem, and deposited in extra- and intercellular spaces as hydrated amorphous silica ($\text{SiO}_2 \cdot n\text{H}_2\text{O}$) (Ma et al. 2006; Yamaji and Ma 2011; Exley 2015; Hodson 2019). Biosilicification has occurred in land plants for over 400 million years (Trembath-Reichert et al. 2015), and numerous plant functions have been associated with this mechanism (Epstein 1994, 2009; Cooke and Leishman 2016; Hartley and DeGabriel 2016; Debona et al. 2017; Coskun et al. 2019). In particular, silicification in plants increases their resistance to water stress (Meunier et al. 2017), pathogens and herbivore attack (Massey and Hartley 2006; Massey et al. 2007; Frew et al. 2016), reduces the soil-to-plant translocation of toxicants (Gong et al. 2006; Coskun et al. 2019), and probably interferes with the recognition process occurring upon pathogen infection (Frew et al. 2018; Coskun et al. 2019; Hall et al. 2019; Leroy et al. 2019). The beneficial effects of Si in graminoid crops (e.g. wheat, rice, maize, sugarcane) can enhance agricultural productivity and food security, particularly in marginal areas (Savant et al. 1999; Datnoff et al. 2001; Tubana et al. 2016).

Through the weathering of Si-bearing minerals, a part of Si is dissolved in the soil solution or is reversibly adsorbed onto mineral surfaces, and therefore made available for plant uptake. The concentration of available Si depends on the nature and particle-size of minerals (Cornelis et al. 2011, 2014; Meunier et al. 2018; de Tombeur et al. 2020b), and on the degree of Si biocycling (Bartoli 1983; Alexandre et al. 1997; Lucas 2001; Cornelis and Delvaux 2016; Vander Linden and Delvaux 2019; de Tombeur et al. 2020a). Available Si concentrations decrease with increasing degree of soil weathering and relative content of poorly-soluble 1:1 clay minerals and quartz (Savant et al. 1999; de Tombeur et al. 2020c). As a consequence, highly-weathered tropical soils

are characterized by low available Si concentrations (Meunier et al. 2018; de Tombeur et al. 2020c). On these highly desilicated soils, Si-accumulating crops are often supplied with Si fertilizers to counterbalance Si exports from harvest (Guntzer et al. 2012; Keller et al. 2012), to reduce disease and increase yield (Datnoff et al. 2001; Liang et al. 2015; Klotzbücher et al. 2017). However, access to common Si fertilizers (e.g. wollastonite) in remote areas is low. In these locations, the simplest and most accessible way to increase Si availability for plant uptake is to use amendments derived from organic products, such as rice crop residues or Si-rich biochar (Li and Delvaux 2019; Li et al. 2019b).

Silicon-accumulating species have very variable [Si] (e.g. from 1.6% to 10.7% of dry weight for leaves of different rice cultivars in Klotzbücher et al. 2018). Such variations in the degree of leaf silicification may impact their properties, although it remains poorly studied. For instance, silica has long been attributed to have a structural role in leaves (Raven 1983), though the underlying mechanisms remain elusive (Bauer et al. 2011; Cooke et al. 2016). The silicification of the leaf epidermis is thought to improve the erectness of leaves under Si fertilization (Ando et al. 2002; Zañão Júnior et al. 2010; Yamamoto et al. 2012), but there is no clear evidence of a relationship between the two parameters. This however needs to be understood as changes in leaf erectness can impact light interception (Ando et al. 2002), and in turn the photosynthetic and growth rates. In addition, several studies showed that Si addition increases leaf toughness (Yamamoto et al. 2012; Kido et al. 2015; Simpson et al. 2017; Johnson et al. 2019). However, these studies performed tearing and shearing tests, while the impact of epidermal silicification on the force required to punch through leaves remains unknown. Finally, because silica deposits are energetically less demanding than the synthesis of C-based compounds (Raven 1983), they represent a cheaper alternative to other plant traits performing structural and defensive functions. Indeed, tradeoffs with

phenolic compounds (Cooke and Leishman 2012; Frew et al. 2016; Johnson and Hartley 2018; Waterman et al. 2020) and with C-based structural components (Schoelynck et al. 2010; Klotzbücher et al. 2018; Schaller et al. 2019; de Tombeur et al. 2020c) have been reported. On this basis, silicification could impact the leaf mass per area unit (LMA), which also exerts a major control on leaf toughness and deterring herbivory (Peeters 2002; Onoda et al. 2011; Kitajima et al. 2012, 2016), but this remain unexplored. This is important since the construction of thinner/less dense leaves at the expense of a high degree of epidermal silicification would reduce the leaf C costs of cultivated grasses, and could ultimately explain the yield increase observed after Si fertilization (e.g. Tubana et al. 2016).

