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Environmental Technology & Innovation 39 (2025) 104270

Contents lists available at ScienceDirect

Environmental Technology & Innovation

journal homepage: www.elsevier.com/locate/eti

Açaí waste and gypsum rapidly enhance ecological functioning in non-weathered, filter press bauxite residue: A one-year field experiment in the humid tropics of Northern Brazil

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ARTICLE INFO

Keywords:
Bauxite residue
Rehabilitation
Vegetation
Microbial activity
Plant uptake
Ecological restoration

ABSTRACT

In our study, the average annual precipitation is approximately 2085 mm, with an average temperature around 26.6°C. In such climate, organic amendments, like açaí seed waste, decompose relatively fast, thus promptly supplying nutrients, and humified organic matter, while promoting microbial activity essential for initiating soil formation processes. Although, the speed and direction of these processes depend on climatic conditions (e.g. rainfall intensity and distribution) their fluctuations are not expected to be such that they will greatly affect the outcome. Also, frequency and abundance of rainfall may vary in the humid tropics thereby affecting the leaching rate of sodium and other soluble contaminants from the BR following gypsum application. This leaching process is important for the rehabilitation of BR as it facilitates the reduction of alkalinity and salinity—critical factors limiting plant growth in untreated BR (Courtney and Kirwan, 2012; Bray et al., 2018; Miura et al., 2024). Intense rainfall events may cause erosion or loss of amendments applied superficially, thus potentially reducing their effectiveness if appropriate erosion controls are not implemented. In the experimental set up the amendments were well mixed into the soil and the test plots were situated in horizontal terrain to limit surface water runoff and erosion. Important in this respect is that, in some experimental plots, where neither açaí seed waste nor gypsum were applied, a slow infiltration of water into the soil was noted (associated with poor aggregation and structure), commonly leading to water ponding in the plots. Bauxite residue (BR), remaining after alumina production, presents high alkalinity and salinity and to its low fertility hindering ecological restoration. This experiment occurred in Northern Brazil's humid tropics, between August 2021 to August 2022, where we evaluated the effects of adding açaí seed waste (8 % w/w) and gypsum (5 %, 8 %, and 13 % w/w) on plant growth, contaminants uptake and microbial activity in BR. Results showed significant aboveground net primary production of *Urochloa brizantha* up to 1.46 kg/m^2, within 6 months while no growth was observed on unamended BR or on BR amended with gypsum alone. Furthermore, the amendments significantly enhanced microbial and enzyme activities as indicated by increases in

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https://doi.org/10.1016/j.eti.2025.104270
Received 23 October 2024; Received in revised form 20 April 2025; Accepted 11 May 2025
Available online 13 May 2025

dehydrogenase, β-glucosidase, and average well color development (AWCD) values, indicating improved microbial metabolic functions essential for ecological restoration. Our study is among the first to show that non-weathered filter-press BR in the humid tropics can be remediated effectively and within a year by a combination of açaí seed waste and gypsum. Our results provide a promising approach for large-scale remediation and ecological restoration of bauxite residue storage facilities (BRSF) in tropical regions, contributing to sustainable land management practices with significant biomass production thereby supporting the broader goals of environmental rehabilitation and sustainable development.

1. Introduction

Bauxite residue (BR), a waste material from alumina production based on the Bayer Process, is a highly alkaline, saline, sodic material with a low natural fertility (Power et al., 2011; Di Carlo et al., 2019). The alumina industry produces approximately 150 million BR yearly, with Brazil being the world's third-largest BR producer, responsible for 9 % of global production (Xue et al., 2019). Typically, BR is stored in Bauxite Residue Storage Facilities (BRSFs), and despite continuous attempts, its reuse (metal extraction, cement industry, bricks, etc.) is limited to approximately 3 % worldwide (Ujaczki et al., 2018; Power et al., 2011; Chao et al., 2022). Historically, BRSFs contain BR, which has been dewatered with drum filter technology (also referred to as red mud) with less than 65 % solid content. To address environmental and geotechnical concerns (Reddy et al., 2021; Kinnarinen et al., 2015), the BR dewatering process has evolved to filter press technology, resulting in a solid content of $>$ 70 % and dry stacking storage. Currently, there is an increasing demand for sustainable, ecologically-based technologies to develop geotechnically stable and productive soils from BR in which potential contaminants are stabilized (Xue et al., 2019).

A continuous vegetation cover is a major step to achieve safe closure of BRSFs (Xue et al., 2016; Phillips and Courtney, 2022). However, the adverse properties of BR are obstacles for its use as a growth medium (Huang et al., 2022). The establishment of vegetation provides not only reduction in dust emission, but also supports the development of a new ecosystem and contributes to landscape aesthetics (Bray et al., 2018; Courtney and Xue 2019). In general, the aluminum industry has difficulties sourcing topsoil for the closure of BRSFs due to its high demand of volume that is necessary to cap the storage facilities. Where topsoil is not available, the addition of amendments to BR to initiate soil development is required. Recent studies found that amendments such as gypsum, compost, manure, fertilizers, and sewage sludge (Courtney and Timpson 2004; Di Carlo et al., 2019a; Bray et al., 2018; Zhu et al., 2017; Courtney and Harrington, 2012) may produce the desired effect. In general, the combination of gypsum and organic materials has been shown to be key to accelerating the development of soil fertility in BR. Enhancing the fertility of BR using amendments to sustain a vegetation cover for BRSFs has been validated in several studies over the years (Jones et al., 2010; Banning et al., 2014; Zhu et al., 2017; Santini et al., 2019; Miura et al., 2023). However, few studies have been done on remediation processes in non-weathered, filter-pressed BR as opposed to studies on BR produced by drum filter technology, stored for several years (Dillon and Courtney, 2024; Jiang et al., 2025; Zhu et al., 2024) Furthermore, while many studies offer extensive insights into the remediation of BR subjected to prolonged leaching in temperate climate regions, there is a notable scarcity of research focusing on the remediation of non-weathered BR in humid tropical environments. Many researchers emphasize gradual physical and chemical changes caused by amendments like gypsum and organic material. Yet, the influence on microbiological development and microbial diversity in *in-situ* non-weathered and filter pressed BR in relation to improved abiotic soil properties, remains largely unexplored.

Microorganisms perform crucial ecological functions, essential in biogeochemical cycling of elements. As such they are important for the development of soil fertility in BR (Wu et al., 2023). While decomposing organic matter, microorganisms may produce organic acids and CO_2, thus reducing the alkalinity of BR (Santini et al., 2019). Furthermore, microbial communities have the potential to excrete extracellular polymeric substances that may bind mineral particles into stable aggregates, thus fostering the development of a stable soil structure (Tian et al., 2020). Also, it is recognized that high microbial diversity is crucial for maintaining multifunctionality in ecosystems (Santini et al., 2016).

