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Silicon can attenuate glyphosate-induced stress in young *Handroanthus albus* by improving photosynthetic efficiency and decreasing cellular electrolyte leakage

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The extensive use of glyphosate, while effective in weed control, poses significant risks to non-target plant species such as *Handroanthus albus* (yellow Ipe), an important species in tropical sustainable forestry. This study aimed to assess the impact of glyphosate on young Ipe plants and to investigate the protective role of silicon (Si) supplementation. Increasing glyphosate concentrations were found to induce heightened cellular electrolyte leakage and reduced concentrations of photosynthetic pigments in young Ipe leaves. Glyphosate exposure also compromised photosynthetic efficiency, resulting in decreased leaf biomass production. Conversely, supplemental applications of Si, applied via root and foliar routes, significantly increased Si accumulation in young Ipe leaves and mitigated the adverse effects of glyphosate. Silicon treatment decreased electrolyte cellular leakage by enhancing antioxidant defenses, particularly through elevated flavonoid and anthocyanin levels, and preserved photosynthetic efficiency. Si-treated plants maintained higher chlorophyll *a* concentration and exhibited improved photochemical efficiency, even under moderate rates of glyphosate stress. Consequently, Si application led to increased leaf dry mass, particularly at moderate glyphosate concentrations, highlighting its role in enhancing the resilience of young Ipe plants to moderate herbicide-induced stress. Incorporating Si into sustainable forestry practices could enhance the resilience of key species, supporting reforestation and afforestation efforts in glyphosate-prone environments.

Keywords Beneficial element, Herbicide stress, Stress Attenuation

Glyphosate, a widely used herbicide, has played a crucial role in modern agriculture, especially in controlling a broad spectrum of weeds. Its application extends beyond traditional agricultural systems, frequently used in mitigating weed competition within young forests and reforestation initiative[1]. Nevertheless, the pervasive use of glyphosate has caused considerable concern due to its potential to increase cellular electrolyte leakage stress

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and impair photosynthetic processes in non-target plant species[2]. Among these, *Handroanthus* spp. showed susceptibility to glyphosate-induced toxicity[3].

Handroanthus albus, an important ornamental and timber species native to Brazil, is commonly referred to as yellow Ipe and is liked for its ornamental appeal, superior timber quality, and role in sustainable forestry practices[4]. As a native species from Brazil, Ipe significantly contributes to biodiversity conservation and the rehabilitation of degraded ecosystems. Its resilience and adaptability help in reforestation and afforestation initiatives, thereby advancing sustainable land management. However, the susceptibility of *Handroanthus* spp. to glyphosate-induced stress presents a considerable challenge to these efforts[5]. While glyphosate is efficient in managing competitive weed species, its application can inadvertently affect nearby native forest areas, posing a risk to young Ipe trees by disrupting cellular stability, reducing photosynthetic pigments, and impairing overall photosynthetic efficiency[6].

The susceptibility of forests to glyphosate-induced stress necessitates the exploration of effective attenuative strategies, with the application of silicon (Si) emerging as a particularly promising approach. Although not traditionally recognized as an essential element, Si has attracted significant attention for its role in enhancing plant resilience to several abiotic stresses[7]. The beneficial effects of Si are evident in plants exposed to environmental extremes such as drought[8–10] heavy metals[11] nutritional disorder[12–14] and herbicides like glyphosate, as reported in tomato plants[15]. Silicon confers its protective effects through multiple mechanisms, including the stabilization of cellular structures[16] enhancement of antioxidant defense system[17] maintenance of nutritional efficiency and homeostasis[18,19] and improving photosynthetic efficiency[20,21]. Despite the growing evidence supporting the protective role of Si in various crop species, its impact on forest species, including Ipe, remains understudied.

Although *Handroanthus albus* is classified as a non-Si-accumulator[22] recent studies have demonstrated the potential benefits of Si application even in species that do not naturally accumulate significant Si levels[23]. The application of soluble Si sources and Si-nanoparticles has shown promising results in enhancing plant resilience to various environmental stresses[24]. These forms of Si are more readily absorbed and utilized by plants, leading to improved stress tolerance, even in non-accumulator species[23].

Given the critical role of *Handroanthus albus* in sustainable forestry and its susceptibility to glyphosate-induced stress, this study developed two key hypotheses. The first hypothesis is that glyphosate exposure will detrimentally affect young Ipe plants by compromising cellular stability, reducing photosynthetic pigments, and impairing photosynthetic efficiency. The second hypothesis is that the application of Si can attenuate these adverse effects by enhancing cellular strength, protecting photosynthetic pigments, and improving overall photosynthetic efficiency. The objective of this research was to evaluate the impact of increasing glyphosate concentrations on young Ipe plants and to assess the efficacy of Si as an attenuator of glyphosate-induced stress in young Ipe plants.

Materials and methods

Growth conditions

The experiment was conducted using one-year-old Ipe trees in a greenhouse at the Federal Institute of Education, Science, and Technology of São Paulo, Barretos, SP, Brazil. Greenhouse conditions were continuously monitored throughout the experimental period using a thermos-hygrometer (KTJ Thermometer® MSC Industrial), with recorded average relative humidity at 46.6 ± 23%, maximum temperature at 34.7 ± 5.4 °C, and minimum temperature at 17.1 ± 4.3 °C.

The young Ipe trees were procured from a specialized nursery that produces forest seedlings and were delivered in plastic bags designed for safe transport and transplanting. Upon arrival, the seedlings were transplanted into 8 dm^3 polyethylene pots filled with soil sourced from a non-agricultural site. The chemical properties of the soil were analyzed following the methods described by Raij et al.[25], with the following results: pH ($CaCl_2$) 5.48; organic matter 13.93 g dm^{-3}; phosphorus (Resin) 5.74 mg dm^{-3}; sulfur 2.98 mg dm^{-3}; calcium 10.95 $mmol_c$ dm^{-3}; magnesium 5.90 $mmol_c$ dm^{-3}; potassium 1.38 $mmol_c$ dm^{-3}; and hydrogen + aluminum 15.23 $mmol_c$ dm^{-3}. Silicon concentration was determined according to Kondörfer et al. (2004)[26], showing a value of 3.0 mg dm^{-3}.

Based on fertilization recommendations for forest species from the Atlantic Forest[27] no lime was required, and each pot received 17 g dm^{-3} of N, as urea; 6 g dm^{-3} of P, as single phosphate; and 18 g dm^{-3} of K, as potassium chloride.

Irrigation maintained soil moisture at 80% of the field capacity in each pot, using either water (-Si) or a silicon solution (+ Si). This level was chosen to ensure adequate water availability for plant growth while avoiding excessive moisture that could compromise root aeration. To determine field capacity, a pot filled with 10 dm^{-3} of dry soil was weighed, then saturated with water until drainage began. The pot was covered with aluminum foil to prevent evaporation, and after 24 h, it was reweighed. Field capacity was calculated by subtracting the weight of the dry soil from the weight of the saturated soil. Weekly measurements of pot weight were taken to assess evapotranspiration, and the necessary amount of water (-Si) or silicon solution (+ Si) was applied to maintain 80% of the field capacity.

Experimental design and treatment application

The study used a randomized block design with a 5 × 2 factorial arrangement with four replicates and one plant per replicate, totaling 40 experimental units. The experimental treatments included five rates of glyphosate (Roundup WG®): 0 (Control), 800, 1400, 2000, and 2600 g equivalent acid (g e.a.) per hectare, applied either with or without silicon (2mM).

The glyphosate concentrations used in this study were based on the manufacturer's recommended rate for weed control (2000 g e.a. ha^{-1}). The lower rates (800 and 1400 g e.a. ha^{-1}) were selected to simulate small and large spray drift scenarios, respectively, while the highest rate (2600 g e.a. ha^{-1}) was chosen to mimic an overapplication

situation. Since glyphosate is not intended for direct foliar application on forest species, these treatments were designed to reflect potential off-target exposure that may occur during herbicide use in operational settings.

Glyphosate was sprayed one week after the sixth Si foliar application. Glyphosate ammonium salt, with a concentration of 792.5 g kg^{-1} (720 g e.a.), was used as the herbicide. The glyphosate was sprayed directly onto the aerial parts of the young Ipe plants using a manual sprayer, ensuring thorough coverage of all leaves until the solution began to drip. Each plant received 23 sprays, totaling 8.28 mL of glyphosate solution per plant. Nanosilica (Si concentration: 168,3 g L^{-1}, specific surface area: 300 m^2 g^{-1}; particle size: 8,5–9,7 nm, pH: 12) was used as the Si source. A concentration of 2 mM was chosen to prevent Si polymerization, which can occur at concentrations exceeding 3 mM[28]. Silicon was applied via fertigation, beginning one day after transplanting and continuing daily throughout the experiment. The pH of the Si solution was adjusted to 6.0 ± 0.5 using either 0.1 M HCl or 0.1 M NaOH.

