



Rhizosphere management by biochar and leaching improved plant performance in fresh bauxite residue sand

Mehran Rezaei Rashti ^a, Maryam Esfandbod ^{a,*}, Ian R. Phillips ^b, Chengrong Chen ^{a,**}

^a Australian Rivers Institute, School of Environment and Science, Griffith University, Nathan, Qld, 4111, Australia

^b Department of Agriculture and Fisheries, Agri-Science Queensland, Toowoomba, Qld, 4350, Australia

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ABSTRACT

High sodicity and Low nutrient retention in bauxite-processing residue sand (BRS) disposal areas restrict sustainable vegetation management in this highly alkaline environment. Although previous investigations have reported the beneficial effect of organic amendments on BRS rehabilitation, little is known about the underlying mechanisms of this complicated process, particularly after supplementary leaching of biochar amended BRS. We have investigated the coupled effect of supplementary leaching process, biochar [aged acidic (AC) vs alkaline pine (PC)] amendment and di-ammonium phosphate (DAP) fertilisation on rhizosphere nutrient dynamic and ryegrass performance in a 116-day glasshouse study. Biochar amendment in DAP + PC and DAP + AC treatments significantly increased and decreased pH (0.3–0.5 units) in all BRS rhizosphere and root-free zones, respectively. The application of alkaline and acidic biochars to BRS have reduced ammonia volatilisation (25–80%) and increased nitrogen retention (9–72%) in comparison with DAP treatment. Supplementary leaching had no significant effect on BRS rhizosphere pH, but reduced the EC values by ca. 62% in biochar-amended treatments. The leachates' pH remained unchanged (ca. 8) throughout the experiment, while their EC reduced in AC (6 folds) and PC (9 folds) amended treatments, with lower reduction in rhizosphere than root-free zones. The interaction of applied biochars and plant roots generally decreased nutrient leaching from rhizosphere in comparison with root-free zones. Sodium was the dominant cation in the leachate of all treatments and cumulative abundance of exchangeable cations were in the order of $\text{Na}^+ > \text{Ca}^{2+} > \text{K}^+ > \text{Mg}^{2+} > \text{Al}^{3+}$. The reduction of BRS salinity has increased plant biomass (ca. 47%) in biochar amended treatments by improving the capacity of fresh BRS rhizosphere for plant establishment.

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1. Introduction

The increasing demand followed by fast development of alumina industry (from 177 Mt in 2005 to 270 Mt in 2016) in recent years, has caused a significant challenge for sustainable remediation of its by-products (Xu et al., 2018). The extraction of alumina from bauxite ore, using sodium hydroxide under high temperature and pressure (Bayer process), yields nearly two tonnes of highly alkaline (pH 11–13), saline and dispersive bauxite-processing residue waste per tonne of produced alumina, which has very low nutrient retention capacity and microbial activity. This residue is

usually deposited in close distance from alumina refineries. Alcoa of Australia (Alcoa) separates bauxite-processing residue into the fine (red mud, <150 µm) and coarse (residue sand, >150 µm) fractions before transportation to the storage areas. Bauxite-processing residue sand (BRS) is used to construct the outer embankments of the storage areas, which encompass the produced red mud. Progressive rehabilitation of the residue sand embankments, through development of a soil-like medium to support sustainable vegetation cover, considered as one of the main parts of closure process in residue storage areas (Zhu et al., 2018). This strategy would increase physical stability of storage areas, suppress dust emission and reduce the risks associated with discharge of alkaline drainage to surrounding environment.

Sustainability of vegetated native plant species (tolerant to high salinity and sodicity) mainly depend on water and nutrient dynamics in BRS harsh environment (Goloran et al., 2014; Gwenzi et al., 2011). Incorporation of organic amendments such as

* Corresponding author.

** Corresponding author.

E-mail addresses: m.rezaeirashti@griffith.edu.au (M. Rezaei Rashti), m.esfandbod@griffith.edu.au (M. Esfandbod), Ian.Phillips@daf.qld.gov.au (I.R. Phillips), c.chen@griffith.edu.au (C. Chen).

biosolid, manure, compost, plant materials and biochar to BRS has been found to improve BRS properties by increasing its aggregate stability (Jones et al., 2011; Zhu et al., 2016), cation exchange capacity, nutrient availability (Goloran et al., 2015; Thiyagarajan et al., 2011), water holding capacity (Courtney et al., 2009; Munshower, 1994; Zhu et al., 2017) and microbial activity (Jones et al., 2010). These physicochemical improvements are likely to help the ecological rehabilitation of BRS disposal areas (Jones et al., 2011). Water leaching process is also recommended as a promising strategy to ameliorate the high salinity and alkalinity of bauxite-processing residue (Zhang et al., 2011) and high sodium (Na^+) concentration has been reported in bauxite-processing residue leachate (Xiang-feng Kong et al., 2018a). Taking full advantage of the available water resources, such as local rainfall and recovery of produced leachate for alumina extraction process, would further justify the practical implication of leaching process in BRS disposal areas. However, little is known about the effect of organic amendments' incorporation to BRS on its leachate's chemical composition and potential environmental impacts.

The presence of plants in any restoration system also has a significant effect on the chemistry and biology of the surrounding environment. Plants' roots and their rhizosphere exudates facilitate aggregation of BRS particles, which consequently improve local microbial biomass and activity. Process based understanding of the interaction between organic amendments and plant roots, in rhizosphere area, is critical for the adoption of sustainable management strategies in the BRS storage areas. Therefore, the main objectives of the present study were to: (1) investigate the effect of biochar amendments on unweathered BRS's nutrient loss, leachate chemical composition and rehabilitation performance; and (2) assess the coupled effects of biochar application and supplementary leaching process on sustainable plant establishment in highly alkaline BRS. The underlying hypothesis was that biochar amendment to BRS would decrease nutrient losses through leaching process and consequently increase plants biomass and nutrient uptake.