Enzyme activities serve as proxies for microbial decomposition rates and are routinely used to assess restoration processes in degraded land, including tailings (R. Courtney et al., 2014; Šnajdr et al., 2013; Courtney et al., 2014). Extracellular enzymes, produced by microorganisms, affect ecosystem processes because of their essential roles in degradation, transformation and mineralization of organic matter (Luo et al., 2017). Dehydrogenase and β-glucosidase activities are widely recognized as reliable indicators of soil microbial activity, due to their integral roles in soil biochemical processes (Courtney et al., 2014; Eivazi and Tabatabai, 1988; Nannipieri et al., 2003). Dehydrogenase enzymes are involved in the oxidative phosphorylation pathway within microbial cells, which is a fundamental process of cellular respiration. β-glucosidase, on the other hand, plays a crucial role in the degradation of cellulose and other carbohydrates, which are major components of organic matter in soils (Nannipieri et al., 2012). The average well color development (AWCD), often used to test community-level physiological profiles, using Biolog EcoPlates, is a key index of microbial carbon metabolism activities, indicating the capacity of soil microorganisms to utilize carbon sources (Gomez et al., 2006). Recent studies have successfully utilized AWCD to assess microbial activities in amended bauxite residue (Dong et al., 2023; Wu et al., 2024).

Historically, BRSFs, primarily consisting of red mud, have been left for 5–20 years before planting vegetation (Haynes and Zhou 2019; Zhu et al., 2016). Thus, most studies are conducted on BR that has undergone extensive weathering (Di Carlo et al., 2019a; Santini and Fey 2018; Bray et al., 2018). However, recent international standards such as the Global Industry Standard on Tailing Management (ICMM, 2020), addressing environmental, social, and safety concerns, are encouraging a faster development of

vegetation on BR tailings. This shift prompts the industry to take proactive steps in the process towards closure of BRSFs.

In a recent paper we evaluated the development of the physicochemical characteristics of amended and non-amended, non-weathered, filter-pressed bauxite residue BR in Barcarena, Northern Brazil, for 12 months. Amendments involved in-situ mixing with gypsum, and açaí seed waste (Miura et al., 2024). Here we present the results of a parallel study, from the same field experiment, focusing on enzyme activities, microbial functional diversity, and plant biomass production in both unamended and amended BRSFs. (Table 1)

The objectives of the present study were:

1) To quantify net primary production of *Urochloa brizantha* (palisade grass), in non-weathered, filter pressed bauxite residue using gypsum and açaí seed waste as amendments, either alone or in combination
2) To determine the effects of amendments of gypsum and acai seed waste on microbial metabolic functions (AWCD and enzyme activities)
3) To identify the relationships between key abiotic parameters, plant growth and microbial activity
4) To assess the influence of gypsum and açaí seed waste amendments on plant biomass and element concentration of sodium (Na), aluminium (Al), arsenic (As) and vanadium (V).

We hypothesized that:

a) 12 months following in-situ combined amendments with gypsum and açaí seed waste to BR, showed the establishment of a robust *Urochloa brizantha* vegetation, which was planted 6 months earlier. Amendments with either gypsum or açaí seed waste alone did not result in enhanced germination and growth of *Urochloa brizantha.*
b) Establishment of *Urochloa brizantha* in response to the combined amendment of BR with gypsum and açaí seed waste was associated with enhanced microbial activity.
c) The application of gypsum and açaí seed waste amendments to bauxite residue will significantly reduce the concentration of toxic elements (sodium, aluminium, arsenic, and vanadium) in the plant tissues.

2. Material and methodology

2.1. Bauxite residue and amendment materials

Non-weathered filter-press BR was sourced from the unload shed facility at the Alunorte alumina refinery in Barcarena, Pará, Brazil. For this study, we will refer to the bauxite residue up to one year into the experiment as non-weathered BR. Waste from açaí seeds, a traditional berry, endemic to northern Brazil, was collected from a processing plant (Ecobiomassa, Barcarena) where the pulp is extracted from açaí seeds. The açaí seed waste was mechanically crushed (~2 mm) before being mixed with BR. Agronomic gypsum was procured from Gesso Integral, located in Grajaú, state of Maranhão, Brazil. Total concentrations in BR and gypsum of sodium (Na), magnesium (Mg), Al, phosphorus (P), potassium (K), calcium (Ca), iron (Fe), vanadium (V), and arsenic (As) were determined following microwave digestion using HF, HCl, and HNO_3 (6:2:2). Extracts were analyzed by ICP-OES (5110 DW, Agilent) and ICP-MS (8800 Triple Q, Agilent). In açaí waste, the concentrations of these elements were determined after perchloric acid digestion (Martinie and Schilte, 1976) in a microwave digester ultraCLAVE (Milestone, Italy). Total concentration of BR, gypsum and açaí seed waste are represented in Table S1 of supplementary material.

2.2. Experimental design

The experiment was carried out at the Bauxite Residue Storage Facility (BRSF) of the Alunorte alumina refinery in Barcarena, state of Pará, Brazil (S 1°33'04'', W 48°43'10''). The climate in Barcarena is classified as Tropical Monsoon (Am), with an average annual precipitation of 2085 mm and an average annual temperature of 26.6°C. During June and July of 2021, a test block area of 450 m^2 (30x15m) with a depth of about 1 m was established. Contour drains with trenches surrounding the test block were constructed to prevent surface runoff entering the block from adjacent areas. Individual plots, approximately 9 m^2 each (Fig. 1 and Fig. S1 – Supplementary Material), were demarcated using 40 cm high density plastic sheets with about 15 cm protruding from the soil. The treatments, which were replicated three times, were randomly allocated across these plots (Completely Randomized Design – CDR). In August 2021, gypsum and açaí seed waste were manually mixed into the top 20 cm layer of BR at specified weight-to-weight percentages (Fig. S1). Soil samples were collected at a depth of 0–20 cm depth after 12 months for microbiological activity analysis, using a soil auger. Composite soil samples consisted of 3 mixed subsamples per plot spaced approximately 30 cm apart. After collection,

Table 1
Treatment codes for the field experiment at Alunorte's BRSF, Barcarena, Brasil. The concentration of amendments is expressed in % (w/w) on a dry-weight equivalent basis.