To address the relatively low efficiency of Si uptake by the roots of forest species[22] six foliar Si applications were also performed. These applications were applied every three days, starting 25 days before the glyphosate treatment. Each plant received approximately 8.28 mL (16.56 µM of Si) of silicon solution (2 M) per application, totaling about 50 mL (99.36 µM of Si) per plant across all six applications.

The use of both root and foliar applications was necessary because *Handroanthus albus* is classified as a non-Si-accumulator suggesting it has limited capacity to absorb silicon via the root system[22]. Therefore, the combined approach was adopted to enhance Si uptake and enable a more accurate assessment of its physiological effects under glyphosate stress.

The application of glyphosate and foliar Si was conducted between 6 and 7 AM under optimal greenhouse conditions, with temperatures below 20°C and air humidity above 80%. These conditions are considered ideal for maximizing foliar absorption[29].

Assessments of cellular electrolyte leakage, flavonoid and anthocyanin index

One week after glyphosate application, cellular electrolyte leakage was analyzed. Ten leaf discs were collected from the second fully developed leaf and placed in a beaker with deionized water for two hours. The initial electrical conductivity (EC1) was measured using a conductivity meter (AK51, Akso, Brazil). Subsequently, the samples were autoclaved at 121°C for 20 min. After cooling, the final electrical conductivity (EC2) was recorded. Electrolyte leakage was quantified by calculating the ratio of EC1 to EC2 and multiplying the result by 100.

The flavonoid and anthocyanin indices in the middle of the second fully matured leaf were measured using the Multiple pigment Meter (MPM-100, OPTI-Sciences, USA).

Pigment production and photosystem efficiency

The content of chlorophyll *a*, chlorophyll *b*, carotenoids, pheophytin *a*, and pheophytin *b* was assessed seven days after glyphosate application. Leaf discs were collected from the middle third of the blade of the second fully developed leaf and immediately weighed to determine fresh mass between 0.025 and 0.030 g. The sample was then depigmented using 80% acetone solution. Pigment analysis was conducted using a DU640 spectrophotometer (Beckman, USA) at specific wavelengths following the method described by Lichtenthaler[30].

Measurements for photosystem efficiency were taken between 7 and 9 a.m. using a portable fluorometer (OS30P+, Opti-Sciences INC. USA) on the third fully developed leaf. Initial fluorescence (F_0), maximum fluorescence (F_m), and variable fluorescence (F_v) were determined. Based on these readings, the photochemical efficiency of photosystem II (F_v/F_m), the efficiency of energy conversion of absorbed light (F_v/F_0), and the maximum yield of non-photochemical excitation (F_0/F_m) were calculated[31].

Photoassimilate production

Analysis was performed using the methanol, chloroform and water solution (MCW – 60% methanol, 25% chloroform, 15% water), in which the samples were left to settle for 48 hours[32]. After this period, 4 mL of the supernatant was extracted and transferred to a 15 mL Falcon tube, to which 1 mL of chloroform and 1.5 mL of deionized water were added and left to settle for another 24 h.

To evaluate total soluble sugars, 0.5 mL of the supernatant was mixed with 0.5 mL of 5% phenol and 2 mL of sulfuric acid, and the absorbance was read at 490 nm using a spectrophotometer (model B442, Micronal, Brazil)[32].

A 1 mL of the supernatant was combined with 0.5 mL of sodium citrate (0.2 M), 0.2 mL of ninhydrin solution (5% in monomethyl ether of ethylene glycol), and 0.1 mL of KCN solution (0.0002 M) to determine total free amino acids. The mixture was heated to 100 °C for 20 min, then cooled under running water. After cooling, 1 mL of 60% ethanol was added, and the absorbance was measured at 570 nm[32].

A 0.1 mL of the supernatant was combined with 0.1 mL of 30% potassium hydroxide (KOH) and 2 mL of sulfuric acid containing anthrone to measure sucrose. This mixture was vortexed, heated to 100 °C for 10 min, and cooled to room temperature before measuring the absorbance at 490 nm[32].

Leaf biomass production

Two weeks after the glyphosate application, the young Ipe plants were harvested. The leaves were washed with water, detergent solution (0.1%), hydrochloric acid solution (0.3%), and then rinsed with deionized water to remove any residual chemicals. After thorough washing, the samples were placed in a forced-air circulation oven (TE-394/3-MP, Tecnal, BR) set at 65 ± 5°C until a constant mass was reached. The dry mass of the leaves was then determined using a semi-analytical scale.

Silicon determination

After drying, the leaves were ground to fine powder using a knife mill for silicon analyses. The Si concentration was quantified following alkaline digestion[26] and determined by colorimetric analysis using ammonium molybdate[33]. Silicon accumulation was calculated by multiplying the dry mass of the leaf material by the Si concentration to determine the total amount of Si stored in the leaf tissues.

Statistical data processing

Statistical analysis was performed using Python (version 3.9.7; Python Software Foundation). The Shapiro-Wilk test was used to assess the normality of data distribution ($p > 0.05$). The Levene test was conducted to ensure homogeneity of variances between groups ($p > 0.05$). An analysis of variance (ANOVA) was performed to detect significant differences between groups ($p < 0.05$). Tukey post-hoc test was used to identify specific differences between groups where ANOVA results were considered significant at $p < 0.05$. Principal component analysis (PCA) was also performed based on the covariance matrix to analyze interrelationships between variables and elucidate variance in terms of inherent dimensions. Hierarchical clustering analysis was conducted using Euclidean distance and the single linkage method for cluster formation.

Results

Silicon concentration and accumulation in leaves of young Ipe plants under glyphosate and silicon treatment

The application of Si administered both via root and foliar routes, significantly increased Si concentration (Fig. 1a) and accumulation (Fig. 1b) in leaves of young Ipe plants. Silicon concentration was influenced by both Si and glyphosate treatments, although no significant interaction between these factors was observed (Fig. 1a). Notably, increasing glyphosate rates did not alter Si concentration in Si-treated plants, which remained around 3.3 g kg^{-1}. However, in plants not treated with Si, Si concentration decreased as glyphosate rates increased, reaching 1.7 g kg^{-1} at the highest glyphosate rate (2600 g e.a. ha^{-1}). Across all glyphosate rates tested, plants treated with Si consistently exhibited higher Si concentration in the leaves, in comparison to plants not treated with Si.