2. Materials and methods

2.1. Bauxite-processing residue sand and biochars physicochemical properties

The unweathered and untreated (fresh) BRS was collected from Alcoa of Australia (Alcoa) Kwinana Residue Storage Area ($32^\circ 11' \text{S}$, $115^\circ 49' \text{E}$), Western Australia. The BRS was air dried and sieved ($<2 \text{ mm}$) prior to the experiment. It has contained 98% sand, 1% silt and 1% clay with initial pH of 11.3 (1:5 water), EC of 34 ds cm^{-1} , and water holding capacity (WHC) of 254 g kg^{-1} . The sieved BRS was amended with gypsum at 1% (w/w basis), rewetted to 60% WHC and incubated for two weeks (Alcoa's rehabilitation prescription). After incubation, the gypsum amended BRS was leached with distilled water equivalent to average annual rainfall of the Kwinana area (i.e. 758 mm; five leaching events). Preliminary experiments have shown that this process is necessary for the preparation of bauxite-processing residue sand and without leaching, the fresh BRS is unlikely to support plant growth and vegetation establishment. Two biochars with contrasting physicochemical characteristics, namely acidic aged eucalyptus biochar (AC) and Alkaline pine biochar (PC), were used in this experiment. The AC biochar was produced during the wild fire in 1969 at Peachester State Forest ($26^\circ 50' \text{S}$, $152^\circ 53' \text{E}$) Sunshine Coast hinterland of Queensland, Australia, with pH of 3.1 (1:5 water). The PC biochar was produced at 700°C in a well-controlled furnace, under oxygen free condition,

with final resident time of 1 h and pH of 8.6 (1:5 water). Detailed properties of the BRS and applied biochars are reported in Table A1.

2.2. Pot trial design

The cylindrical polyethylene pot designed for this experiment (12 cm diameter and 12.5 cm height) was comprised of three concentric compartments namely: rhizosphere (0–3 cm radius from centre), root-free zone 1 (3–4 cm radius from centre) and root-free zone 2 (4–6 cm radius from centre). The compartments were separated (but not isolated) using weaved stainless still frames covered with $40\text{-}\mu\text{m}$ polyethylene mesh (Fig. A1). Detachable transparent polyethylene gas chambers were also designed for NH_3 volatilisation measurement. The pots had separate leachate collection tubes installed in rhizosphere and root-free zones (combined leachate from root-free zone 1 and root-free zone 2) and gas chambers could be connected to the pot using an elastic rubber band and high vacuum silicon grease to provide an airtight condition during gas sampling events. The rhizosphere in this study is defined as the area that is in close contact with growing plants' roots and their exudates. The root-free zone 1 is defined as the area that is not in contact with growing plants' roots but affected by their exudates, while the root-free zone 2 is defined as the area that is not affected by growing plants' roots and their exudates.

The study was conducted with four main treatments in six replicates namely: (1) Control (CK): BRS (1580 g pot^{-1}) without adding N fertiliser and biochar; (2) DAP: BRS (1580 g pot^{-1}) + $177.2 \text{ mg N pot}^{-1}$, equivalent to $574.2 \text{ kg N ha}^{-1}$ as Di-ammonium phosphate; (3) DAP + AC: BRS (1580 g pot^{-1}) + Di-ammonium phosphate + AC (the same N rate of DAP plus 26.33 g pot^{-1} , equivalent to 85.0 ton ha^{-1} AC in the top 30 cm); and (4) DAP + PC: BRS (1580 g pot^{-1}) + Di-ammonium phosphate + PC (the same N rate of DAP plus 13.17 g pot^{-1} , equivalent to 42.5 ton ha^{-1} PC in the top 30 cm). The AC and PC biochars (passed through 2 mm sieve) were added to BRS at a ratio of 1:20 (i.e. 5% on a v/v basis) and mixed thoroughly by end-over-end shaking for 24 h to make a uniform mixture.

The moisture content of each treatment then adjusted to 25% WHC (WHC = 25.4% moisture for BRS, 29.1% moisture for BRS + AC and 30.3% moisture for BRS + PC), using distilled water and Hoagland solution (120.4 ml per pot containing $235 \text{ mg L}^{-1} \text{ K}$, $200 \text{ mg L}^{-1} \text{ Ca}$, $31 \text{ mg L}^{-1} \text{ P}$, $64 \text{ mg L}^{-1} \text{ S}$, $48 \text{ mg L}^{-1} \text{ Mg}$, $0.5 \text{ mg L}^{-1} \text{ B}$, $5 \text{ mg L}^{-1} \text{ Fe}$, $0.5 \text{ mg L}^{-1} \text{ Mn}$, $0.05 \text{ mg L}^{-1} \text{ Zn}$, $0.02 \text{ mg L}^{-1} \text{ Cu}$ and $0.01 \text{ mg L}^{-1} \text{ Mo}$, without N source); the latter to provide essential nutrients for plant growth. Considering that biochar amendment has changed the WHC of fresh BRS, different treatments would have slightly different moisture content (25%–30% moisture) while the percentage of WHC remain the same in all treatments. Treated samples were pre-incubated for one week at 25°C , after which the moisture of treatments were re-adjusted to 50% WHC with distilled water and Di-ammonium phosphate (DAP) solution for control and fertilised treatments, respectively. The prepared samples then transferred to pots (50% W/W in rhizosphere, 17% W/W in root-free zone 1 and 33% W/W in root-free zone 2) and 30 Wimmera rigid ryegrass (*Lolium rigidum*) seeds were sown in the rhizosphere compartment of each pot. Following germination of ryegrass seeds, 20 plants were left in each pot. The pots were maintained at 50% WHC (by adding distilled water every three days) and $22 \pm 2^\circ \text{C}$ for the first eleven weeks of the experiment to help the establishment of ryegrass plants prior to inducing supplementary leaching (Fig. A2). At the end of this period, three replicates of each treatment went through additional five leaching events with one week intervals (190 mm per pot in each event, equal to average three

months rainfall in the Kwinana Refinery, Western Australia) using distilled water (defined as “leached”) and the other three replicates remained unchanged (defined as “unleached”). This process finally resulted in an experiment with eight treatments in three replicates. The pot trial was continued for five weeks and terminated after 116 days. The plants' above- and below-ground biomass were harvested at the end of experiment.