Burkina Faso relies heavily on Si-accumulating crop species such as rice and maize (Traoré et al. 2015; MAAH 2020), grown predominantly on highly desilicated soils, but with poor access to common Si fertilizers. Through a pot experiment in controlled conditions, we sought to determine if the application of biochar on a highly-desilicated soil from Burkina Faso (1) can be a Si source for local rice varieties, (2) can impact the patterns of leaf silicification, and (3) can impact other leaf traits with structural and defensive functions. We hypothesized that biochar from high-Si accumulating rice would result in higher plant Si accumulation of the rice crop compared to low-Si accumulating cotton-based biochar. Secondly, we hypothesized that a higher degree of Si accumulation will result in an increase of leaf silicification, resulting in straighter leaves. Thirdly, we hypothesized that a higher degree of leaf silicification could affect the LMA and the force required to punch leaves, but without conjecture on the direction of the response.

Materials and methods

Soil selection and sampling

The soil selected for the experiment was a Ferric Lixisol developing on a granitic parent material and showing a ferruginous crust between 20 and 40 cm depth. This soil type is highly represented in Burkina Faso (about 40% of the total surface; SP/CONEDD 2010). The soil has a sandy loam texture (73% sand, 15% silt and <12% clay), a low CEC (2.9 cmol_c kg⁻¹), a soil pH-H₂O of 6.1 and significantly lower soil pH-KCl (4.8). The mineralogy is dominated by quartz and kaolinite (Table 1), which is typical for highly desilicated soils. This is corroborated by the low Si concentrations extracted with CaCl₂ (11.2 mg kg⁻¹) compared to literature (de Tombeur et al. 2020b for a review). Soil samples were collected in January 2019 near Koumbia, a village located in province of Tuy, in southwest Burkina Faso (11°13'N, 3°43'W). The weather is characterized as a Sudanian climate, with a rainy season between May and September. The mean annual rainfall varies between 1000 and 1200 mm and the mean monthly temperature between 22°C and 35°C. Four subplots under typical cotton-maize rotation were randomly selected to collect the soil required for the pot experiment. The soil was collected above the ferruginous crust.

Silicon amendments

We studied two potential biochar-based Si amendments that can easily be produced by local farmers, making them more accessible than conventional Si fertilizers. Biochar was produced from two crop residues, rice husks (rice biochar) and cotton stalks (cotton biochar), at the University of Nazi Boni, Bobo-Dioulasso, Burkina Faso. The biochar was produced through pyrolysis at approximately 450°C during 80 minutes using a conventional Top-Lit UpDraft (TLUD) oven. Characteristics of both biochars were determined according to the European Biochar Certificate

(EBC) by Eurofins Umwelt. Both biochars were compared to wollastonite, a conventional Si fertilizer (Datnoff et al. 2001), provided by Vanderbilt Chemicals, LLC (W10).

Pot experiment

The experiment was conducted in a greenhouse at Gembloux Agro-Bio Tech, ULiège (Belgium). Three Si-based amendments were applied (rice biochar referred as R, cotton biochar referred as C and wollastonite referred as W), with two input rates of 5 and 30 t ha⁻¹: R5, R30, C5, C30, W5 and W30. The control (no Si fertilization) is referred as 'T'. The lower application rate of 5 t ha⁻¹ corresponds to what can be commonly found in biochar experiments in tropical agro-ecosystems (Nair et al. 2017). However, given typical rice husk yields are approximately ~600 kg ha⁻¹ (Bandaogo 2014) which produce about ~100-300 kg ha⁻¹ of biochar (pyrolysis yield about 30%), amending 5 t ha⁻¹ of rice husk biochar at once is unrealistic for farmers and a distribution of the amendment over the years should therefore be considered. The higher rate was included to examine the potential impacts of higher Si inputs to soil and accumulation in leaf tissues. Rice cultivar FKR 45 N was selected as it is commonly cultivated in Burkina Faso (Traoré et al. 2015). It has a growth cycle of 95 days. The experiment was carried out in pots (10.5*10.5*22 cm), in a randomized complete block design. The seeding rate was 70 to 90 kilograms per hectare, which represents one plant per pot. Dibbling seeding was performed, followed by a thinning after 10 days to select the healthiest plant. 150 kg ha⁻¹ of NPK (14-23-14) and 100 kg ha⁻¹ of urea (46% N) were applied after respectively 20 and 35 days of experiment, which is a typical fertilization pattern in Burkina Faso. Pots were watered daily with 150 mL of tap water.

Soil analyses

Soil physico-chemical characterization

Soil pH-KCl was determined using 1M KCl (mass ratio soil:solution 1:5). The amount of bioavailable nutrients (P, Ca, Mg, K) in soil were assessed using ammonium acetate-EDTA 1M extractable solution (Lakanen and Erviö 1971), in which elements were measured by atomic absorption spectroscopy (Ca, Mg, and K) or by spectrophotometry (P). Total C and N contents were determined with a CN analyzer.