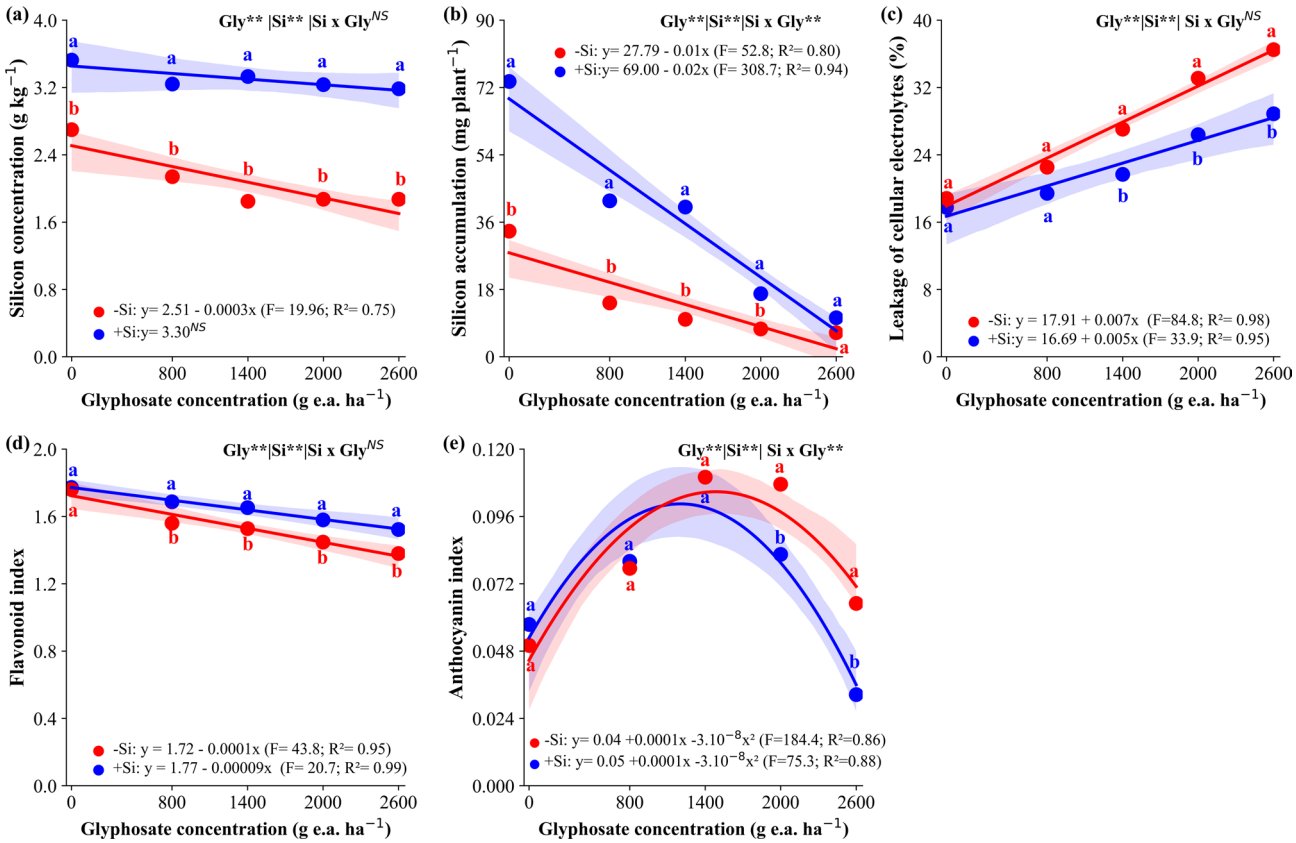


Fig. 1. Silicon concentration (a), silicon accumulation (b), leakage of cellular electrolytes (c), flavonoid index (d), and anthocyanin index (e) of young Ipe leaves cultivated under glyphosate (Gly) rates and absence or presence of silicon (Si). *, ** and ^{ns}- Significant by 1 and 5% and non-significant by the F test. Error bars represent the standard error of the mean (SE). Letter compares the absence and presence of Si at the same glyphosate concentration.

A significant interaction between Si supply and glyphosate rates was observed in Si accumulation (Fig. 1b). In plants not treated with Si, Si accumulation also declined linearly, reaching 1.79 mg per plant at 2600 g e.a. ha^{-1}. Similarly, in plants treated with Si, Si accumulation decreased linearly with increasing glyphosate rates, reaching 6.94 mg per plant at the highest glyphosate rate. Despite this decline, Si accumulation remained consistently high in plants treated with Si across all glyphosate rates, except at the highest rate (2600 g e.a. ha^{-1}), where no significant difference in Si accumulation was observed between treated and non-treated plants.

Cellular electrolyte leakage of young Ipe plants under glyphosate and silicon treatment

Cellular electrolyte leakage was affected by both glyphosate and Si treatments, with no significant interaction between these factors (Fig. 1c). Electrolyte leakage increased linearly with rising glyphosate rates, peaking at 29.7% in plants treated with Si and 36.1% in plants not treated with Si at the highest glyphosate rate. Silicon application effectively reduced electrolyte leakage at glyphosate rates of 1400, 2000, and 2600 g e.a. ha^{-1}, with no significant difference observed at lower rates (0 and 800 g e.a. ha^{-1}).

Flavonoid and anthocyanin index of young Ipe plants under glyphosate and silicon treatment

Both Si and glyphosate treatments significantly influenced the flavonoid (Fig. 1d) and anthocyanin (Fig. 1e) indices in young Ipe plants. The flavonoid index was affected by both glyphosate rates and Si treatment, with no interaction between these factors. As glyphosate concentration increased, the flavonoid index decreased linearly, reaching 1.54 in plants treated with Si and 1.46 in plants not treated with Si at 2600 g e.a. ha^{-1}. Silicon application enhanced the flavonoid index across all glyphosate rates tested, except at 0 g e.a. ha^{-1}.

An interaction between Si and glyphosate was observed for the anthocyanin index in young Ipe plants (Fig. 1e). In Si-treated plants, the anthocyanin index exhibited a quadratic increase with rising glyphosate rates, peaking to 0.105 at 1348 g e.a. ha^{-1} of glyphosate. Conversely, plant not treated with Si reached a maximum anthocyanin index of 0.099 at 1324 g e.a. ha^{-1}. At the highest glyphosate rates (2000 and 2600 g e.a. ha^{-1}), the presence of Si reduced the anthocyanin index, while no significant difference was observed at the other glyphosate rates.

Photosynthetic pigments in leaves of young Ipe plants under glyphosate and silicon treatments

The concentration of photosynthetic pigments in young Ipe leaves was significantly affected by Si and glyphosate treatments (Fig. 2). A significant interaction was observed between these treatments for chlorophyll *a*

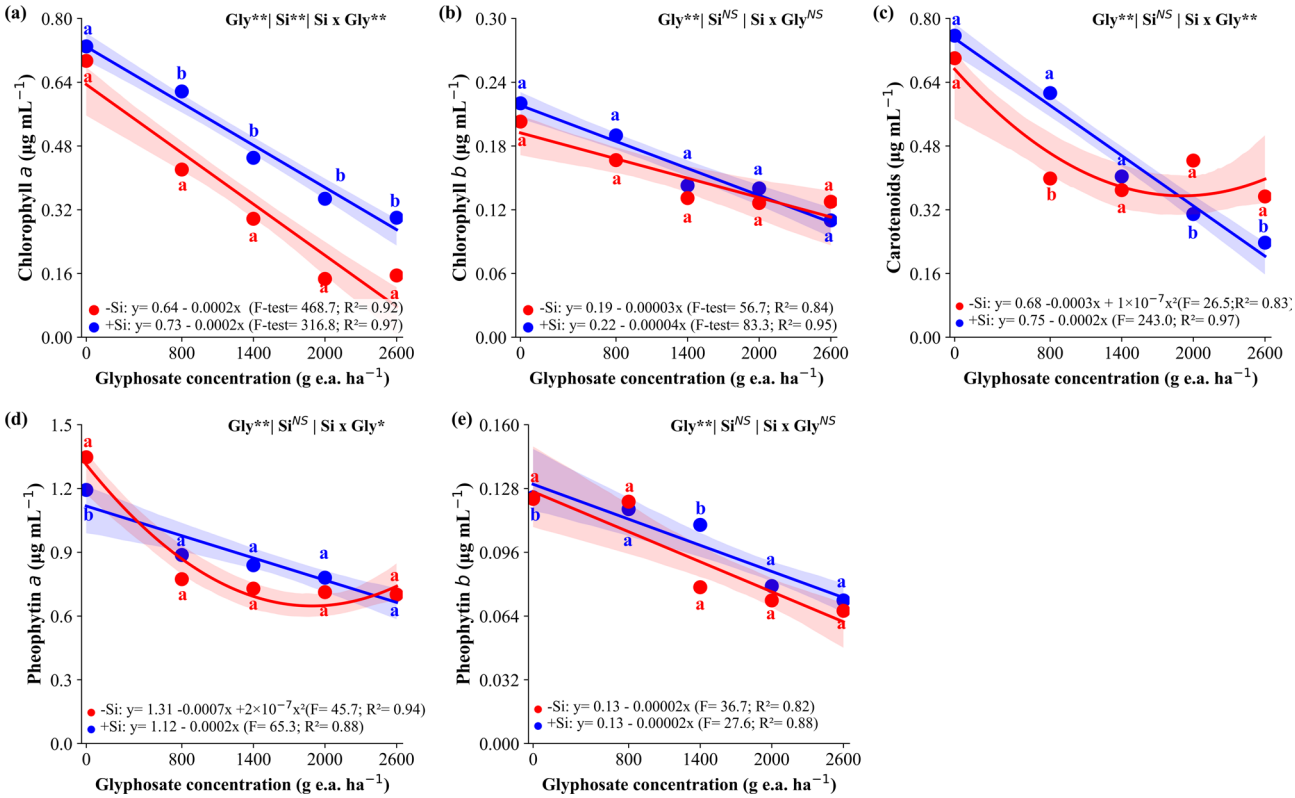


Fig. 2. Chlorophyll *a* (a), chlorophyll *b* (b), carotenoids (c), pheophytin *a* (d), and pheophytin *b* (e) of young Ipe leaves cultivated under glyphosate (Gly) rates and absence or presence of silicon (Si). *, ** and ^{ns}- Significant by 1 and 5% and non-significant by the F test. Error bars represent the standard error of the mean (SE). Letter compares the absence and presence of Si at the same glyphosate concentration.

concentration (Fig. 2a). In plants treated with Si, chlorophyll *a* concentration decreased linearly with increasing glyphosate rates, reaching as low as 0.21 μg mL^{-1} at the highest glyphosate rate. Similarly, in plants not treated with Si, chlorophyll *a* concentration also decreased linearly, reaching as low as 0.12 μg mL^{-1} at 2600 g e.a. ha^{-1}. Silicon application increased chlorophyll *a* concentration in all glyphosate treatments. However, in the absence of glyphosate (0 g e.a. ha^{-1}), no significant difference was observed between treated and non-treated plants, suggesting that Si benefits are more evident under stress conditions.