2.3. Bauxite-processing residue sand, leachate and plant analysis

The NH_3 volatilisation was measured using the sponge trap method modified by Chen et al. (2010). Ammonia trapping solution was a 1:3 ratio mixture of glycerol and concentrated sulfuric acid. In the first two weeks of the experiment, samples were collected after incubation periods of 1, 4, 7 and 14 days, respectively. After this initial two weeks, the sponges were incubated for 16 h on the 2nd and 5th days of each week to monitor NH_3 volatilisation for the remainder of the experiment. The captured ammonia was extracted with 2 M KCl (1:10 ratio) using an end-over-end shaker for 1 h and filtering through Whatman 42 filter paper. The NH_4^+ -N concentration in the supernatant was determined by SmartChem[®]200 Discrete Chemistry Analyser (WESTCO Scientific Instruments Inc.).

The plants' above- and below-ground biomass were measured for each treatment by the end of experiment. The root lengths were estimated by a modified line intersect method (Tennant, 1975). Total C (TC) and N (TN) contents of plants and BRS treatments were measured by dry combustion using a LECO CN analyser (TruMac NO. 830-300-400). The pH and EC values in BRS treatments (1:5 distilled water) and leachate samples were measured using a glass electrode (Rayment and Lyons, 2011). The mineral N (NO_3^- -N and NH_4^+ -N) concentration in BRS treatments and leachate (pH of leachate was reduced to 3 by adding concentrated hydrochloric acid (HCl) prior to chemical analysis) samples were determined by SmartChem[®]200 Discrete Chemistry Analyser (WESTCO Scientific Instruments Inc.). The concentration of major bioavailable nutrients (P, K, Ca, Mg, Na and Al) in the leachate samples were measured using an inductively coupled plasma optical emission spectrometer (ICP-OES; Perkin Elmer; Optima 8300). Microbial biomass C (MBC) and N (MBN) of treatments were determined using the chloroform fumigation-extraction method (Vance et al., 1987) by applying an EC factor of 2.64 and an EN factor of 2.22. The concentration of samples' dissolved organic C (DOC) and N (DON), as well as their inorganic C (IC) contents were measured using a TOC/N analyser (Shimadzu TOC-V_{CSH/CSN}). The BRS, leachate and plant analysis included three replicates from each treatment and the results were expressed on an oven-dry basis.

2.4. Data processing and statistical analysis

Statistical analysis was performed by analysis of variance using the IBM SPSS Statistics 23 software package (IBM Corp. Released, 2013. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.). The differences at $P \leq 0.05$ between experimental treatments using LSD test considered statistically significant and all variables were tested for normality of distribution using Kolmogorov-Smirnov test.

3. Results and discussion

3.1. pH and EC of bauxite-processing residue sand and leachate solution

pH is one of the main regulating factors of N loss in highly

alkaline environments such as BRS disposal areas (Chen et al., 2010). Successful revegetation of residue disposal areas is highly dependent on reducing residual alkalinity (Liao et al., 2018), as high pH in the rhizosphere reduce solubility and availability of nutrients for plant uptake (Gahoonia, 1993; Hedley et al., 1982). Comparing different treatments under leached and unleached conditions indicated that biochar application in DAP + PC and DAP + AC treatments significantly ($P < 0.05$) increased and decreased pH in all BRS layers, respectively (Table 1). Although leaching showed to have no significant effect on pH at rhizosphere and root-free zone 1, but reduced ($P < 0.05$) the pH at root-free zone 2, that was rarely affected by plant roots and their exudates. The leaching events significantly ($P < 0.05$) reduced EC of rhizosphere and root-free zones in all treatments. This is consistent with findings of Jones et al. (2015), who reported a substantial reduction in EC after leaching events in BRS, as infiltration of BRS by rainfall to lower soluble salt concentrations is one of the basic methods to neutralise residue (Ho et al., 1985). However, comparing EC of different BRS layers in treatments without biochar amendment (CK and DAP) showed an increase in EC from rhizosphere toward root-free zones, while no significant differences were observed between different layers in biochar-amended treatments (DAP + AC and DAP + PC).

The pH of leachate samples collected from rhizosphere and root-free zones did not show a general trend during five leaching events (Fig. 1). This observation indicated that fertiliser and biochar applications had no significant effect on the buffering capacity of BRS under leached condition. However, this is in contrast with observations of Jones et al. (2012) who reported a decline in leachate pH by time. The reason may be attributed to the pre-treatment of BRS in our study, before initiating the supplementary leaching experiment, as gypsum pre-treatment would result in precipitation of soluble alkalinity as calcium carbonates. It has been also demonstrated by Grafe et al. (2011) that dissolution of solid alkalinity such as desilication product (release of hydroxyl ions into the pore water) may provide pH buffering and increases pH between leaching intervals. The presence of alkaline minerals in BRS, such as cancrinite, hydrogarnet and tri-calcium aluminate may act as an alkaline storage and buffer the leachate solution to pH around 8 (Kong et al., 2008b; Xue et al., 2018). On the other hand, the EC of leachate samples collected from both rhizosphere and root-free zones in current experiment showed a significant ($P < 0.05$) decrease throughout the leaching events (from day 77–105), in all treatments, with higher values in root-free zones than rhizosphere.