Silicon kinetic release

The pool of available Si, referred as Si_{CC} , was estimated through a kinetic 0.01M $CaCl_2$ extraction (Haymsom and Chapman 1975; Sauer et al. 2006; Georgiadis et al. 2013; Li et al. 2019b), in triplicates, with a solid:liquid ratio of 5g:50 mL. The solution was hand-shaken twice a day (Sommer et al. 2013) to avoid quartz abrasion (McKeague and Cline 1963). At each time step (6h, 24h, 8 days and 32 days), the solution was centrifuged at 4750g for 5 minutes and the supernatant was filtered. The pH of the 32-day extracts was measured. Each extract was then acidified by addition of 50 μ l of ultrapure 65% HNO_3 , and stored in darkness at 4 °C prior to analysis. Silicon was measured by ICP-OES.

We normalized the Si_{CC} concentrations by the total Si content of each soil amendment, according to the following equation (Li et al. 2019b):

$$RE_{Si} = \frac{(Si_{cc}treatment - Si_{cc}control)}{Total Si treatment} * 100$$

where RE_{Si} is the Si_{CC} release efficiency, $Si_{cc}treatment$ and $Si_{cc}control$ are the contents of Si released from each treated soil and control soil, respectively, after 32 days of $CaCl_2$ extraction and $Total Si treatment$ is the total content of Si of each treated soil. All terms are expressed in mg.

179 *Plant analyses*

180 *Leaf silicon and nutrients concentration*

181 All leaves from each plant were harvested, rinsed with distilled water, dried at 50°C for 48h, and
182 ground to powder. Leaf Si, Ca, Mg, P and K concentrations were determined by ICP-OES after
183 calcination at 450 °C for 24 hours (ash content), followed by a fusion in a graphite crucible at 1000
184 °C with 0.4 g of Li-tetraborate and 1.6 g Li-metaborate (Chao and Sanzalone 1992; Kowalenko
185 and Babuin 2014; Nakamura et al. 2020a), and the dissolution of fusion beads in 10% HNO₃. Leaf
186 carbon and nitrogen concentrations were quantified with a CN analyzer.

187 *Physical extraction of leaf phytoliths*

188 Phytoliths were extracted through wet digestion (adapted from Kelly 1990; Fraysse et al. 2009;
189 Corbineau et al. 2013). For each amendment, 0.1g of dried leaf material was taken from each five
190 replicates and mixed to obtain a sample of 0.5g. The sample was transferred to a glass beaker with
191 10% HCl at 80°C to remove carbonates. After three rinsing cycles with distilled water, 65% HNO₃
192 was gradually added in order to remove the majority of organic tissue. After rinsing, 65%
193 HNO₃/30% H₂O₂ mixture (2:1) was gradually added to the beaker, maintained at 80°C, until the
194 reaction was no longer active and the residue no longer colorful. The residue in the beaker was
195 carefully rinsed with deionized water and transferred into polypropylene tubes for centrifugation
196 at 3700 rpm for five minutes. This washing was repeated three times. The residue was oven-dried
197 at 50°C during 48h.

198 *SEM observation and X-ray microanalysis*

199 For each treatment, one leaf sample was randomly selected from one of the five plant replicates
200 and mounted on a glass slide using double-sided carbon tape and bridged with silver paint, before

being carbon-coated in a Balzers MED010 evaporator (de Tombeur et al. 2020c). Images were obtained through a backscattered-electron detector in a FEI ESEM-FEG XL-30 working at 30 kV accelerating voltage and fitted with a Bruker 129 eV X-ray detector for elemental microanalysis. The Si distribution on leaf surfaces was obtained on both the abaxial and adaxial sides of the leaves by elemental Si mapping acquired on the Si $K\alpha,\beta$ peak at 1.74 keV. To compare the Si concentrations on the leaf surfaces between treatments, we randomly positioned 7 squares on each image (200*200 pixels) in which we counted the percentage area of yellow pixels (representing the Si signal on the elemental mappings) (de Tombeur et al. 2020c). The phytolith samples extracted from plant material were spread on glass slides covered with double-sided carbon tape, gold-coated and observed under the same conditions as the leaves.

Plant trait measurements

Plant height was measured at the end of the experiment. Leaf arc was measured on the third-highest leaf as the distance between the midpoint of the line joining the apex to the point of blade insertion and the midpoint on the adaxial surface of the leaf (Zanão Júnior et al., 2010; Fig. S1 for a schematic representation). Leaf Mass per Area (LMA; $1/SLA$) was calculated as the ratio of leaf dry mass to leaf area ($g\ m^{-2}$), on the third-highest leaf.