Chlorophyll *b* (Fig. 2b) and pheophytin *b* (Fig. 2e) concentrations were negatively affected by glyphosate treatments, with no significant effect of Si application on these pigments. As glyphosate rates increased, chlorophyll *b* decreased linearly, reaching 0.116 μg mL^{-1} in plants treated with Si and 0.112 μg mL^{-1} in plants not treated with Si at 2600 g e.a. ha^{-1}. Similarly, pheophytin *b* decreased linearly with increasing glyphosate rates, reaching as low as 0.72 μg mL^{-1} in plants treated with Si and 0.69 μg mL^{-1} in plants not treated with Si at 2600 g e.a. ha^{-1} of glyphosate.

An interaction between Si and glyphosate treatments was observed for carotenoid concentration (Fig. 2c). In plants treated with Si, carotenoid levels decreased linearly with rising glyphosate rates, reaching 0.23 μg mL^{-1} at the highest glyphosate rate. In plants not treated with Si, carotenoid levels followed a quadratic trend, with a minimum concentration of 0.46 μg mL^{-1} observed at a glyphosate rate of 1,500 g e.a. ha^{-1}. Silicon treatment increased carotenoid concentration only at 800 g e.a. ha^{-1} of glyphosate but decreased at higher glyphosate concentrations (2,000 and 2,600 g e.a. ha^{-1}).

An interaction was also observed between Si and glyphosate treatments for pheophytin *a* concentration (Fig. 2d). In plants treated with Si, pheophytin *a* concentration decreased linearly with rising glyphosate rates, reaching 0.60 μg mL^{-1} at the highest tested glyphosate rate. In plants not treated with Si, pheophytin *a* concentration exhibited a quadratic decrease, reaching a minimum of 0.70 μg mL^{-1} at 1,750 g e.a. ha^{-1} of glyphosate. Silicon application reduced pheophytin *a* content only at 0 g e.a. ha^{-1} of glyphosate.

Photosynthetic efficiency in leaves of young Ipe plants under glyphosate and silicon treatments

Photosynthetic efficiency (Fig. 3) was assessed through measurements of initial fluorescence (F_0), maximum fluorescence (F_m), photochemical effiiency of photosystem II (F_v/F_m), efficiency of energy conversion of absorbed light (F_v/F_0) and maximum yield of non-photochemical excitatio (F_0/F_m.).

An interaction between Si and glyphosate treatments was observed for F_0 (Fig. 3a). In plants treated with Si, increasing glyphosate rates led to a linear rise in F_0, reaching a maximum of 330 at the highest glyphosate rate. In plants not treated with Si, F_0 also increased linearly with rising glyphosate rates, reaching 425 at 2,600 g e.a. ha^{-1}. Notably, Si application decreased the F_0 only at a glyphosate of 2,600 g e.a. ha^{-1}.

Conversely, no significant interaction between the treatments was observed for F_m (Fig. 3b). Increasing glyphosate rates resulted in a linear decrease in F_m for both treated and non-treated plants, reaching 878.51 and 758.13, respectively. Plants treated with Si showed consistently higher F_m values across all glyphosate rates, except at the highest rate of 2,600 g e.a. ha^{-1}.

For F_v/F_m (Fig. 3c), a similar interaction was found. In plants treated with Si, F_v/F_m decreased linearly with increasing glyphosate rates, reaching 0.64 at the highest glyphosate rate. In contrast, plants not treated with Si exhibited a steeper linear decrease, with F_v/F_m values dropping to 0.49 at the same glyphosate rate. Silicon application significantly improved F_v/F_m in all glyphosate treatments, except at 0 g e.a. ha^{-1}, indicating that its protective effects on photosystem II efficiency are more evident when herbicide-induced stress is present.

Independent effects of Si and glyphosate treatments were observed for F_v/F_0 (Fig. 3d), with no interaction between these factors. Increasing glyphosate rates led to a linear decrease in F_v/F_0, reaching 1.64 in plants treated with Si and 0.88 in non-treated plants at the highest glyphosate rate. The application of Si consistently increased F_v/F_0 values across all glyphosate rates.

An interaction was also observed between Si and glyphosate treatments for F_0/F_m (Fig. 3e). In plants treated with Si, increasing glyphosate rates caused an increase in F_0/F_m up to 0.37 at the highest glyphosate rate. In plants not treated with Si, increasing glyphosate rates increased F_0/F_m, reaching 0.51 at 2,600 g e.a. ha^{-1}. Silicon application effectively reduced F_0/F_m across all glyphosate rates, indicating improved photosynthetic efficiency in plants treated with Si.

Photoassimilates products in leaves of young Ipe plants under glyphosate and silicon treatments

The effects of Si and glyphosate treatments on photoassimilates products (Fig. 4), including total free amino acids, total soluble sugars, and sucrose, were analyzed, with no significant interaction between Si and glyphosate treatments observed.

Increasing glyphosate rates resulted in a linear increase in total free amino acids (Fig. 4a), reaching 0.66 mg g^{-1} fresh weight in plants treated with Si and 0.83 mg g^{-1} fresh weight in non-treated plants at the highest glyphosate rate. The presence of Si reduced total free amino acids content at all glyphosate rates.

Total soluble sugars (Fig. 4b) decreased linearly with increasing glyphosate rates, reaching 15.0 mg g^{-1} dry weight in plants treated with Si and 13.5 mg g^{-1} dry weight in non-treated plants at glyphosate rate of 2,600 g e.a. ha^{-1}. Silicon application increased total soluble sugars only at lower glyphosate concentrations (0, 800, and 1,400 g. e.a. ha^{-1}).

Sucrose also decreased linearly with increasing glyphosate concentration, reaching 2.51 mg g^{-1} and 2.08 mg g^{-1} at 2600 g e.a. ha^{-1} of glyphosate for plants treated with Si and non-treated plants, respectively. Silicon application increased sucrose content at 1,400 g e.a. ha^{-1} of glyphosate.