3.2. Nitrogen losses through NH_3 volatilisation and leaching process

Ammonia volatilisation is one of the main pathways of N loss in alkaline environments (Hayashi et al., 2008). However, N loss through NH_3 volatilisation in the current experiment was not affected by leaching events as the majority (up to 95%) of NH_3 loss, from all treatments, occurred within the first three weeks of the study and before the first leaching event at day 77 (Table A2). Biochar amendment in DAP + AC and DAP + PC treatments decreased ($P < 0.05$) cumulative NH_3 volatilisation by 80% and 25% compared with DAP treatment, respectively. This can be related to the biochar-induced shifts in BRS pH and $\text{NH}_3/\text{NH}_4^+$ adsorption capacity. Chen et al. (2010) and Philips and Chen (2010) demonstrated that pH reduction is the most effective approach to reduce NH_3 volatilisation, while rehabilitation of BRS through acidification is economically and operationally unfeasible. The high pH along with lack of organic amendment may reduce NH_4^+ adsorption by

Table 1
The pH and EC at rhizosphere and root-free zones.

Treatment		pH (RZ)	pH (RFZ1)	pH (RFZ2)	EC $\mu\text{S cm}^{-1}$ (RZ)	EC $\mu\text{S cm}^{-1}$ (RFZ1)	EC $\mu\text{S cm}^{-1}$ (RFZ2)
Unleached	CK	8.26 b*	8.29 b	8.34 b	250.3 a	286.3 ab	348.0 a
	DAP	8.19 bc	8.16 bc	8.18 c	266.3 a	305.7 a	297.7 ab
	DAP + AC	7.76 e	7.81 d	7.89 e	266.7 a	207.4 c	240.9 b
	DAP + PC	8.54 a	8.57 a	8.54 a	248.0 a	231.0 bc	248.0 b
Leached	CK	8.10 cd	8.12 c	8.12 cd	80.8 d	79.3 e	82.2 d
	DAP	8.04 d	8.13 bc	8.12 cd	82.9 cd	84.2 de	82.4 d
	DAP + AC	8.14 bcd	8.10 c	8.09 d	102.7 b	106.0 d	106.4 c
	DAP + PC	8.24 b	8.21 bc	8.28 b	95.1 bc	95.9 de	100.1 cd

* Means followed by different letters within a column indicate significant differences between the treatments at $P < 0.05$. CK = Control; DAP = Di-ammonium phosphate; DAP + AC = Di-ammonium phosphate + acidic aged biochar; DAP + PC = Di-ammonium phosphate + pine biochar; WHC = Water holding capacity; RZ = Rhizosphere; RFZ1 = Root-free zone 1; RFZ2 = Root-free zone 2.

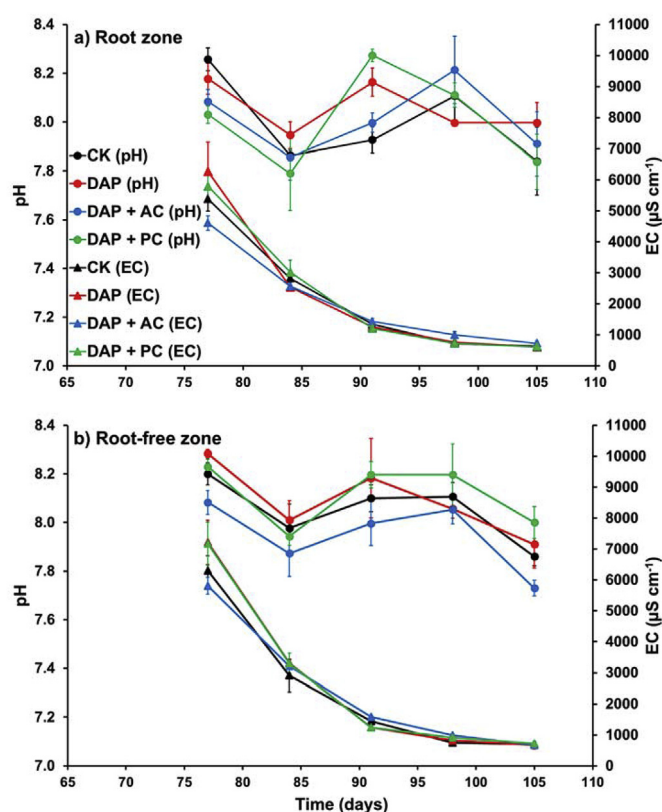


Fig. 1. pH and EC of leachate from root zone (a) and root-free zone (b) during the leaching events. Vertical bars are standard error of three replicates. CK = Control; DAP = Di-ammonium phosphate; DAP + AC = Di-ammonium phosphate + acidic aged biochar; DAP + PC = Di-ammonium phosphate + pine biochar.

Table 2
The NO_3^- -N and NH_4^+ -N concentrations at rhizosphere and root-free zones.