Reference	Gypsum 0 %	Gypsum 5 %	Gypsum 8 %	Gypsum 13 %
BR	Br	BrG	BrG2	BrG3
BR + Açaí 8 %	BrA	BrGA	BrG2A	BrG3A

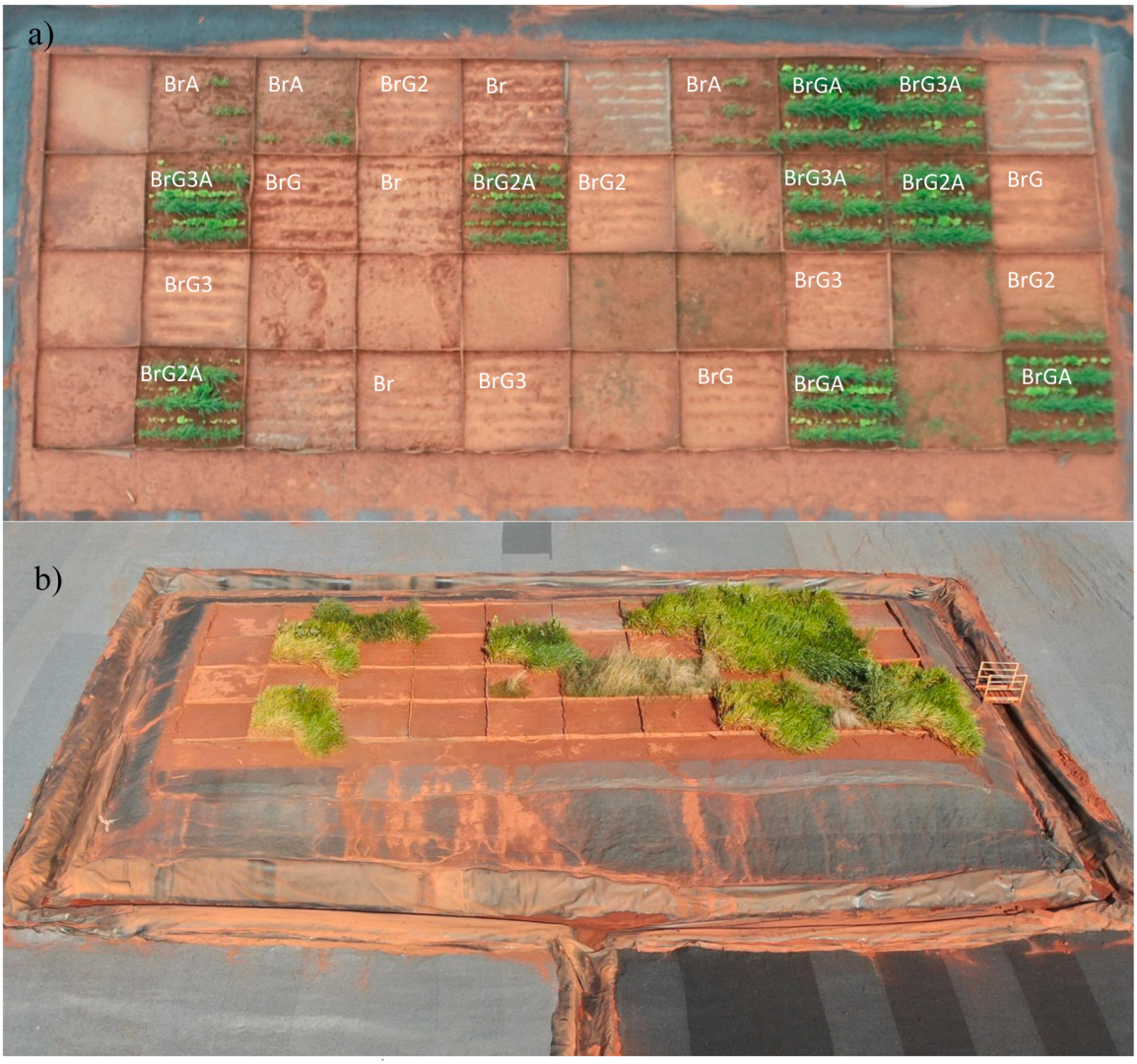

Fig. 1. Aerial photo taken on March 16th, 2022, 40 days after seeding Urochloa brizantha (a) and right before harvest in August, 2022 (b). The treatments, replicated three times, were randomly allocated across these plots (Completely Randomized Design – CDR). The codes represent the different treatments as explained in Table 1.

samples were stored in plastic bags, transported to the laboratory, and kept refrigerated at 4°C pending analysis. By February 2022, the plots were planted with *Urochloa brizantha*. This species was selected because of its high adaptability to low-fertility soils and its ability to grow rapidly. Both characteristics make it widely used in remediation and environmental rehabilitation projects worldwide (de Fátima Pedroso et al., 2018; de Souza Costa et al., 2021; So et al., 2022; Zanchi et al., 2021; Zhou et al., 2024). *Urochloa brizantha* is commonly used in Brazil for erosion control, and it proliferates rapidly. It is a robust, fast-growing grass, usually 60–120 cm high, with deep roots (which may extend to 2 m) and short rhizomes. Three rows were sown roughly 3 cm deep and spaced 80 cm apart, with a density of 75 seeds per linear meter, while no fertilizer was added. Precipitation data were available from the Alunorte weather station, utilizing a Campbell TB4MM system located at BRSF.

2.3. Plant samples

Biomass production, contaminants and nutrient concentrations in *Urochloa brizantha* were determined at each plot. To assess aboveground plant biomass, one row was randomly selected and then completely harvested six months after sowing. The harvested material was air-dried for 72 hours before being weighed. Composite plant samples were collected at each plot by taking one sub-sample of leaves from each row of plants. After collection, samples were stored in plastic bags, transported to the laboratory, and kept refrigerated at 4°C pending analysis. Approximately 0.2–0.25 g of air-dried plant material, which was milled and sieved to a fine powder (approximately 0.5 mm), and weighed into PTFE digestion vessels. To this, 2 mL of deionized water was added, and the

mixture was vortexed for homogenization. Subsequently, 5 mL of double-distilled nitric acid was added to the wetted samples before digestion using the Ultraclave (Milestone) digestion program designed specifically for plant samples. After digestion, once cooled to approximately 20°C, the samples were diluted using deionized water to a volume of 50 mL prior to analysis. Elemental analysis was conducted using ICP-OES Agilent 5110 VDV for macro elements and ICP-MS Agilent 8800 triple Q for arsenic (As) and vanadium (V). To ensure accuracy and assess recovery rates, two certified reference plant materials were digested alongside the samples: NCS DC 73349 (bush branches and leaves) and NIST 1515 (apple leaves). Detailed information of quality control is described in Supplementary Material.