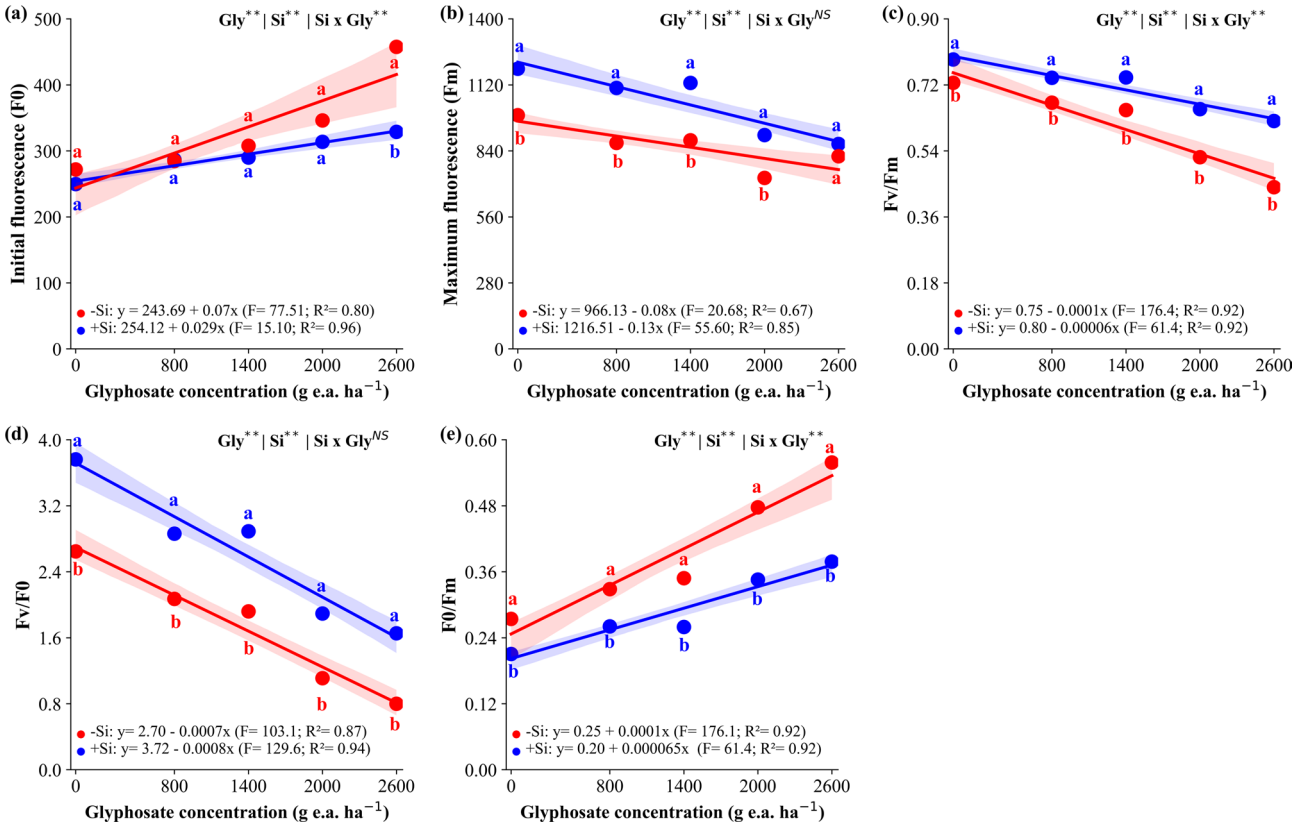


Fig. 3. Initial fluorescence (F0) (a), maximum fluorescence (Fm) (b), photochemical efficiency of photosystem II (Fv/Fm) (c), efficiency of energy conversion of absorbed light (Fv/F0) (d), maximum yield of non-photochemical excitation (F0/Fm) (e) of young Ipe leaves cultivated under glyphosate (Gly) rates and absence or presence of silicon (Si). *, ** and ^{ns}- Significant by 1 and 5% and non-significant by the F test. Error bars represent the standard error of the mean (SE). Letter compares the absence and presence of Si at the same glyphosate concentration.

Leaf dry matter production and visual symptoms of young Ipe plants under glyphosate and silicon treatments

An interaction between Si and glyphosate treatments was observed for plants development, measured through dry matter content (Fig. 4d). In plants treated with Si, leaf dry matter decreased linearly, reaching 4.4 g per plant at the highest glyphosate concentration. In non-treated plants, dry matter also decreased linearly, reaching 3.1 g per plant at 2,600 g. e.a. ha^{-1}. Silicon application effectively mitigated glyphosate toxicity in young Ipe plants at concentrations of 0, 800 and 1,400 g. e.a. ha^{-1}, increasing dry matter production compared to Si-untreated plants.

Visual symptoms of glyphosate toxicity in young Ipe leaves were assessed (Fig. 5). In the absence of Si, leaves displayed progressive injury correlating with the increasing glyphosate concentration. Initial symptoms at 800 g. e.a. included mild yellowing, advancing to the development of brown spots at 1,400 g e.a., significant necrosis and yellowing at 2,000 g e.a., and severe necrosis, curling, and discoloration at 2,600 g e.a. Conversely, plants treated with Si showed reduced symptoms of glyphosate toxicity across all concentrations. Leaves of plants treated with Si maintained better health, characterized by minor yellowing and fewer brown spots at lower glyphosate concentrations, and reduced severity of necrosis and discoloration at higher concentrations.

Hierarchical clustering analysis (HCA)

Plants treated with Si generally exhibited higher Si concentration and accumulation, as indicated by the darker red color in the columns for the corresponding treatments, particularly at 800 and 1,400 g e.a. ha^{-1} (Fig. 6). In contrast, high glyphosate concentrations without Si, such as 2,000 and 2,600 g e.a. ha^{-1}, displayed elevated levels of electrolyte leakage and F_0/F_m, as indicated by the red shading in these columns, signifying increased oxidative stress and photosystem disruption. These treatments also showed lower levels of chlorophyll *a*, chlorophyll *b*, and carotenoids, depicted by blue shading, suggesting a reduction in photosynthetic pigment content due to glyphosate toxicity.

Notably, plants treated with Si clustered together, particularly at moderate glyphosate concentrations, 800 and 1,400 g e.a. ha^{-1}, reflecting the effects of Si against glyphosate-induced injury. In contrast, non-treated plants at higher glyphosate levels, 2,000 and 2,600 g e.a. ha^{-1}, separated, highlighting the exacerbation of stress response in the absence of Si.

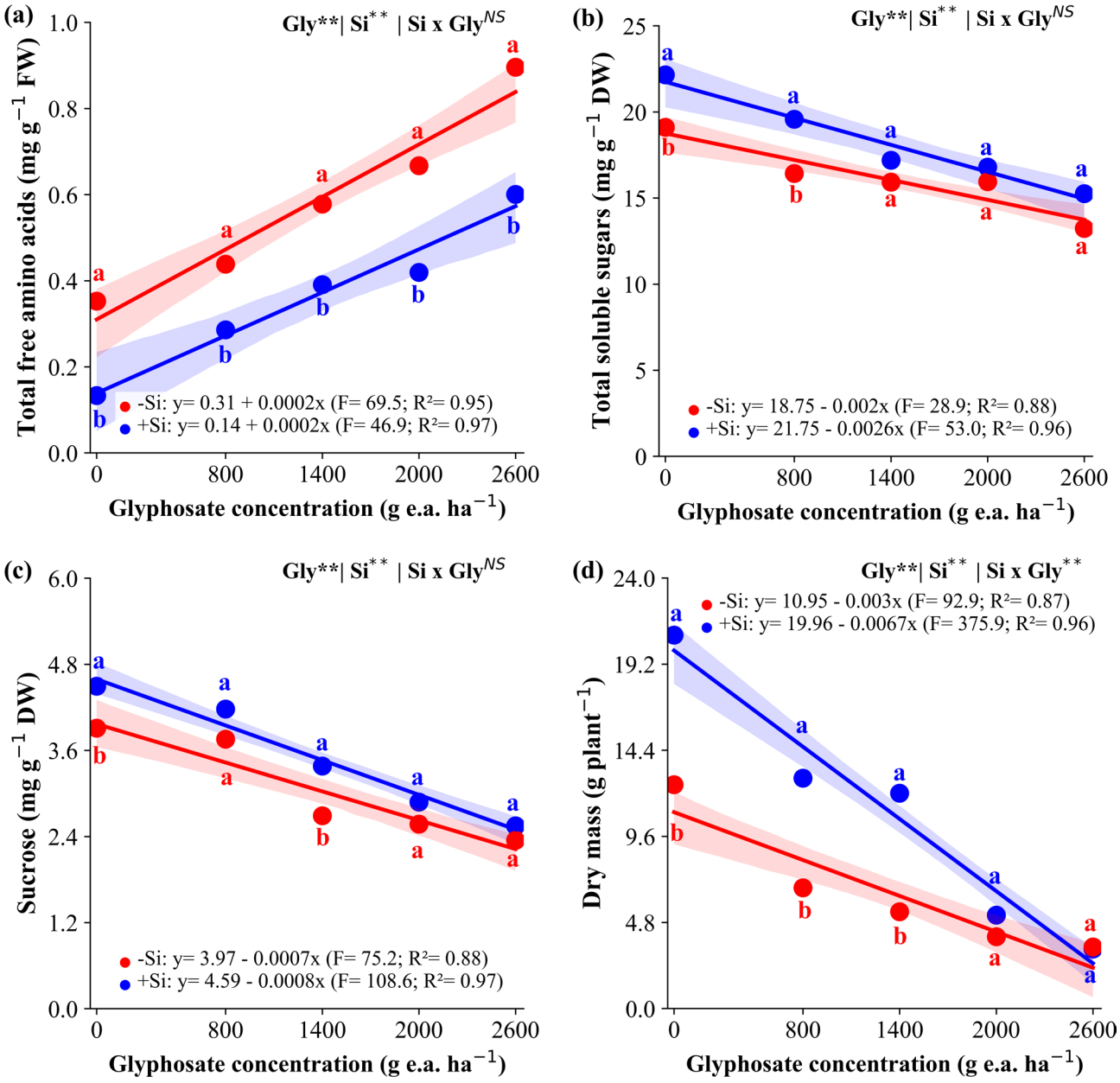


Fig. 4. Total free amino acids (a), total soluble sugars (b), sucrose (c), leaf dry mass (d) of young Ipe leaves cultivated under glyphosate (Gly) rates and absence or presence of silicon (Si). *, ** and ^{ns}- Significant by 1 and 5% and non-significant by the F test. Error bars represent the standard error of the mean (SE). Letter compares the absence and presence of Si at the same glyphosate concentration.

Principal component analysis (PCA)

The PCA biplot illustrates the relationships among various treatments and measured variables in young Ipe plants subjected to glyphosate and Si treatments (Fig. 7). The first principal component (Dim1) explains 69.1% of the variance, while the second component (Dim2) accounts for 7.1%. The biplot reveals distinct clustering of treatment groups. Plants not treated with glyphosate (0 g e.a. ha^{-1}), both with and without Si, are positioned on the left side of the biplot. Plants treated with Si show strong associations with variables such as Si accumulation, chlorophyll a, dry mass, and photosynthetic efficiency (F_v/F_m, F_v/F_0). In contrast, plants treated with the highest glyphosate concentrations (2,600 g e.a. ha^{-1}) cluster in the far-right quadrant, particularly those without Si, indicating severe stress characterized by increased electrolyte leakage, F_0, and F_0/F_m. Silicon application appears to mitigate some of this stress, as evidenced by the closer proximity of Si-treated plants to the center of the biplot.