Treatment		NO_3^- -N mg kg^{-1} (RZ)	NO_3^- -N mg kg^{-1} (RFZ1)	NO_3^- -N mg kg^{-1} (RFZ2)	NH_4^+ -N mg kg^{-1} (RZ)	NH_4^+ -N mg kg^{-1} (RFZ1)	NH_4^+ -N mg kg^{-1} (RFZ2)
Unleached	CK	0.06 c*	0.06 c	0.27 c	5.01 b	5.30 ab	5.07 ab
	DAP	10.24 b	12.82 b	13.31 b	6.26 a	5.80 a	5.89 a
	DAP + AC	29.84 a	26.83 a	27.42 a	5.00 b	4.38 c	5.10 ab
	DAP + PC	30.01 a	28.66 a	32.19 a	5.11 b	5.64 a	5.38 ab
Leached	CK	0.01 c	0.01 c	0.01 c	4.56 bc	4.53 bc	4.12 b
	DAP	0.01 c	0.01 c	0.01 c	5.08 b	3.91 c	4.60 ab
	DAP + AC	0.01 c	0.01 c	0.01 c	3.86 c	4.32 c	4.17 b
	DAP + PC	0.01 c	0.01 c	0.01 c	4.60 bc	4.30 c	4.63 ab

* Means followed by different letters within a column indicate significant differences between the treatments at $P < 0.05$. CK = Control; DAP = Di-ammonium phosphate; DAP + AC = Di-ammonium phosphate + acidic aged biochar; DAP + PC = Di-ammonium phosphate + pine biochar; WHC = Water holding capacity; RZ = Rhizosphere; RFZ1 = Root-free zone 1; RFZ2 = Root-free zone 2.

BRS and consequently increase N loss through volatilisation. Therefore, application of biochars, especially with acidic characteristics, can be considered as an alternative rehabilitation strategy in highly alkaline BRS.

Comparing different treatments under leached and unleached conditions (Table 2) indicated that, although biochar application (DAP + AC and DAP + PC) in unleached condition significantly ($P < 0.05$) increased NO_3^- retention in all layers compared with DAP treatment, but could not effectively protect NO_3^- from being washed out of BRS profile under leaching events. This behaviour is resulted in similar NO_3^- concentration in all layers of different treatments by the end of experiment. Chen et al. (2010) and Jones et al. (2015) also indicated the high mobility of NO_3^- in predominantly negative charged BRS, especially in the wet months of the year. The results showed that NH_4^+ concentration in all layers was only affected by leaching events, while the differences between treatments were not statistically significant (except DAP treatment in rhizosphere that had higher NH_4^+ concentration than other treatments). Leaching process has significantly ($P < 0.05$) decreased NH_4^+ concentrations in all BRS layers when compared with unleached condition.

The high leaching potential of BRS would result in rapid transport of weakly adsorbed cations and anions from the plants' rhizosphere area (Phillips and Chen, 2010). The leachates N concentration showed higher ($P < 0.05$) NO_3^- loss from DAP + AC treatment in both rhizosphere and root-free zones than DAP + PC and DAP treatments (Fig. 2). However, the differences between NO_3^- concentrations in rhizosphere and root-free zones within each treatment were not statistically significant ($P > 0.05$). In contrast to NO_3^- observations, biochar application significantly ($P < 0.05$) reduced NH_4^+ leaching from DAP + AC and DAP + PC treatments compared with DAP. There were also no significant differences between NH_4^+ concentrations in rhizosphere and root-free zones

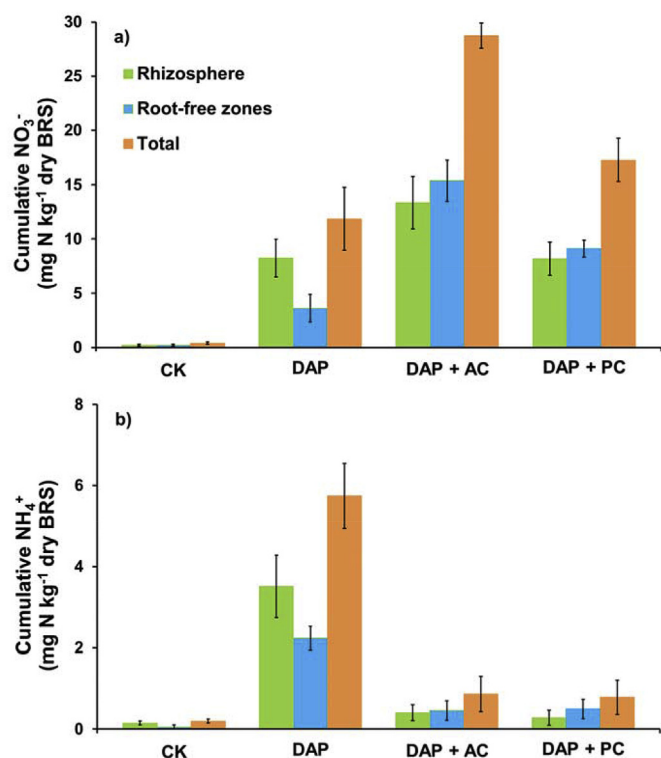


Fig. 2. Cumulative NO₃-N (a) and NH₄⁺-N (b) concentrations in leachates collected from rhizosphere and root-free zones. Vertical bars are standard error of three replicates. The value reported for root-free zones is the combined concentration of both root-free zone 1 and root-free zone 2. CK = Control; DAP = Di-ammonium phosphate; DAP + AC = Di-ammonium phosphate + acidic aged biochar; DAP + PC = Di-ammonium phosphate + pine biochar.

within each treatment. The low NH₄⁺ concentration in leachates of DAP treatment may be related to the low availability of NH₄⁺ for leaching process due to the high volatilisation rate of DAP treatment in the early days after mineral fertiliser application.