2.4. Measurement of enzyme activity

Field-moist soil samples were sieved (2 mm) and stored at 4 ºC prior to enzyme determination (Paul 2014). Dehydrogenase activity was estimated by adding 2 mL of triphenyltetrazolium choride (TTC) to 5 g soil followed by incubation at 30ºC for 24 hours. The TTC is reduced to triphenyl formazan (TPF) in the presence of active dehydrogenase enzymes. Next, it is extracted with acetone and determined colorimetrically at 546 nm (Thalmann 1968). β – glucosidase activity was measured as described by (Alef and Nannipieri 1995). Here, 1 g samples of sieved soil were incubated for 1 h at 37 ºC with p-nitrophenil- β-d-glucosidase and toluene in a pH 6.0 modified universal buffer. Following addition of $CaCl_2$ and Tris buffer (pH12) the β – glucosidase activity was determined colorimetrically at 400 nm.

2.5. Community-level physiological profiles

The Average Well Colour Development (AWCD) values represent the overall metabolic activity of the microbial communities. We applied the Biolog EcoPlates (ECO MicroPlate, Biolog, USA) to assess the Community-Level Physiological Profiles (CLPPs) of the aerobic culturable heterotrophic microbial communities, according to the protocol by Floch (Garland and Mills 1991). Each plate contains 31 different carbon sources, categorized into carbohydrates, amino acids, carboxylic acids, polymers, phenolic acids, and amines. Microbial suspensions were prepared from each treatment and inoculated into the wells of the Biolog plates. The salt contained in the wells (Tetrazolium Violet) serves as an electron acceptor for bacterial respiration and transforms into purple formazan as bacteria metabolize the various carbon substrates. These dilutions were adjusted to ensure consistent concentrations of microbial cells across all samples, based on the growth of culturable bacteria. The microplates were incubated at ≅ 30 °C in a humid atmosphere and optical densities (OD) were measured at 590 nm using a microplate reader (Infinite®M200, Tecan, Switzerland). The initial well OD were subtracted from the well OD at 144 h to correct for inherent colors from the substrates and soil residue mixture. If the corrected OD exceeded the arbitrary threshold of 0.1, we considered the growth in the well as positive and used this positive value to compute four indicators after 144 h of incubation (Eqs. (5)–(8).

$$\text{AWCD AWCD} = \Sigma\,(Ci - \text{R})/\text{n} \tag{3}$$

$$Pi = (Ci - R)/\Sigma(Ci - R) \tag{4}$$

$$\text{Shannon index}: \quad H = \Sigma pi * \ln(P_i) \tag{5}$$

$$\text{Simpson index}: \quad D = 1 - \Sigma(P_i^2) \tag{6}$$

$$\text{Pielou index}: \quad J = (-\Sigma P_i * \ln P_i)/\ln S \tag{7}$$

$$\text{McIntosh index}: U = \sqrt{\Sigma(N_i^2)} \tag{8}$$

Here, C_i is the absorbance reading for well i, R is the control well absorbance. P_i is the proportion of the total number of individuals that species i contributes, and S is the total number of species. Pi is the proportion of individuals of the species relative to the total community. Ci is the count of the number of individuals of the species in the community. Ni provides a measure of community diversity emphasizing the dominance or evenness of species distribution.

2.6. Statistical analysis

All statistical analyses were conducted using R studio for Windows. The AWCD analysis included 31 carbon sources, reflecting the microbial community's metabolic diversity and intensity. Kruskal-Wallis analysis was applied to determine the significance of the difference in AWCD and diversity between treatments. Correlation analysis was conducted to assess the relationships between residue properties, diversity index and enzyme activities.

3. Results

3.1. Amendment effects on plant growth and element uptake

Two weeks after sowing *Urochloa brizantha*, the first signs of germination were observed, and by the end of the experiment, six months after sowing, a significant accumulation of biomass was recorded (Fig. 1, Fig. S2). This study is among the first to report substantial biomass production in non-weathered and filter-press bauxite residue amended with açaí seed waste and gypsum just a few months after their application. Also, few publications have reported plant biomass from residue field trials.

The addition of açaí seed waste resulted in significant production of *Urochloa brizantha* biomass (on average up to 1.5 kg dry matter per m^2; Table 2, Fig. 1), particularly if added in combination with gypsum. Specifically, BrG2A and BrG3A (receiving 8 % and 13 % gypsum, respectively), but not BrGA (with 5 % gypsum), significantly outperformed BrA. By contrast, neither unamended Br nor gypsum-only amended treatments (BrG, BrG2, and BrG3) showed plant growth (Table 1).

3.2. Amendment effects on enzyme activities

Fig. 2 illustrates the enzyme activities in the different treatments. The activities of dehydrogenase (Fig. 2a) and β-glucosidase (Fig. 2b) increased significantly when açaí seed waste was added to BR ($p < 0.05$), whereas no notable changes were observed when only gypsum was used ($p > 0.05$). For dehydrogenase activity, this increase was to about 100 μg g^{-1} hr^{-1} and for β-glucosidase, the increase was to about 1000–2500 μg g^{-1} hr^{-1}. However, for this enzyme, such a strong increase in activity did require gypsum in addition to açaí seed waste.

3.3. Microbial activities and metabolism function

One year after beginning of the experiment, BR which had received combined gypsum – açai seed waste amendments (i.e. BrG3A, BrG2A, and BrGA) showed highest AWCD values, indicating higher microbial development compared to other treatments (Table 3). Microbial development data are further detailed in Fig. S3 – Supplementary Material which support the results observed in Table 3 showing consistently higher activity level is organic-amended treatments. The significantly lower AWCD values in Br, BrG3, and BrA suggest low microbial metabolic activity in these treatments. This implies that only the presence of gypsum in combination with açaí seed waste amendments significantly boosted microbial activity.

Table 3 presents various ecological indices with respect to microbial diversity in addition to AWCD for the different treatments. The Richness index (S), indicating the number of different species present, as well as the Shannon index (H) and Simpson index (D), which provide insights into species diversity and evenness, significantly increased in the BrGA, BrG2A, and BrG3A treatments compared to the control Br treatment. Notably, the Richness and McIntosh indices, a measure of diversity that gives more weight to more abundant species, were significantly higher in the BrG3A treatment compared to BrG2A ($p < 0.05$), while the Shannon, Simpson, and Pielou, an evenness measure that assesses how evenly individuals are distributed across the different species showed no significant differences. In the BrGA treatment, both the Richness and McIntosh indices were significantly higher than those in the BrA treatment ($p < 0.05$).