The biplot also highlights the differential effects of Si and glyphosate on photosynthetic pigments and antioxidant indices. Photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, carotenoids, pheophytin *a*, and pheophytin *b*) are negatively correlated with Dim1 and are associated with lower glyphosate concentrations. On the other hand, flavonoid and anthocyanin index are more strongly associated with higher glyphosate levels

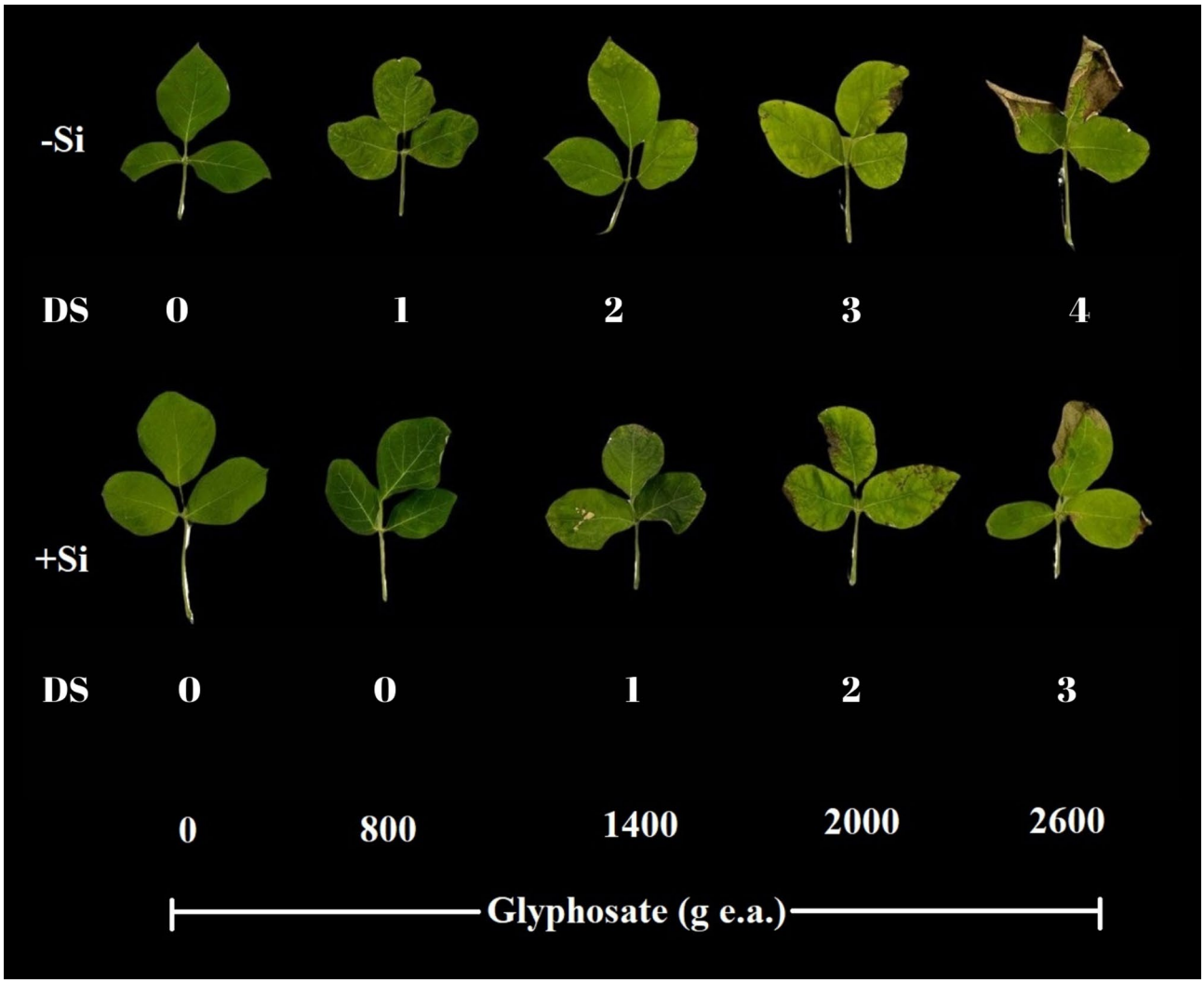


Fig. 5. Visual symptoms of glyphosate toxicity in young *Handroanthus albus* (yellow Ipe) leaves under different glyphosate concentrations (0 to 2600 g e.a. ha^{-1}), with and without silicon (Si) treatment. A damage severity (DS) score was assigned to each leaf based on visible symptom intensity, where 0 represents no visible symptoms, 1 indicates mild yellowing, 2 reflects moderate yellowing with initial brown spotting, 3 denotes advanced chlorosis with necrotic lesions, and 4 corresponds to severe necrosis, curling, and deformation. DS values are displayed beneath each leaf image to provide a standardized, objective assessment of visual damage across treatments.

and Si treatments, underscoring the role of Si in enhancing antioxidant defense compounds of plants. Overall, the biplot demonstrates that Si treatment significantly attenuates the negative effects of glyphosate on young Ipe plants, particularly by improving photosynthetic efficiency.

Discussion

Although specific information regarding the effects of glyphosate on the leaves of young Ipe plants is lacking, it is well-documented that glyphosate can decrease cellular integrity in plant tissues[34]. The primary mode of action of glyphosate is the inhibition of the shikimate pathway[35]; however, it also disrupts mitochondrial functions by impairing normal electron flow within the respiratory electron transport chain[36]. These disruptions can lead to the overproduction of reactive oxygen species (ROS) that is closely associated with cellular damage and dysfunction, including decreased integrity of cellular membranes[37]. This membrane damage often results in increased leakage of vital cellular electrolytes[38]. In this study, it was observed that higher glyphosate concentrations increased electrolyte leakage in the leaves of young Ipe plants (Fig. 1c). Notably, the relationship between elevated glyphosate levels and electrolyte leakage was evident, particularly in the absence of Si (Figs. 6 and 7).

Because of the cellular damage caused by glyphosate, pigment concentrations were significantly affected. Increasing glyphosate rates were linked to a reduction in all pigments studied in young Ipe leaves (Fig. 2). Glyphosate decreases pigment concentration primarily by disrupting the shikimate pathway, which is essential