3.3. Nutrient losses from bauxite-processing residue sand profile

The main soluble alkaline components of BRS include: sodium hydroxide (NaOH), sodium carbonate (Na₂CO₃), sodium bicarbonate (NaHCO₃), sodium aluminate (NaAl(OH)₄), sodium silicate (Na₂SiO₃), potassium hydroxide (KOH), and potassium carbonate (K₂CO₃). The reduction in concentration of these chemical compounds through leaching process would significantly improve the rehabilitation performance of introduced vegetation cover to unweathered BRS (Clark et al., 2015; Kinnarinen et al., 2015). The cumulative concentrations of several element of interest present in considerable contents in collected leachate samples were measured to identify potential pathways of reduction in nutrient loss associated with biochar amendment to BRS (Table 3). The application of complex organic compounds such as biochar is expected to increase cation exchange capacity of treated BRS. The interaction of applied biochars and plant roots generally decreased nutrient leaching from rhizosphere compared with root-free zones in DAP + AC and DAP + PC treatments. However, ryegrass roots and their exudates increased nutrient losses from rhizosphere area in DAP and CK treatments in comparison with their root-free zones. Sodium was the dominant soluble cation in the leachate of all treatments, indicating the potential of leaching process to promote sodicity reduction in fresh BRS. The amendment of both AC and PC biochars to BRS significantly ($P < 0.05$) reduced Na leaching compared with DAP and CK treatments, while only PC application decreased ($P < 0.05$) P, Ca and Mg losses in comparison with DAP treatment. On the other hand, PC application showed significantly higher ($P < 0.05$) K and Al concentrations in collected leachates than DAP + AC and DAP treatments during leaching events. The cumulative abundance of exchangeable cations in the leachate samples were in the order of Na⁺ > Ca²⁺ > K⁺ > Mg²⁺ > Al³⁺, which is consistent with the study of Xue et al. (2018) who reported the dominance of sodium and calcium cations in the bauxite-processing residue slurry. The higher concentration of an element in leachate of a biochar-amended treatment indicates that biochar application increased the bioavailability of that nutrient in BRS. Therefore, it can be concluded that PC application reduced the bioavailability of P, Ca and Mg in the solution phase, while AC

Table 3
The cumulative nutrient concentrations in leachates collected from rhizosphere and root-free zones.

Leached elements		CK	DAP	DAP + AC	DAP + PC
P*	Rhizosphere	0.01c ***	0.07 a	0.05 ab	0.03 bc
	Root-free zones**	0.01 c	0.04 ab	0.05 a	0.04 b
	Total	0.02 c	0.11 a	0.10 a	0.07 b
K	Rhizosphere	3.9 b	7.8 b	4.0 b	13.4 a
	Root-free zones	2.7 c	4.3 b	5.4 b	22.1 a
	Total	6.6 c	12.1 b	9.4 b	35.5 a
Ca	Rhizosphere	39.9 b	85.8 a	82.1 a	34.8 b
	Root-free zones	26.2 c	46.2 b	78.4 a	42.0 b
	Total	66.1 b	132.0 a	160.5 a	76.8 b
Mg	Rhizosphere	1.5 ab	1.7 a	1.7 a	0.8 b
	Root-free zones	1.0 b	0.9 b	1.6 a	1.0 b
	Total	2.5 ab	2.6 ab	3.3 a	1.8 b
Na	Rhizosphere	347.0 a	372.6 a	200.4 b	167.4 b
	Root-free zones	206.8 ab	186.9 b	200.8 b	256.2 a
	Total	553.8 a	559.5 a	401.2 b	423.6 b
Al	Rhizosphere	0.07 a	0.07 a	0.05 a	0.07 a
	Root-free zones	0.05 b	0.04 b	0.03 b	0.09 a
	Total	0.12 b	0.11 b	0.08 b	0.16 a

* Cumulative concentrations of five leaching event reported according to mg kg⁻¹ dry BRS weight. **The value reported for root-free zones is the combined concentration of both root-free zone 1 and root-free zone 2. ***Means followed by different letters within a row indicate significant differences between the treatments at $P < 0.05$. CK = Control; DAP = Di-ammonium phosphate; DAP + AC = Di-ammonium phosphate + acidic aged biochar; DAP + PC = Di-ammonium phosphate + pine biochar.

application decreased K and Al bioavailability during the experimental period. The variations in leaching behaviour of nutrients can be related to the differences in their chemical forms and texture (Harter, 1991). Jones et al. (2011) also reported an increased concentration of extractable P, K and Mg after biosolids and poultry manure application to residue sand. However, the low P content of the treatments' leachate in the current experiment may also be related to the high P fixation capacity of BRS due to its high concentration of Fe and Al oxides (Huang et al., 2008). Aluminium rhizotoxicity is considered as one of the main limiting factors of plant growth in fresh bauxite-processing residue (Carter et al., 2008; Woodard et al., 2008), however the findings of the current experiment indicated that AC amendment to BRS may has a potential to reduce the toxic levels of water-soluble Al in the early stages of BRS rehabilitation.

3.4. Total, dissolved and microbial biomass C and N concentrations of bauxite-processing residue sand profile

The microbial growth and activity in fresh BRS has been reported to be extremely low due to limited C availability. However, the rapid development of a diverse microbial community in BRS observed by Banning et al. (2011) suggest that rehabilitation of residue sand embankments may not be limited by the lack of microorganisms able to survive in this alkaline environment. The improvement of microbial activity in BRS is an important factor in promoting a functioning belowground ecosystem, cycling of nutrients through organic pools and consequently increasing fertility of the residue sand (Jones et al., 2010). The effect of leaching process on microbial biomass C concentration in the current experiment was not consistent between different treatments (Table 4). While leaching events have significantly decreased ($P < 0.05$) MBC concentrations in all different layers of DAP treatment, there were no significant differences observed on MBC concentrations in CK, DAP + AC and DAP + PC treatments. The MBC concentrations generally decreased from rhizosphere toward root-free zone 2 in all treatments. In a similar way, leaching events significantly decreased ($P < 0.05$) MBN concentrations in different layers of all treatments (except CK, which showed no differences under leached and unleached conditions). The DAP treatment had highest ($P < 0.05$) MBN concentration followed by DAP + PC, DAP + AC and CK treatments by the end of experiment.