The adaxial surface of the highest leaf was used to perform punching tests (Onoda et al. 2011). Tests were performed using an SMS® TA-XT2 texture machine. Biomechanical properties were derived from force-displacement curves using the software SMS® Exponent (version 6.1.16.0). We used a flat-ended, sharp-edged cylindrical steel punch with a 3 mm diameter ($7.07\ mm^2$ punch area), and a die mounted onto the moving head of the test machine. The die moved downwards at a constant speed. The punch was applied halfway along the longitudinal axis of the leaf and halfway

between the midrib and the leaf margin. The force was divided by the circumference of the punch to give the force to punch (kN m^{-1}) (Onoda et al. 2011).

Statistical analyses

Means were compared through one-way analyses of variance (ANOVA), followed by *post hoc* multiple comparison (Tukey's Least Significant Difference [LSD] tests), after verifying that the conditions of the model were satisfied. Letters indicate results that were significantly different at the 95% level of confidence. Potential correlations were tested with Pearson's chi-square tests. Statistical analyses were performed using the software MiniTab®18.1.

Results

Biochar properties

The rice husks and cotton stalks contained 69 and 2 g kg^{-1} Si respectively which, after pyrolysis, led to biochars with Si concentrations of 198 and 4 g kg^{-1} (Table 2). The Si concentration of wollastonite was 240 g kg^{-1} . The concentrations of other nutrients were far more similar between both biochars, with the exception of Ca which was higher in cotton biochar (14 g kg^{-1} for cotton biochar and 2 g kg^{-1} for rice biochar) and Fe which was higher in rice biochar (13 g kg^{-1} for rice biochar and 1 g kg^{-1} for cotton biochar).

Effect of amendments on soil properties

Compared to the control, only W30 significantly increased pH-KCl (Table 3). Biochar amendment significantly increased the OC and N contents in soils. All the amendments (except R5 and W30) increased bioavailable Mg concentrations, while only biochar-treated soils increased the concentration of bioavailable P (except R5). The only amendment that impacted bioavailable K

concentration was C30. All the amendments increased bioavailable Ca concentration, except for R5.

After 32 days of extraction, the Si_{CC} concentrations of the treated soils were significantly higher compared to the control (11.2 mg kg^{-1}) (Fig. 1). Concentrations were the lowest in the cotton biochar amendments (12.0 and 14.5 mg kg^{-1} for C5 and C30), intermediate in the rice biochar amendments (16.4 and 26.9 mg kg^{-1} for R5 and R30), and the highest in the wollastonite amendments (143.7 and 189.2 mg kg^{-1} for W5 and W30). Although the pH-KCl measured in soils increased only for W30 (Table 3), pH measured in the 32-day CaCl_2 extractions increased significantly: $T < R5 < C5 < C30 < R30 < W5 < W30$ (Table S1), and were positively correlated with Si_{CC} concentrations (Fig. S2).

Leaf Si and nutrients concentration

Leaf [Si] varied strongly among the Si-based amendments (Fig. 2). The concentration in the control plants was the lowest at $19.0 \pm 3.0 \text{ g kg}^{-1}$. Concentrations of plants grown with both cotton biochar amendments and W5 were similar to the control, and ranged between 20 and 30 g kg^{-1} . Leaf [Si] more than doubled in R5 and W30 with 48.7 ± 2.0 and $52.5 \pm 3.2 \text{ g kg}^{-1}$ respectively. R30 resulted in the highest leaf [Si] at about three times the control: $70.4 \pm 2.3 \text{ g kg}^{-1}$. Considering only the biochar amendments and the control, leaf [Si] was positively correlated with Si extracted with CaCl_2 for 32 days (Fig. 3). The leaf concentrations of macronutrients showed large variations within the same treatment (Fig. S3). Only W30 increased leaf [N] compared to the control, while R30, C30, W5 and W30 increased leaf [P] (Fig. S3). No amendments increased leaf [K], leaf [Ca] (but it decreased for W5) or leaf [Mg] (but it decreased for R30, W5 and W30) (Fig. S3).

Leaf surface silicification and leaf phytoliths structure

The degree of silica deposition on the adaxial leaf epidermis was larger for both rice biochar and wollastonite amendments compared to the control and the cotton biochar amendments (Fig. 4a, b). While rows of dumbbell-shaped cells were entirely silicified in all amendments (silica cells; Kumar and Elbaum 2018), silica was deposited more heavily in the areas between those rows, for both wollastonite and rice biochar amendments (Fig. 4a). The same trend was observed for the abaxial surface (Fig. 4b; Figs. S4 and S5 for images).

The average size of phytoliths was significantly higher for all rice biochar and wollastonite amendments compared to the control (Fig. 5). Phytoliths of at least 500 μm in length, and with many conjoined silicified cells, were commonly found in these 4 amendments, but they were absent from the control and cotton biochar amendments.