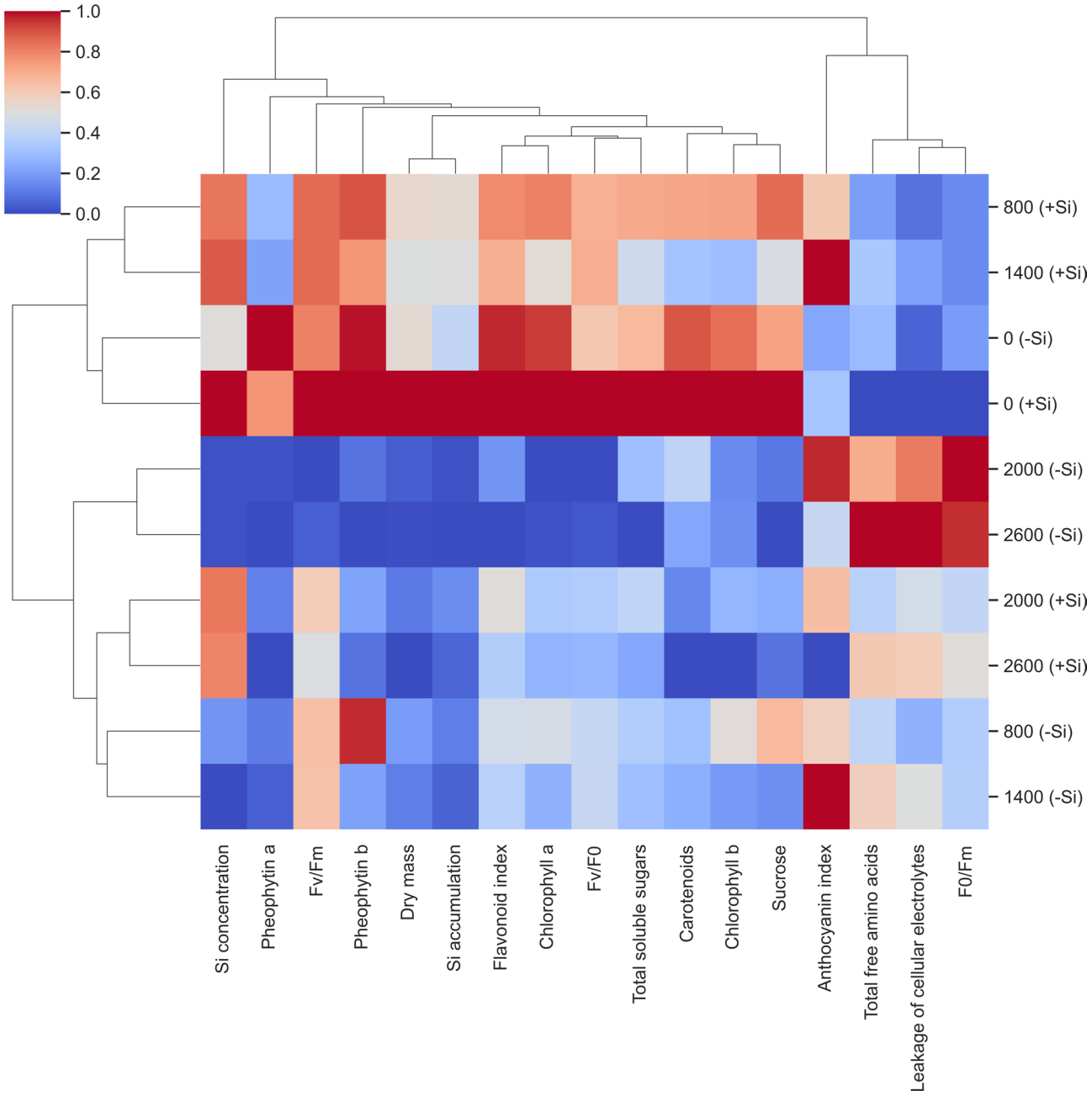


Fig. 6. Hierarchical clustering heatmap of response variables of *Handroanthus albus* cultivated under glyphosate rate (0, 800, 1400, 2000 and 2600 g e.a. ha^{-1}) in the absence (-Si) and presence of silicon (+Si).

for the biosynthesis of aromatic amino acids and other phenolic compounds[39]. These aromatic amino acids play an important role in biosynthetic pathway of pigments[40]. Additionally, glyphosate-induced oxidative stress further damages cellular structures, including chloroplasts, thereby exacerbating pigment loss[41].

Photosynthetic efficiency (Fig. 2) is another important process adversely affected by glyphosate, as evidenced by the strong correlation between higher glyphosate rates and F_0 and F_0/F_m (Figs. 6 and 7). In tree species, glyphosate can damage photosystem II (PSII) reaction centers[42] which are essential for the initial steps of light absorption and energy conversion in photosynthesis[43,44]. The observed increase in F_0 in the leaves of young Ipe plants (Fig. 2a) indicates that more energy is being dissipated as fluorescence due to the impaired function of PSII, reflecting a reduced capacity for photochemistry. The F_0/F_m ratio, which reflects the efficiency of PSII in converting absorbed light into chemical energy[45] further supports this observation. Under normal conditions, a low F_0/F_m ratio indicates that most of the absorbed light is being dissipated through non-photochemical quenching mechanisms[46] as the plant attempts to protect itself from further injury under glyphosate-induced stress.

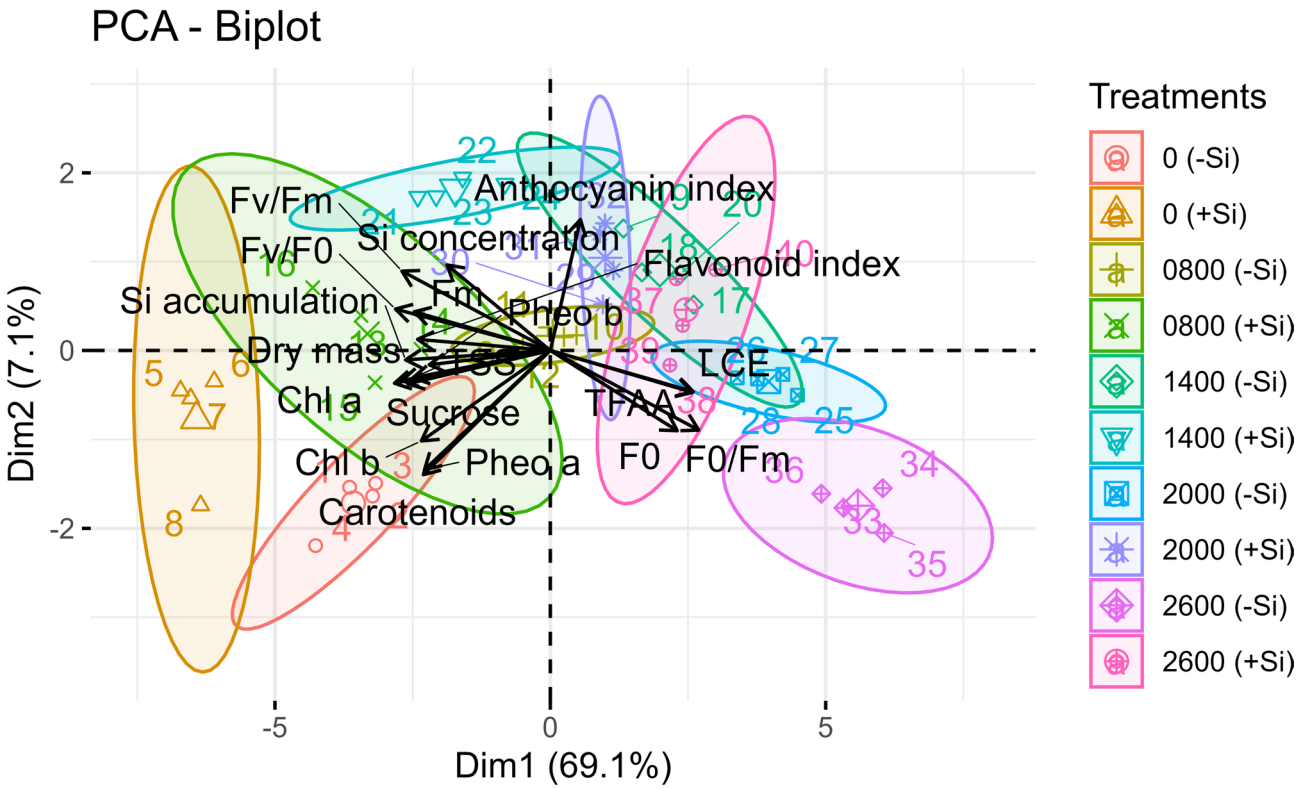


Fig. 7. Principal component analysis of response variables of *Handroanthus albus* cultivated under glyphosate rate (0, 800, 1400, 2000 and 2600 g e.a. ha^{-1}) in the absence (-Si) and presence of silicon (+ Si). Response variables: Si concentration, Si accumulation, Si concentration, leakage of cellular electrolytes (LCE), flavonoid index, anthocyanin index, chlorophyll *a* (Chl a), chlorophyll *b* (Chl b), carotenoids, pheophytin *a* (Pheo a), pheophytin *b* (Pheo b), initial fluorescence (F0), maximum fluorescence (Fm), photochemical efficiency of photosystem II (Fv/Fm), efficiency of energy conversion of absorbed light (Fv/F0), maximum yield of non-photochemical excitation (F0/Fm), total free amino acids (TFAA), total soluble sugars (TSS), sucrose, dry matter.

The detrimental effects of increasing glyphosate rates can also be observed through reductions in F_m (Fig. 2b), F_v/F_m (Fig. 2c), and F_v/F_0 (Fig. 2d). Under normal conditions, F_m represents the maximum fluorescence when all PSII reaction centers are fully reduced, suggesting maximum absorption of light energy primed for photochemical reactions[45]. However, with glyphosate, the ability of PSII reaction centers to fully reduce when exposed to light diminishes, leading to a decrease in F_m (Fig. 2b). This reduction indicates that fewer PSII reaction centers are fully operational, reflecting a decline in the overall photochemical efficiency of the young Ipe plants, as previously reported for other plants[47].

The F_v/F_m ratio serves as an indicator of the efficiency with which PSII converts absorbed light into chemical energy under optimal conditions[45]. Glyphosate-induced stress and damage to the PSII reaction centers lead to a decrease in the F_v/F_m ratio (Fig. 2c), signaling compromised efficiency in driving photochemical reactions. As glyphosate stress increases, the PSII reaction centers become progressively impaired, reducing their capacity to use absorbed light for photochemical processes. This injury is further reflected in the decline of the F_v/F_m ratio, which indicates a reduced overall efficiency of PSII (Fig. 2d). As the reaction center struggles to handle absorbed light energy, the photosynthetic performance of plants diminishes, and its vulnerability to photooxidative damage under glyphosate exposure increases[45].