The DOC concentration in rhizosphere and root-free zones of DAP + AC, DAP and CK treatments generally decreased by leaching process, while its concentration significantly ($P < 0.05$) increased in DAP + PC treatment (Table 5). Comparing different treatments showed that CK had highest ($P < 0.05$) DOC concentrations under both leached and unleached conditions followed by DAP + AC, DAP + PC and DAP treatments. Similarly, leaching process

decreased ($P < 0.05$) DON concentrations in all applied treatments and different layers within each treatment (except CK, which showed similar DON concentrations with and without leaching practice). The highest DOC and lowest DON contents in CK treatment showed the N limitation for microorganisms and plants growth, while lowest DOC and highest DON contents in biochar-amended treatments indicated the C limitation for the growth of microorganisms and plants, which is consistent with the observed MBC and MBN concentrations of these treatments. The alkaline BRS is able to react with atmospheric CO_2 to produce carbonates, which potentially increase the IC concentration over time. However the effect of leaching process on IC concentration in different treatments was not consistent, with increasing effect in DAP and DAP + PC and decreasing effect in CK and DAP + AC treatments. The highest concentration of IC in leached and unleached conditions were observed in DAP and CK treatments, respectively.

The leaching process significantly ($P < 0.05$) decreased total N content in all fertiliser and biochar applied treatments (DAP, DAP + AC and DAP + PC), while the differences between total N concentration under leached and unleached conditions were not statistically significant in CK treatment (Table 6). The combined application of AC with mineral fertiliser showed the highest ($P < 0.05$) total N concentration among all treatments, while CK had the lowest ($P < 0.05$) total N content by the end of experiment. The total C concentration of different treatments were not generally affected by leaching process (except a significant decrease ($P < 0.05$) in root-free zones of DAP + AC). However, similar to the total N, the highest and lowest ($P < 0.05$) total C contents in all rhizosphere and root-free zones observed in DAP + AC and CK treatments, respectively.

3.5. Plant biomass and nitrogen uptake as affected by biochar amendment and leaching process

Leaching process was able to reduce the salinity of BRS to levels lower than the critical threshold for limiting plant growth in saline soils. Leaching has significantly ($P < 0.05$) increased ryegrass shoot biomass, with highest biomass was observed in DAP + AC treatment followed by DAP + PC, DAP and CK treatments, respectively (Table 7). This is in agreement with the findings of Kaur et al. (2016) that observed a higher biomass production form kikuyu grass under leached condition and acidification of BRS. Goloran et al. (2015, 2014) also reported a significant inverse relationship between pH and biomass production of plants grown in BRS. Leaching would also reduce the phytotoxic compounds in alkaline bauxite-processing residue, such as high contents of $\text{HCO}_3^-/\text{CO}_3^{2-}$ and aluminate ions (Courtney and Kirwan, 2012), resulting a sustainable environment for optimum plant growth. In a similar way, leaching process has increased ($P < 0.05$) treatments' root biomass

Table 4
Microbial biomass C and N concentrations at rhizosphere and root-free zones.

Treatment	MBC mg kg ⁻¹ (RZ)	MBC mg kg ⁻¹ (RFZ1)	MBC mg kg ⁻¹ (RFZ2)	MBN mg kg ⁻¹ (RZ)	MBN mg kg ⁻¹ (RFZ1)	MBN mg kg ⁻¹ (RFZ2)
Unleached						
CK	3.6 d*	2.0 e	1.1 e	0.6 e	0.4 f	0.3 f
DAP	83.0 a	62.4 a	59.1 a	12.7 a	9.7 a	7.1 a
DAP + AC	29.1 c	15.2 d	10.0 de	9.0 b	7.8 b	5.2 b
DAP + PC	32.3 c	25.5 c	24.2 bc	8.0 b	6.1 c	4.4 bc
Leached						
CK	10.2 d	8.0 e	5.4 e	0.6 e	0.4 f	0.3 f
DAP	65.1 b	52.3 b	33.6 b	6.1 c	4.4 d	3.5 cd
DAP + AC	29.4 c	24.5 c	18.2 cd	3.2 d	2.4 e	1.1 ef
DAP + PC	36.5 c	28.6 c	24.3 bc	4.5 d	3.4 de	2.3 de

* Means followed by different letters within a column indicate significant differences between the treatments at $P < 0.05$. CK = Control; DAP = Di-ammonium phosphate; DAP + AC = Di-ammonium phosphate + acidic aged biochar; DAP + PC = Di-ammonium phosphate + pine biochar; WHC = Water holding capacity; MBC = Microbial biomass C; MBN = Microbial biomass N; RZ = Rhizosphere; RFZ1 = Root-free zone 1; RFZ2 = Root-free zone 2.

Table 5

Dissolved organic C and N and inorganic C concentrations at rhizosphere and root-free zones.

Treatment	DOC mg kg ⁻¹ (RZ)	DOC mg kg ⁻¹ (RFZ1)	DOC mg kg ⁻¹ (RFZ2)	DON mg kg ⁻¹ (RZ)	DON mg kg ⁻¹ (RFZ1)	DON mg kg ⁻¹ (RFZ2)	IC mg kg ⁻¹ (RZ)	IC mg kg ⁻¹ (RFZ1)	IC mg kg ⁻¹ (RFZ2)
Unleached CK	15.4 a*	16.8 a	20.3 a	0.2 d	0.3 c	0.4 c	92.8 b	95.2bc	95.3 a
DAP	1.7 d	2.7 d	2.3 e	6.3 c	9.8 b	8.6 b	67.3 d	73.7 f	67.7 c
DAP + AC	9.8 bc	14.2 ab	8.0 c	21.3 a	26.1 a	23.4 a	75.2cd	88.4cd	82.9 b
DAP + PC	1.0 d	1.7 d	3.0 e	11.6 b	11.8 b	10.3 b	82.5 c	82.2de	79.1 b
Leached CK	11.8 b	10.4bc	10.8 b	0.2 d	0.3 c	0.4 c	78.4 c	79.2ef	76.6bc
DAP	1.2 d	1.7 d	1.7 e	0.1 d	0.1 c	0.1 c	111.6a	106.3a	100.3a
DAP + AC	2.4 d	2.3 d	2.0 e	0.1 d	0.1 c	0.1 c	68.5 d	63.8 g	66.3 c
DAP + PC	7.4 c	6.4 cd	5.3 d	0.1 d	0.1 c	0.1 c	104.2a	98.2 ab	102.8a