Impact of Si accumulation on rice leaf traits

Leaf [ash] was positively correlated with leaf [Si], which is negatively correlated to leaf [C] (Fig. 6a, b). Plant height was greater than the control for R5, W5 and C30, lower for W30, not significantly different for R30 and C5, and was unrelated to leaf [Si] (Fig. 6c). Plant biomass was significantly lower for R30 and W30 (Table 4) and was negatively correlated with leaf [Si] (Fig. 6d). The LMA was lowest in the Si-rich leaves R5, R30 and W30 (Table 4) and was negatively correlated to leaf [Si] (Fig. 6e). The leaf arc and the force to punch were negatively correlated with leaf [Si] (Fig. 6f, g). Compared to leaf Si, the other nutrients were poorly correlated with the traits discussed above (Fig. S6). Plant biomass was negatively correlated to leaf [N] and positively correlated to leaf [Mg], the force to punch was negatively correlated to leaf [P], and leaf arc was positively correlated with leaf [Mg] (Fig. S6).

Discussion

Amendments significantly increased the available Si concentration in the studied desilicated soil, corroborating previous studies using biochar (Houben et al. 2014; Li et al. 2018, 2019b; Wang et al. 2018) and wollastonite (Tavakkoli et al. 2011; Haynes et al. 2013; Babu et al. 2016). The more pronounced Si_{CC} increase under the rice biochar amendments compared to the cotton biochar amendments is explained by higher Si concentrations in the pyrolyzed feedstock (Table 2). After normalizing Si_{CC} concentrations by the total Si content of the amendment (RE_{Si} ; Table 5), the ability to release Si is higher for cotton biochar than for rice biochar. This may occur because the silica is present as minute deposits, scattered throughout the organic matrix of cotton-biochar (Fig. S7), which results in higher surface area per unit Si and therefore higher solubility than the larger phytoliths present in rice. The higher Si_{CC} concentrations, by an order of magnitude, in wollastonite compared to the biochar amendments has previously been observed by Li et al. (2018). This cannot be entirely explained by a higher total Si content, as wollastonite RE_{Si} was significantly higher than rice biochar RE_{Si} (for both input rates) and to cotton biochar RE_{Si} for the highest input rate of 30 t ha^{-1} (Table 5). Besides different inherent dissolution rates between phytoliths and wollastonite, the organic matrices of biochars could contribute to slowing down the dissolution of phytoliths (Frayse et al. 2006, 2010; Nakamura et al. 2020b), which could explain the higher Si_{CC} concentrations in soil amended with wollastonite. Finally, the positive relationship between Si_{CC} and pH_{CC} (Fig. S2) has been observed elsewhere (Miles et al. 2014; Meunier et al. 2018; Li et al. 2019b; de Tombeur et al. 2020b), and suggests that the liming effect of biochar amendments may contribute to increased phytolith dissolution (Frayse et al. 2009; Li et al. 2019b).

The 32-day $CaCl_2$ extractions accurately estimated the plant-available Si for biochar-treated soils, supporting recent studies (e.g. Li et al. 2019, Wu et al. 2019) (Fig. 3). Although wollastonite

released Si at a much faster rate than biochars, leaf [Si] of R5 and R30 were 1.9- and 1.3-fold higher than the W5 and W30 respectively. In addition, leaf [Si] with the rice biochars amendments were the highest: 2.5 and 3.7 fold higher than the control for the application rates of 5 and 30 t ha⁻¹ respectively. These results confirm that phytolith-rich biochar is an efficient slow-release Si amendment for desilicated soils (Li and Delvaux 2019; Wang et al. 2019), with cascading positive effects for plant growth (Zama et al. 2018; Leksungnoen et al. 2019; Li et al. 2019a; Huang et al. 2020; Wang et al. 2020). Rice-husk biomass is an agriculture residue that is readily available for biochar production in Burkina Faso (FAO 2014), and this work provides strong evidence that the production of biochar from this residue is a very promising way to recycle Si-rich agricultural wastes from rice fields to increase Si plant-availability in soils of Burkina Faso. To ensure maximum sustainability for the use of biochar, the input feedstock and pyrolyzing unit must be as close to the field as possible, owned by the local farmers.

Rice biochar increases the degree of leaf silicification, but does it impact physical traits?

The application of rice biochar markedly increased the degree of rice leaf silicification (up to 70 g kg⁻¹ of Si for R30, or about 15% of silica by dry weight), which are extremely high levels (Hodson et al. 2005). The impact of this high degree of silicification on leaf and plant traits is discussed below.