Fig. 3 details the carbon utilization intensity of different microbial communities. Treatments with higher AWCD values, such as BrG3A, showed higher utilization intensity across these carbon sources, indicating a broader metabolic capability. The lower utilization rates in Br and BrG3 suggest limited metabolic activity and diversity. The BrG treatment only utilized polymers (82 %) and carboxylic acids (18 %) as carbon source (Fig. 3b). The addition of açaí seed waste enhanced the utilization intensity of amines, aminoacids, carbohydrates, carboxylic acids and phenolic acids, whereas it decreased the utilization intensity of polymers. The contribution of various organic sources to carbon utilization in treatments amended by açaí seed waste followed the order: polymers (20 % - 34 %), carboxylic acids (21 % - 27 %), aminoacids (10 % - 23 %), amines (10 % - 18 %), carbohydrates (5 % - 10 %) and phenolic compounds (2 % - 12 %) (Fig. 3b).

3.4. Correlations between soil biological and chemical properties

In a recent study, we presented soil chemical and soil physical characteristics of all the treated plots, one year after starting the experiment and six months after sowing (Miura et al., 2024). Data are summarized in Tables S2 and S3 and Fig. S3 (Supplementary material). In brief, results showed that the combined addition of gypsum and açaí seed waste reduced pH levels from 12.0 to 7.7–8.1. Also, electrical conductivity (EC) reduced significantly, reaching values below 4mS/cm. The extractable sodium percentage (ESP) values decreased from 98.8 % in non-weathered BR to 18.0 % in BR with 8 % gypsum, and to 8.4 % in treatment with 8 % gypsum and 8 % açaí seed waste. Treatments with gypsum (BrG, BrG2, BrG3, BrGA, BrG2A, and BrG3A) showed significantly lower sodium adsorption ratio (SAR) declining from 325 in Br to 0,66 in BrG2A. Gypsum treatment also decreased the fraction of unstable aggregates of BR from ~70 % to ~ 40 %. Treatments with açaí seed waste resulted in higher organic carbon (OC). Total nitrogen (TN) levels increased significantly in all treatments receiving açaí seed waste.

A correlation analysis of soil physico-chemical parameters (pH, SAR, ESP, EC, OC, TN and aggregate stability), and biological parameters (Shannon index, AWCD and biomass) is presented in Fig. 4. Here, we selected the Shannon index to represent species diversity because it has been widely used to estimate microbial diversity in ecological restoration (Wu et al., 2024b, 2020; You et al., 2019). Biomass was positively correlated with TN (0.81), AWCD (0.71) and Shannon index (0.68), whereas it was negatively correlated with pH (-0.87), ESP (-0.45) and SAR (-0.44). The AWCD was positively correlated with aggregate stability (0.64) and TN (0.51),

Table 2
Mean biomass of Urochloa brizantha (kg/m2) and its concentration of Ca (g/kg), K (g/kg), P (g/kg), N (g/kg), Na (g/kg), Al (g/kg), As (mg/kg) and V (mg/kg). Treatments followed by the same letters do not differ significantly at $p < 0.05$.

Treatments	Kg/m²			g/kg																	mg/kg							
	Biomass		Std	Ca		Std	K		Std	P		Std	N		Std	Na		Std	Al		Std	As		std	V		std	
Br	0		-	-		-	-		-	-		-	-		-	-		-	-		-	-		-	-		-	
BrG	0		-	-		-	-		-	-		-	-		-	-		-	-		-	-		-	-		-	
BrG2	0		-	-		-	-		-	-		-	-		-	-		-	-		-	-		-	-		-	
BrG3	0		-	-		-	-		-	-		-	-		-	-		-	-		-	-		-	-		-	
BrA	0.50	b	1.7	2	b	1.1	20.7	a	2.39	0.8	a	0.04	13.9	a	4.6	0,70	b	0,20	0.55	b	0.06	0.17	b	0.01	2,33	b	0,17	
BrGA	1.31	ab	4.8	4.4	ab	1.9	23.0	a	1.00	0.7	a	0.18	9.30	a	0.4	0,11	a	0,10	0.26	a	0.06	0.08	a	0.02	1,11	a	0,21	
BrG2A	1.46	a	0.9	3.7	ab	0.3	21.0	a	4.35	1.1	a	0	10.9	a	2.2	0,18	a	0	0.31	a	0.12	0.09	a	0.04	1,27	ab	0,52	
BrG3A	1.34	a	1.3	5.7	a	0.9	18.3	a	3.51	0.8	a	0.37	10.9	a	4.2	0,15	a	0	0.38	ab	0.09	0.12	ab	0.01	1,77	ab	0,4	

Significantly ($p < 0.05$) lower concentrations of Na, Al, and As were observed in *Urochloa* biomass of treatments where açaí seed waste and gypsum were added in combination as compared to the treatment with açaí seed waste only (BrA) (Table 2). Also for V, the concentration in *Urochloa* was lower in the combined açaí seed waste and gypsum treatments, but this was only significant for the BrGA treatment.

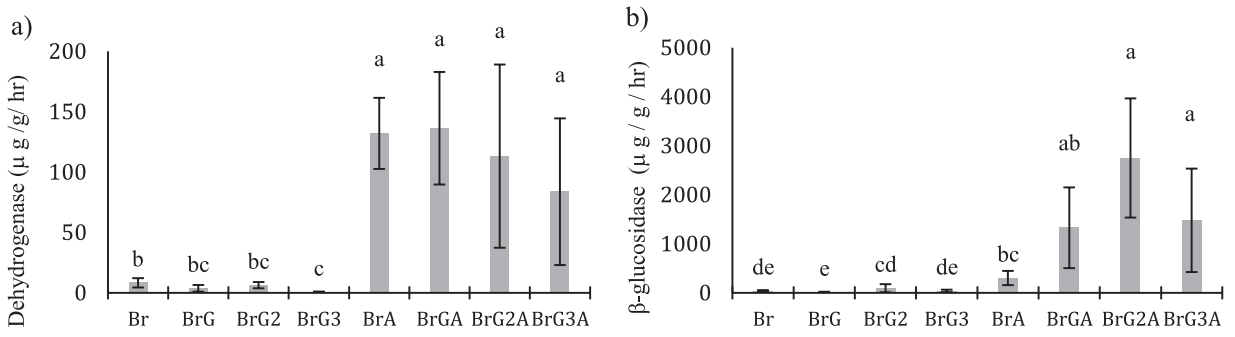

Fig. 2. Dehydrogenase (a) and β-glucosidase (b) in BR with different amendments, 1 year after planting. Different lowercase letters represent significant differences between different treatments ($P < 0.05$). The data are shown as the means ± standard deviations ($n = 3$). Error bars are standard deviations.

but negatively with pH (-0.77) and ESP (-0.63). Microbial diversity represented here by the Shannon index showed positive correlation with TN (0.63), and aggregate stability (0.45), whereas it was negatively correlated with pH (-0.82) and ESP (-0.5).