Glyphosate-induced injury to PSII not only impairs the production of photosynthetic compounds but also alters their composition by increasing the accumulation of free amino acids (Fig. 3a) and reducing the levels of soluble sugars (Fig. 3b) and sucrose (Fig. 3c). Under normal conditions (0 g e.a. ha^{-1} of glyphosate), free amino acids are used in the synthesis of sucrose and other carbohydrates, which are essential for energy storage and transport within the plant[48]. However, when glyphosate inhibits photosynthesis by damaging PSII, sucrose production declines (Fig. 4c), leading to a reduced availability of carbon substrates necessary for the synthesis of carbohydrates and soluble sugars. The decline in photosynthetic efficiency and the resulting energy deficit can also trigger a metabolic shift, causing an accumulation of total free amino acids (Fig. 4a). With insufficient sucrose and energy to support growth-related processes such as protein synthesis, the plant reallocates its resources toward nitrogen-based metabolism, thereby increasing the pool of free amino acids as a stress response[49]. Consequently, the synthesis of complex proteins and carbohydrates can be reduced.

This combined effect of glyphosate increasing oxidative stress, impaired photosynthesis, and altered metabolic process, can decrease the biomass production of leaves in young Ipe trees (Fig. 4d). As glyphosate damages PSII,

the ability of plants to produce photosynthetic products, such as sucrose, is significantly reduced (Fig. 4c). This decline in sucrose availability limits the energy supply necessary for growth and decreases the substrates required for synthesizing essential carbohydrates and proteins. Additionally, the increased glyphosate application rates led to cellular damage and disrupted normal metabolic functions. This stress response, characterized by the accumulation of free amino acids (Fig. 4a), indicates a shift in plant metabolism away from growth-related processes toward survival mechanisms. With less energy available and a focus on managing stress rather than growth, there was an overall decline in leaf biomass production (Fig. 4d).

The application of soluble or nanoparticulate Si sources, delivered via root uptake along with supplemental foliar application, has showed to effectively enhance Si concentration and accumulation in non-accumulator plants of Si[19,23,24,50] as also observed in young Ipe plants (Fig. 1a, b). Once absorbed, Si provides protective benefits to leaf tissue, mitigating glyphosate-induced injury, particularly under moderate glyphosate exposure (Fig. 6). The protective effects of Si are primarily mediated through two key pathways: the substantial reduction of cellular electrolyte leakage, and the preservation of photosynthetic efficiency.

Silicon is extensively recognized for mitigating oxidative stress which enhances of both enzymatic and non-enzymatic antioxidative mechanisms[17,51]. In this study, we observed that the antioxidant indices, particularly the flavonoid and anthocyanin levels, exhibited a stronger association with elevated glyphosate concentrations in conjunction with Si treatment (Figs. 6 and 7). These findings underscore the critical role of Si in augmenting the antioxidant defense mechanism of plants, thereby contributing to their resilience under glyphosate stress through a reduction in cellular electrolyte leakage.

Flavonoids represent a diverse group of polyphenolic compounds that play a crucial role in plant defense, particularly through their potent antioxidant activity[52]. These compounds are proficient in neutralizing ROS, thereby mitigating oxidative stress in plants[53]. Through the donation of hydrogen atoms, flavonoids convert ROS into more stable and less reactive molecules, effectively reducing oxidative stress[52]. In young Ipe leaves, the presence of Si was observed to increase flavonoid concentrations across all glyphosate rates (Fig. 1d), suggesting that this response may be a critical stress-attenuating mechanism of Si in young Ipe plants under glyphosate-induced stress[54]. In this study, a similar pattern was observed for both anthocyanins (Fig. 1e) and carotenoids (Fig. 2b), where a significant reduction in the concentration of these pigments was noted in plants treated with Si at the highest glyphosate rates. This decline suggests that while anthocyanins and carotenoids are integral to the antioxidant defense system of plants, their efficacy may be diminished under extreme oxidative stress.

The role of Si in alleviating oxidative stress and decreasing cellular electrolyte leakage is intrinsically linked to the photosynthetic performance. In young Ipe trees, plants treated with Si within the group of plants not exposed to glyphosate (0 g e.a. ha^{-1}) exhibited strong associations with elevated chlorophyll *a* concentration, and photosynthetic efficiency, as evidenced by F_v/F_m and F_v/F_0 ratios (Figs. 6 and 7). Specifically, Si application effectively attenuated the degradation of chlorophyll *a* in response to increasing glyphosate concentrations (Fig. 2a), a phenomenon likely attributable to the high susceptibility of chlorophyll *a* to stress conditions[55].

The beneficial effects of Si in enhancing photosynthetic efficiency are evident through the maintenance of Fm (Fig. 3b), F_v/F_m (Fig. 3c), and F_v/F_0 (Fig. 3d) in young Ipe leaves subjected to glyphosate-induced stress. These parameters are critical indicators of the functional status of photosystem II and overall photosynthetic performance[56]. The ability of Si to preserve these fluorescence parameters under stress has been documented across various plant species[13]. This protective effect can be attributed to the stabilization of chloroplast structures, and to the improvement of energy transfer within photosystem II. Silicon contributes to the stabilization of chloroplast structures, likely through the deposition of Si in cell walls and membranes[57] which enhances their structural integrity and shields them from disintegration typically induced by environmental stress. Moreover, Si facilitates improved energy transfer within photosystem II by maintaining the integrity of photosynthetic protein complexes and pigments[58] thereby ensuring the efficient flow of excitation energy between chlorophyll molecules and reaction centers. This preservation of structural and functional integrity results in increased photosynthetic efficiency under glyphosate-induced stress.

The combined beneficial effects of Si culminated in a marked increase in leaf dry mass production in young Ipe plants under glyphosate-induced stress, particularly at low and moderate application rates (Fig. 4d). This enhancement in biomass is attributed to the ability of Si to fortify cellular structures, which reduces cellular electrolyte leakage (Fig. 1c). The maintenance of higher chlorophyll *a* (Fig. 2a) and the preservation of photosystem II functionality (Fig. 3) are crucial for supporting continued metabolic activity and growth, even in the presence of glyphosate-induced stress. In addition, Si maintained normal metabolic functions, with lower total free amino acids (Fig. 4a), and higher total soluble sugars (Fig. 4b) and sucrose (Fig. 4c) production. However, at higher glyphosate rates, the protective effects of Si were insufficient to sustain leaf dry mass production, indicating a threshold beyond which the mitigating capacity of Si becomes limited. This finding shows the effectiveness of Si in enhancing biomass production under moderate glyphosate-induced stress while also highlighting the challenges posed by extreme glyphosate exposure. The findings from this study have important implications for sustainable forestry management, particularly in regions where glyphosate is frequently used. The demonstrated protective role of Si suggests that its application may enhance the resilience of sensitive tree species, such as *Handroanthus albus*, to herbicide-induced injuries and stresses. However, translating these results to field conditions requires additional research to develop simplified, cost-effective application strategies and to validate physiological benefits at operational scales.

It is important to note that *Handroanthus albus* is classified as a non-accumulator of Si, suggesting that it has limited capacity to absorb silicon via the root system[21]. Therefore, the combined use of fertigation and multiple foliar sprays in this study were necessary to maximize Si uptake and allow for physiological assessment under glyphosate-induced stress. While this protocol proved effective in a controlled environment (Fig. 1b), it may be impractical for large-scale forestry due to labor and cost limitations. Future studies should explore more feasible

approaches, such as comparing foliar versus direct root application methods, testing different Si sources, and identifying optimal concentrations for field use.

Although the use of nanosilica improved plant physiological responses, its long-term environmental fate remains poorly understood. Factors such as persistence in the soil, mobility, accumulation in plant tissues, and interactions with soil microbiota warrant further evaluation. Long-term field studies are essential to assess the ecological safety and sustainability of repeated nanosilica use in forestry systems.

Considering these findings, forest managers in glyphosate-prone areas may consider the application of Si, particularly under low and moderate herbicide exposure, as a practical tool to enhance stress resilience in young Ipe seedlings. However, before implementation in routine forestry protocols, these practices should be further validated under field conditions to ensure efficacy, cost-efficiency, and environmental safety.

Conclusion

These findings support our initial hypothesis that silicon plays a protective role in *Handroanthus albus* under glyphosate-induced stress. The observed improvements in membrane stability, pigment retention, and photochemical efficiency suggest that silicon application enhances the physiological resilience of the plant, particularly under low to moderate herbicide exposure.

Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Author contributions

JPSJ, DK and RMP conceived the study and designed the experiments. RAC and LSM conducted the field study. MGC, JKRM and MTSL performed laboratory experiments and contributed to the data collection. DK, AMC and JPSJ contributed to the experimental design and provided critical revisions to the manuscript. MGC helped with statistical analysis, visualization of the data and writing the first draft of the manuscript. All authors discussed the results, contributed to the writing, reviewed the article and approved the final version of the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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