Leaf [Si] and leaf arc were negatively correlated, which confirms that Si accumulation in grasses contributes to leaf straightness. The four amendments (wollastonite, rice biochars) for which leaf arc significantly decreased compared to the control were also those for which silica was more abundantly deposited on both the adaxial and abaxial sides of leaf epidermis, and the size of phytoliths was larger (more conjoined silicified cells) (de Tombeur et al. 2020c; Guerriero et al. 2020). In addition, negative correlations between the percentage area of silica deposits on leaf

surface (Fig. 4) and leaf arc were found for both abaxial ($R^2=0.69$; $n=7$; $p<0.05$) and adaxial sides ($R^2=0.82$; $n=7$; $p<0.01$). These results highlight the key role of the degree of leaf silicification, especially the number of conjoined silicified cells, in the control of leaf erectness. By contrast, rows of silica cells (specialized epidermal cells with the entire lumen filled with silica, forming dumbbell-shaped phytoliths; Kumar and Elbaum 2018) do not seem to play a role in leaf erectness, because they were entirely silicified for all amendments, irrespective of leaf [Si] and leaf arc values. As leaf arc values were not correlated with macronutrients (Fig. S6), we conclude that rice biochar is an effective soil amendment to improve leaf erectness through silicification. This can have a positive influence on plant fitness as changes in leaf erectness can impact light interception (Ando et al. 2002).

Under rice biochar amendments, the LMA decreased by a factor of 1.6 compared to the control, and we observed a strong negative correlation between leaf [Si] and LMA, which, to our knowledge, has not been demonstrated before. Lower LMA is explained either by thinner leaves of the same density, or by less dense leaves of the same thickness. Given silica is denser than C-based compounds and that leaf [Si] and [C] were negatively correlated, thinner leaves seem more likely. The negative correlation may indicate a tradeoff between a high degree of leaf silicification and thicker leaves that perform similar plant functions. Both leaf silica deposits and higher leaf thickness impact leaf water status (Búrquez 1987; McBurney 1992; Gong et al. 2003; Meunier et al. 2017), mechanical properties (Schoelynck et al. 2010; Onoda et al. 2011; Klotzbücher et al. 2018), and potential to deter herbivores feeding (Peeters 2002; Massey et al. 2006). Investing in silica deposits when plant-available Si is enhanced by Si-based amendments rather than the construction of thick leaves could perform functions such as 1) minimizing leaf water loss and/or

2) improving leaf erectness and/or 3) reducing herbivory, and at a lower energetic cost (Raven 1983).

Silica deposits in the leaf epidermis can also increase leaf abrasiveness (Hartley et al. 2015; Hall et al. 2020), which can in turn reduce herbivore feeding (Massey and Hartley 2006; Massey et al. 2007; Hall et al. 2020), and reduce the digestive efficiency of herbivores (Massey et al. 2006). Since the magnitude of silica deposits on leaf surface increased with leaf [Si] (Fig. 4), it seems likely that the application of rice biochar can contribute to reducing biotic stress in rice growing on the desilicated soils of Burkina Faso. Surprisingly, the force required to punch leaves was negatively correlated with leaf [Si], which seems somewhat contradictory given the evidence of defense against herbivores for Si-rich leaves. Although some research showed that Si increases the force required to tear (Yamamoto et al. 2012; Kido et al. 2015; Simpson et al. 2017) and shear (Johnson et al. 2019) leaves, our results suggest that it is not the case for punching, as other leaf parameters may change along with epidermal silicification. In particular, the Si-rich leaves of the present study could possibly be penetrated more easily because of their lower LMA (Onoda et al. 2011). However, the ability of penetrometers to estimate leaf mechanical properties and to mimic herbivores penetration remains controversial (Sanson et al. 2001). In addition, with the main pests of rice in Burkina Faso attacking stems (Ba et al. 2008), further work on the impacts of biochar/Si on stem properties would be valuable.

Finally, although the decrease in LMA and leaf [C] with increasing leaf [Si] suggests that effective Si amendments diminish the leaf C costs of rice crops, plant height was not correlated with leaf [Si] and not strongly impacted by the different treatments (with the exception of W30 for which it significantly decreased due to limited growth), and plant biomass decreased with increasing leaf [Si]. This contrasts with previous studies showing the positive effects of Si on the overall plant

growth (e.g. Zañão Júnior et al. 2010), but is in line with others showing no clear effects of Si addition (e.g. Ando et al. 2002). It is possible that plant height did not increase significantly because of a limited root growth in the pots used for the experiment, or because Si has beneficial effects on plant growth mainly for stressed plants (Fauteux et al. 2006). Since plant height was not markedly affected by Si addition, the decrease in plant biomass with increasing leaf [Si] could be explained by the net decrease in LMA for Si-rich leaves, though the total leaf area was not measured. More generally, the lack of positive effect of both biochars on plant growth is surprising given its global positive effect (Biederman and Stanley Harpole 2013), but has nevertheless already been reported in the literature (Haefele et al. 2011; Güereña et al. 2013; Tammeorg et al. 2014; Reibe et al. 2015). This indicates a need for in-situ studies into the effect of rice biochar on rice yields, keeping in mind that the application of biochar in agroecosystems plays other roles such as C storage and improved nutrient retention through an increase in the cation exchange capacity (Gul et al. 2015), which could have a long-term positive impact on soils of Burkina Faso.