4. Discussion

4.1. Biomass production on non-weathered BR

Biomass production rates in our experiment (non-fertilized with average yields on BrG2A treatments up to 1.5 kg m^{-2} (15 tons/ha)) are comparable to those found in studies that used *Urochloa brizantha* in typical soils from Barcarena (Oxisol – USDA). According to Couto et al. (1999), the average dry matter productivity of *Urochloa brizantha* in the Paragominas region, including Barcarena, ranges from approximately 4.68 tons/ha (without P_2O_5 fertilization) to 19.2 tons/ha, when 200 kg of P per hectare was applied. This indicates that mixing gypsum and açaí seed waste in BR resulted in similar yields as obtained for *Urochloa brizantha* grown on fertilized natural soils in the region. Probably, the significant biomass production rates on BrA and even more so on BrGA, BrG2A and BrG3A in this study (Table 2) depended strongly on N and P provided by the açaí seed waste, while the addition of gypsum further enhanced biomass production due to lower pH and ESP and higher aggregate stability. Biomass production in our study was highly correlated with Total N and soil aggregation, but negatively correlated with pH (Fig. 4). In our experiment, we applied approximately 24 tons of açaí waste per hectare, which resulted in the addition of approximately 18 kg of P and 240 kg of N per hectare. This quantity is still less than that used in the study conducted by Couto et al. (1999).

Significant plant growth on BR was found only after amendment with açaí seed waste either without (BrA) or with gypsum (BrGA, BrG2A and BrG3A; Fig. 1 and Table 1). Among these treatments, BrG2A and BrG3A had significantly higher biomass compared to BrA. Thus, our data indicate that besides an addition of açaí seed waste at a rate of 8 %, the addition of 8 % (or more) gypsum may further enhance biomass production of *Urochloa brizantha*. This additional effect of biomass production by gypsum can be explained by the synergetic effect with açaí seed waste. Gypsum ($CaSO_4{\cdot}2H_2O$) chemically reduces alkalinity and sodicity through the dissolution process, releasing calcium ions (Ca^{2+}) that displace sodium ions (Na^{+}) from exchange sites on clay and mineral surfaces. This chemical reaction rapidly decreases the extractable sodium percentage (ESP) and residue pH, significantly improving residue structure, porosity, and aggregate stability (Bray et al., 2018; Courtney and Kirwan, 2012; Miura et al., 2024). Simultaneously, the addition of açaí seed waste introduces labile organic carbon and essential nutrients (N, P), stimulating robust microbial activity. Microbial decomposition of organic matter chemically generates organic acids (such as acetic and citric acids) and carbon dioxide (CO_2), further neutralizing residual alkalinity and lowering the pH (Santini et al., 2015; Wu et al., 2020; You et al., 2019). Thus, the complementary chemical actions of these amendments significantly accelerate the transformation of initially hostile BR into fertile, biologically active soils suitable for sustainable vegetation establishment and ecological recovery.

Although our findings of the importance of organic amendments on plant growth in BR are in accordance with earlier work (Courtney and Harrington, 2012; Elisa Di Carlo et al., 2019a; Phillips and Courtney, 2022) this is among the first studies to successfully use açaí seed waste as organic amendment in the humid tropics on fresh, filter press BR.

4.2. Microbial activity development in amended, non-weathered BR

Although açaí seed waste plays a fundamental role in the development of microbial activity and diversity, gypsum further enhanced these processes, indicating its importance for the successful remediation of BR. The enhanced ecological complexity from the combined addition of açaí seed waste and gypsum was associated to increased biomass production. This was clearly demonstrated by the strong correlation between AWCD, the Shannon index, and biomass production observed in this study, as well as by the increased dehydrogenase and β-glucosidase activities. The amendment with açaí waste likely supported microbial communities by supplying resources for their growth. In addition, the gypsum addition played an important role in improving aggregate stability, thus enhancing soil structure, important for an optimal supply of roots and microbes with air and water (You et al., 2019).

Table 3
Ecological indices for amended and unamended BR in microbial communities including Richness (S), Shannon (H), Simpson (D), Pielou (J), McIntosh (U), and AWCD index (the latter after 144 hours incubation). Mean values (n = 3) and standard deviations (Std) are presented, with the statistical significance of differences denoted by letters ($P < 0.05$). The BrG2 treatment was excluded from this analysis due to the samples being compromised during collection.

Treatment	Richness (S)	Std		Shannon (H)	Std		Simpson (D)	Std		Pielou (J)	Std		McIntosh (U)	Std		AWCD	Std	
Br	0.3	0.0	e	0.0	0.0	c	0.0	0.0	b	0.0	0.0	b	0.0	0.1	d	0.00	0.00	c
BrA	8.0	1.4	d	2.0	0.2	b	0.8	0.0	a	1.0	0.0	a	0.9	0.2	cd	0.09	0.03	c
BrG	1.7	0.5	e	0.5	0.3	c	0.3	0.2	b	0.7	0.5	ab	1.7	0.8	c	0.09	0.06	c
BrG2	NA			NA			NA			NA			NA			NA		
BrG3	0.3	0.5	e	0.0	0.0	c	0.0	0.0	b	0.0	0.0	b	0.2	0.3	d	0.01	0.01	c
BrGA	19.3	1.2	ab	2.7	0.0	ab	0.9	0.0	a	0.9	0.0	a	4.3	0.3	b	0.49	0.02	b
BrG2A	16.3	1.2	bc	2.4	0.1	ab	0.9	0.0	a	0.8	0.0	a	3.9	0.2	b	0.40	0.05	b
BrG3A	22.3	1.2	a	3.4	0.9	a	0.9	0.1	a	1.0	0.3	a	6.8	0.1	a	0.87	0.05	a