We conclude that the use of rice biochar as a Si amendment on desilicated soils of Burkina Faso is an efficient method to increase the degree of silicification of rice leaves, which in turn impacts leaf traits likely to contribute positively to plant fitness. This is of special interest as our study demonstrates an efficient way to transfer Si-related fertility from rice field in lowlands to highly desilicated soils of cotton, maize and rice fields in upland positions. Given these promising results, further *in situ* work is now needed to examine if the positive effects of silicification from Si-rich biochar – as well as the overall positive effects of biochar on soil properties (Gul et al. 2015) – can protect plants against pest attacks while increasing plant productivity and crop yields in agroecosystems of Burkina Faso. The long-term impact (10-20 years) of biochar application on

rice Si uptake should also be tested as a more realistic for the local agricultural context, with amendment rates between 200 and 400 kg of biochar per hectare per year.

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Authors contribution

FdT, LC and J-TC planned and designed the research. FdT, LC, DC, FS, VB, DL, HN and J-TC conducted fieldwork. LC and FdT performed experiments. FdT, JC, LC, DC, FS, HN and J-TC analyzed data. FdT led the writing of the manuscript, with substantial inputs from all the authors.

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Tables

Table 1 – Physico-chemical characteristics of the Lixisol used for the experiment. Standard-errors are indicated in brackets (n=3). The term ‘CEC’ stands for ‘cation exchange capacity’ and ‘Si_{cc}’ is the Si concentration extracted with CaCl₂ 0.01M after 32 days (see the ‘Silicon kinetic release’ section in the Materials and Methods).

Particle-size distribution ^a (%)			pH-KCl ^b	pH-H ₂ O ^b	CEC ^c	Si _{cc}	Mineralogy ^d				
Sand	Silt	Clay			cmol _c kg ⁻¹	mg kg ⁻¹	Quartz	Kaolinite	K-feldspars	Anatase	Mica/illite
73.3 (1.6)	14.5 (0.9)	12.2 (0.6)	4.8 (0.0)	6.1 (0.3)	2.9 (0.0)	11.2 (0.4)	72%	23%	2%	2%	1%

^aGravimetric sedimentation after OM removal with hydrogen peroxide.

^b1:5 soil to solution ratio. 1M KCl for pH-KCl

^cMetson method (1956)

^dXRD analysis on bulk soil and clay-sized fraction. Carried out by a Bruker D8- Advance Eco diffractometer generating CuK radiation. Minerals identified with EVA 3.2 software and then quantified by TOPAS software.

Table 2 – Characteristics of the biochars used as Si amendments.

	Crop residue ^a	Biochar ^b						
	Si g kg ⁻¹	OC g kg ⁻¹	Si	P	Mg	Ca	K	Fe
Rice husks/biochar	69	409	198	2	2	2	7	13
Cotton stalks/biochar	2	709	4	1	2	14	9	1

^aAlkaline fusion with Li-tetra/metaborate at 1000°C, after calcination. Quantified by ICP-AES.

^bDetermined by European Biochar Certificate (EBC), Eurofins Umwelt. Both biochars fulfill EBC's premium quality requirement for heavy metal content.

Table 3 – Soil pH-KCl and concentrations of OC, total N and bioavailable P, K, Mg and Ca for the different amendments. The standard-errors are indicated under brackets (n=3). The letters represent Fisher HSD groupings ($p \leq 0.05$).

Amendment	Input rate t ha ⁻¹	pH-KCl	OC	Total N	Bioavailable nutrients (mg 100g ⁻¹)			
			g kg ⁻¹	%	P	K	Mg	Ca
Control (T)	-	4.84 (0.03)bc	3.46 (0.10)e	0.30 (0.01)de	0.13 (0.03)c	3.91 (0.35)b	3.68 (0.23)d	19.76 (1.63)e
Rice biochar	5	4.86 (0.13)bc	5.82 (0.41)c	0.35 (0.04)cde	0.18 (0.00)bc	3.96 (0.15)b	5.37 (0.59)bcd	23.22 (1.41)de
Rice biochar	30	4.70 (0.06)c	11.03 (0.40)a	0.45 (0.05)abc	0.22 (0.02)ab	4.94 (0.19)b	6.32 (0.82)abc	25.68 (1.94)cd
Cotton biochar	5	4.71 (0.05)c	7.77 (0.57)b	0.50 (0.05)ab	0.27 (0.03)a	4.31 (0.57)b	7.55 (1.06)a	33.00 (2.49)b
Cotton biochar	30	5.07 (0.12)b	12.68 (1.08)a	0.53 (0.02)a	0.24 (0.02)ab	8.83 (1.01)a	7.05 (0.56)ab	33.00 (0.60)b
Wollastonite	5	5.04 (0.12)b	5.58 (0.84)cd	0.40 (0.06)bcd	0.19 (0.01)bc	3.65 (0.17)b	6.17 (0.92)abc	29.48 (2.43)bc
Wollastonite	30	6.54 (0.03)a	3.97 (0.01)de	0.28 (0.00)e	0.18 (0.00)bc	3.93 (0.08)b	4.55 (0.07)cd	42.84 (0.80)a

Table 4 – Leaf ash and C content, plant height and biomass, leaf mass per area (LMA), leaf arc and force to punch for the different amendments. The standard-errors are indicated in brackets (n=5). The letters represent Fisher HSD groupings ($p \leq 0.05$).