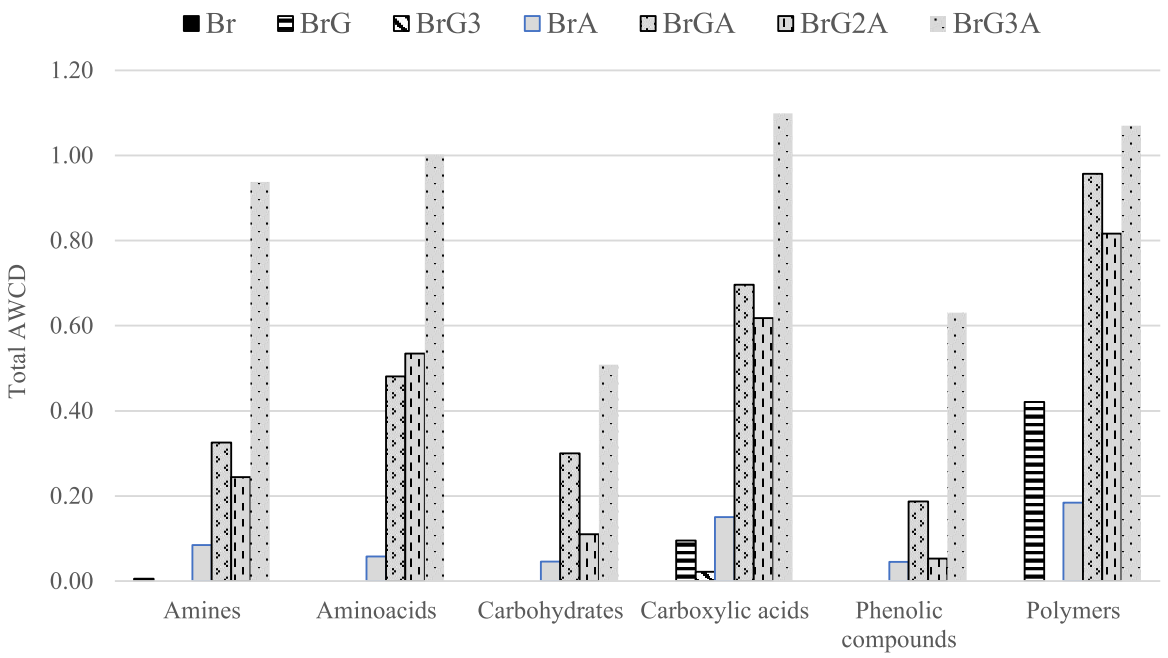

Fig. 3. Carbon utilization intensity, expressed by the AWCD after 144 hours incubation, in bauxite residue under different treatments (n = 3).

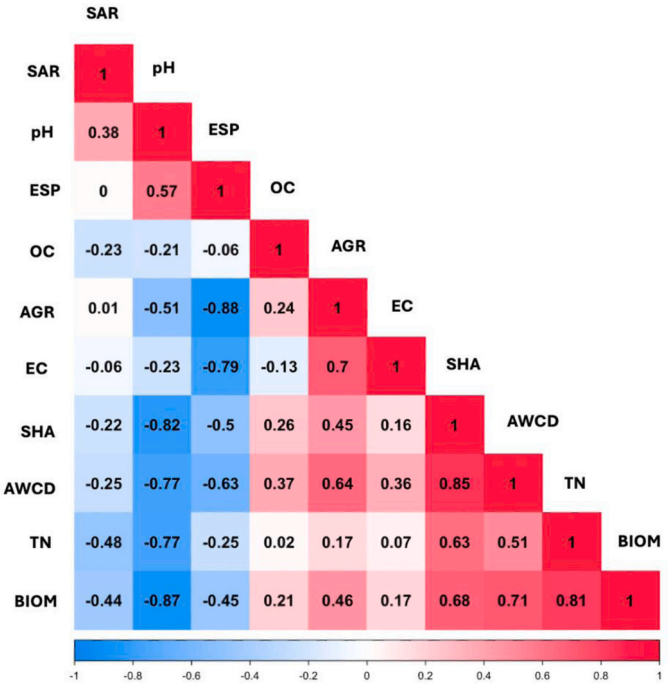

Fig. 4. Correlation matrix for BR properties (pH, SAR, ESP, Organic Carbon – OC, soil aggregation - AGR, EC, total nitrogen – TN), plant biomass – BIOM, Shannon index – SHA and AWCD (n = 3). In the correlation matrix we considered all treatments one year after starting the experiment (6 months after planting). Soil chemical and physical parameters are from the same plots as the ones in this study and were obtained from Miura et al. (2024).

Several studies have investigated the development of microbial activities in BR. For example, Wu et al. (2024) found that straw biomass addition significantly increased AWCD in BR, while Dong et al. (2022) reported that corn biomass addition to BR significantly increased microbial diversity with further increases observed due to the addition of phosphogypsum. To date, most studies have been done on drum-filter derived, older BR storage facilities, while limited work has been done on non-weathered filter press BR. For

example, Scullett-Dean et al. (2023) recently reported that combining the upper 5 cm layer of pre-neutralized BR with microbial inoculum (5 wt% gypsum, 5 wt% topsoil as inoculum, in addition to 7.5 wt% dextrose and 2.25 wt% spent brewing) and 1,67 wt% compost in a bioreactor pit, showed rapid progress towards attainment of multiple remediation targets only one year after starting the experiment. Our in-situ study at the BRSF in Baracarena not only achieved similar chemical results (Miura et al., 2024) but also observed significant development of microbial diversity and vegetation biomass without pre-treatment of BR through bacterial inoculation. This demonstrates that our methodology combining açaí seed waste and gypsum, applied directly to non-weathered BR, is technically feasible and low cost.

Previous remediation studies have employed microbial inoculants, such as specific bacterial or fungal strains, to accelerate the rehabilitation of BR. These microbial inoculants typically require careful isolation, cultivation, and application, significantly increasing operational complexity, preparation time, and associated costs. For example, Anam et al. (2019a) utilized inoculation with *Trichoderma asperellum* to promote plant growth and enhance microbial activity in bauxite residue. Their method necessitated significant pre-experiment preparation and costs associated with isolation, growth, application, and quality control of the microbial inoculant. Similarly, Scullett-Dean et al. (2023) although their approach accelerated remediation within one year, it required extensive microbial culturing and pre-neutralization of bauxite residue with microbial inoculum increasing operational complexity and costs. In contrast, our methodology did not require pre-inoculation or culturing of microbial communities. Instead, our combined application of gypsum and locally sourced açaí seed waste enhanced native microbial communities directly in-situ. This simpler and direct method significantly reduced operational complexity, application time, and associated financial costs, while still achieving comparable or superior outcomes in terms of microbial diversity, enzymatic activity, and vegetation establishment within the same timeframe.

Correlation matrix revealed that both the AWCD and the Shannon index exhibited positive correlations with biomass, soil aggregation, total nitrogen (TN), and organic carbon (OC) (Fig. 4). Similar studies on other soil systems as farmland soil under straw returning, TN and OC have been shown to be key factors influencing microbial carbon metabolism Hao et al. (2019), which the authors attributed to the presence of labile organic carbon fractions, generally regarded as substrates for microbial metabolism and growth (Wu et al., 2024a). The AWCD and Shannon index in our study showed negative correlations with pH and Extractable Sodium Percentage (ESP) (Fig. 4). High sodicity is toxic to both soil microbes and plants (Anam et al., 2019b), and high salt contents (high EC) are considered important obstacles to the establishment of microbial communities in BR (Banning et al., 2011). The decrease in alkalinity and salinity in the amended BR in our study suggests that the addition of gypsum in combination with açaí seed waste to BR most likely promotes microbial enhancement. This is supported by Courtney et al. (2014) who found that phospholipids fatty acid analysis (PLFA) profiles were negatively correlated to Extractable Na, sodicity, pH and salinity whereas they were positively correlated to Ca^{2+}, Mg^{2+}, Total C and Total N.

4.3. Amendment effects on element concentration in plants

Six months after sowing, concentration of Na in *Urochloa brizantha* leaves were below 1 %, which is the toxicity threshold proposed by Ruyters et al. (2011). Similar values of Na concentration from five species of plants leaves were reported by Phillips and Courtney (2022), from 1 to 10 years after amendment in rehabilitated BR sites amended by gypsum, fertilizers and wood mulch. Our study demonstrated that the combination of gypsum and locally sourced açaí seed waste was an effective and rapid treatment to reduce Na concentrations to levels within the acceptable range for the successful development of *Urochloa brizantha* in non-weathered bauxite residue in the humid tropics of Northern Brazil. Specifically, treatments incorporating gypsum alongside açaí seed waste (BrGA, BrG2A, BrG3A) significantly reduced Na uptake in plant tissues, as indicated by lower Na concentrations in the leaves (0.11 – 0.70 g/kg) compared to treatments with only açaí seed waste (BrA), where Na concentration reached 0.7 g/kg. This suggests that the addition of gypsum plays a key role in limiting Na availability and plant uptake, due to the displacement of Na ions from exchange sites by Ca ions derived from gypsum (Miura et al., 2024), followed by Na leaching together with SO_4^{2-}. As shown in Table S2 (Supplementary Material), Na concentrations in plant tissue decreased by over 80 % in the BrGA treatment compared to the control, demonstrating effective reduction of plant toxicity risks. Rehabilitation strategies using gypsum as an amendment are effective in the long term for mitigating plant-available Na (Dillon and Courtney, 2024). In addition, gypsum improves soil structure by promoting the formation of stable soil aggregates, thus facilitating water transport and Na leaching in BR (Di Carlo et al., 2019; Di Carlo et al., 2019a).

Potential plant toxicity from Al, As, and V in alkaline BR has been reported by several authors, primarily based on laboratory experiments (Lehoux et al., 2013; Lockwood et al., 2014; Mišík et al., 2014) However, field experiments assessing concentrations of these elements in plants from BR rehabilitated sites are limited (Dillon and Courtney, 2024), particularly for those conducted on non-weathered filter-press BR. Aluminium toxicity is shown to be one of the major limitations to plant growth in bauxite residue rehabilitation projects (Di Carlo et al., 2019). Although our findings showed that plant Al concentrations were within the range of common, non-toxic concentrations (10 – 1000 mg/kg) as reported by (Pais and Jones Jr, 1997), our values were higher than those detected in previous studies that used gypsum and organic waste as amendments (Courtney and Kirwan, 2012; Di Carlo et al., 2020). The higher Al values in our work may be attributed to pH > 9 recorded in some of our treatments (Miura et al., 2024) and the shorter period of in-situ weathering after storage compared to previous studies which recorded pH values around 8 after 8 years of BR amendment. Water extractable Al showed higher concentration if no gypsum with açaí seed waste had been added (Tables S2 and S3). Despite the challenging pH conditions and elevated plant-available aluminum in the soil even after amendment with gypsum and açaí seed waste, *Urochloa brizantha* demonstrated tolerance against alkaline, non-weathered bauxite residue, confirming it as an excellent choice for rehabilitation projects.

Our results also demonstrated significant reduction in As and V in *Urochloa brizantha* after gypsum application compared to treatments solely amended with açaí seed waste. This is supported by water extraction analysis, where we showed that the

concentration of water extractable As was up to 6 times lower when gypsum was combined with açaí seed waste (Table S3; Miura et al., 2024). Gypsum addition in combination with açaí seed waste drives pH reduction and resulted in enhanced sorption of As and V to iron oxides, thus effectively inhibiting metalloid release to solution (Bray et al., 2018). Closer to neutral pH, V and As are less available to plants (Bray et al., 2018; Lockwood et al., 2014; Mišík et al., 2014).

5. Conclusion

The findings of our in-situ study provide a promising and cost-effective approach for large-scale remediation and ecological restoration of BRSFs in tropical regions, promoting sustainable land management practices. The remediation approach combining locally sourced açaí seed waste and gypsum successfully transformed non-weathered, alkaline, and saline filter-press bauxite residue into productive land, achieving substantial *Urochloa brizantha* productivity of 15 tonnes of dry biomass per hectare within six months, comparable to yields obtained on fertilized local soils. In addition, significantly boosted microbial activity and metabolic functions as well as production of enzymes as dehydrogenase and β-glucosidase, essential for ecological restoration.

As global environmental regulations tighten and social expectations rise, it is crucial for alumina producers to prioritize sustainable land use practices for BR storage facilities. Our findings align with critical objectives of the Global Industry Standard on Tailings Management (GISTM), particularly requirements regarding safe closure and pollutant risk mitigation. The demonstrated practicality, speed, and ecological effectiveness of our remediation strategy provide an economically viable and environmentally robust model for global adoption, particularly within humid tropical regions facing similar tailings management challenges.

CRediT authorship contribution statement

Mulder Jan: Writing – review & editing, Validation, Supervision, Methodology, Conceptualization. **Miura Yuuki Silveira:** Writing – original draft, Software, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Courtney Ronan:** Writing – review & editing, Validation. **Zivanovic Valentina:** Software, Methodology, Formal analysis, Data curation. **Okkenhaug Gudny:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition. **Souza Adriana:** Investigation, Data curation.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Yuuki Silveira Miura reports financial support was provided by Norsk Hydro ASA. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eti.2025.104270.

Data availability

Data will be made available on request.

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