Amendment	Input rate	Leaf ash	Leaf C	Height	Biomass	LMA	Leaf arc	Force to punch
	t ha ⁻¹	%	g kg ⁻¹	cm	g	g m ⁻²	cm	kN m ⁻¹
Control (T)	-	10.7(1.4)c	394(3)b	61.4(2.3)b	1.8(0.1)a	55.3(3.6)a	10.5(0.6)a	0.30(0.02)b
Rice biochar	5	18.8(1.1)ab	364(4)cd	76.4(1.8)a	1.5(0.1)ab	37.1(2.1)c	7.8(0.6)b	0.30(0.01)b
Rice biochar	30	19.1(1.3)ab	353(7)d	65.8(1.4)b	1.2(0.1)b	35.2(2.0)c	4.0(0.2)c	0.22(0.01)c
Cotton biochar	5	10.7(1.0)c	408(4)a	65.9(2.4)b	1.8(0.1)a	43.7(4.3)bc	9.6(0.3)a	0.39(0.03)a
Cotton biochar	30	11.9(1.3)c	407(3)ab	77.1(2.4)a	1.6(0.1)a	41.6(4.2)bc	9.8(0.3)a	0.30(0.02)b
Wollastonite	5	16.2(1.6)b	369(6)c	72.2(2.3)a	1.6(0.1)a	49.2(2.8)ab	7.3(0.6)b	0.30(0.02)b
Wollastonite	30	20.2(1.2)a	376(4)c	48.3(2.0)c	0.7(0.0)c	34.6(2.0)c	5.4(0.6)c	0.22(0.01)c

Table 5 – Silicon release efficiency (RE_{Si}) for the different amendments. The standard-errors are indicated in brackets (n=3). The letters represent Fisher HSD groupings ($p \leq 0.05$).

Amendment	Input rate t ha ⁻¹	RE_{Si} %
Rice biochar	5	1.5(0.1)d
Rice biochar	30	0.8(0.0)d
Cotton biochar	5	10.4(1.1)b
Cotton biochar	30	7.2(0.7)c
Wollastonite	5	30.5(1.2)a
Wollastonite	30	6.8(0.2)c

Figures

Fig. 1 – Kinetic extraction of Si with CaCl₂ 0.01M for the different amendments. The second plot is a zoom of the first plot without the wollastonite treatments. The colors indicate the different treatments. The error bars represent the standard errors (n = 3) and the letters represent Fisher HSD groupings ($p \leq 0.05$) after 32 days of extraction.

Fig. 2 – Boxplot of rice leaf silicon concentration for the different amendments. The central horizontal bar in each box shows the median, the box represents the interquartile range (IQR), the whiskers show the location of the most extreme data points that are still within a factor of 1.5 of the upper or lower quartiles, and the large black points are values that fall outside the whiskers. The small opaque black points represent the data ($n = 5$), and red points indicate the overall mean. The letters represent Fisher HSD groupings ($p \leq 0.05$).

Fig. 3 – Relationship between soil Si extracted with CaCl₂ 0.01M for 32 days and rice leaf Si concentration for the control and the biochar amendments. The error bars represent the standard errors (n= 3 for Si_{cc}; n=5 for leaf [Si]). The coefficient of determination (R^2) and p-value of the regression line are shown. The shaded area represents the 95% confidence intervals.

Fig. 4 – SEM-derived images and Si mapping (EDX) of the leaf adaxial surfaces for the different amendments in (a). Area percentage of yellow pixels (%) on the leaf abaxial and adaxial surfaces for the different amendments in (b). In (a), the red square on T mapping exemplifies the area used to count the yellow corresponding to Si (see materials and methods). The scale bars have the same size and are equivalent to 200 μm for all the images. In (b), the error bars represent the standard-deviations ($n=7$ randomly selected squares by images).

Fig. 5 – Images of phytoliths physically extracted from rice leaves for the different amendments.

The scale bars have the same size and are equivalent to 500 µm for all the amendments.

Fig. 6 – Plots of rice leaf Si concentrations versus: ash concentration in (a), carbon concentration in (b), plant height in (c), plant biomass in (d), leaf mass per area in (e), leaf arc in (f) and force to punch in (g) ($n = 35$). The coefficient of determination (R^2) and p-value of the regressions are shown. Shaded areas represent 95% confidence intervals.