* Means followed by different letters within a column indicate significant differences between the treatments at $P < 0.05$. CK = Control; DAP = Di-ammonium phosphate; DAP + AC = Di-ammonium phosphate + acidic aged biochar; DAP + PC = Di-ammonium phosphate + pine biochar; WHC = Water holding capacity; DOC = Dissolved organic C; DON = Dissolved organic N; IC = Inorganic C; RZ = Rhizosphere; RFZ1 = Root-free zone 1; RFZ2 = Root-free zone 2.

Table 6

Total N and C concentrations at rhizosphere and root-free zones.

Treatment	Total N mg kg ⁻¹ (RZ)	Total N mg kg ⁻¹ (RFZ1)	Total N mg kg ⁻¹ (RFZ2)	Total C g kg ⁻¹ (RZ)	Total C g kg ⁻¹ (RFZ1)	Total C g kg ⁻¹ (RFZ2)
Unleached CK	6.2 c*	6.1 d	6.2 d	1.5 d	1.5 e	1.5 c
DAP	32.5 b	33.0 c	31.6 c	1.4 d	1.4 e	1.4 c
DAP + AC	75.1 a	91.2 a	115.7 a	10.0 a	17.0 a	12.8 a
DAP + PC	40.7 b	45.4 b	46.7 b	6.6 c	6.4 d	6.5 b
Leached CK	5.7 c	5.4 d	6.2 d	1.5 d	1.4 e	1.5 c
DAP	9.1 c	9.6 d	10.2 d	1.4 d	1.3 e	1.3 c
DAP + AC	32.0 b	35.7 c	47.3 b	8.4 ab	10.9 b	8.9 b
DAP + PC	9.4 c	10.2 d	11.6 d	6.9 bc	7.4 c	7.2 b

* Means followed by different letters within a column indicate significant differences between the treatments at $P < 0.05$. CK = Control; DAP = Di-ammonium phosphate; DAP + AC = Di-ammonium phosphate + acidic aged biochar; DAP + PC = Di-ammonium phosphate + pine biochar; WHC = Water holding capacity; RZ = Rhizosphere; RFZ1 = Root-free zone 1; RFZ2 = Root-free zone 2.

Table 7

Plant biomass and N uptake.

Treatment	Shoot* biomass (mg pot ⁻¹)	Root biomass (mg pot ⁻¹)	Shoot/Root biomass ratio	Root length (m pot ⁻¹)	Plant biomass/Root length (mg m ⁻¹)	Plant N concentration (mg g ⁻¹)	Plant N uptake (mg pot ⁻¹)
Unleached CK	191 f**	71 b	2.7 d	22.8 b	11.5 cd	13.2 e	3.4 d
DAP	233 def	52 b	4.5 bc	14.8 c	19.3 a	29.2 b	8.2 c
DAP + AC	402 bc	51 b	7.9 a	22.8 b	19.9 a	34.2 a	15.5 a
DAP + PC	321 cd	51 b	6.3 ab	22.1 b	16.8 ab	29.5 b	10.8 b
Leached CK	211 ef	80 b	2.6 d	30.1 b	9.7 d	12.7 e	3.7 d
DAP	283 de	67 b	4.2 cd	24.4 b	14.3 abc	22.4 c	7.9 c
DAP + AC	657 a	193 a	3.4 cd	51.2 a	16.6 ab	20.9 c	17.8 a
DAP + PC	513 ab	177 a	2.9 d	52.6 a	13.1 bc	17.5 d	12.0 b

* The data reported according to plant dry weight.

** Means followed by different letters within a column indicate significant differences between the treatments at $P < 0.05$. CK = Control; DAP = Di-ammonium phosphate; DAP + AC = Di-ammonium phosphate + acidic aged biochar; DAP + PC = Di-ammonium phosphate + pine biochar; WHC = Water holding capacity.

with higher values in DAP + AC and DAP + PC treatments than DAP and CK treatments. These observations consequently resulted in lower shoot/root biomass ratios in leached than unleached condition.

Leaching process resulted in significantly ($P < 0.05$) higher root length with highest and lowest values at DAP + PC and DAP treatments, respectively. The plant biomass/root length ratio was also higher in N applied treatments than CK by the end of experiment. Although plant N concentration was significantly ($P < 0.05$) lower under leached condition, but the differences between total plant N uptakes under leached and unleached conditions were not statistically significant. Finally, the highest plant N uptake observed in DAP + AC followed by DAP + PC, DAP and CK treatments, with no significant difference between leached and unleached conditions. This observation is in contrast with findings of [Kaur et al. \(2016\)](#) who reported an enhancement in N uptake by kikuyu grass due

to leaching of excess salts and alkalinity from the residue profile. These findings suggest the great potential of supplementary leaching process along with organic material amendments to optimise plants' rhizosphere management for sustainable vegetation performance in fresh bauxite-processing residue sand.

4. Conclusion

Successful revegetation of a bauxite-processing residue disposal area is highly dependent on improving its nutrient retention capacity as well as the proper amelioration of its highly saline and alkaline environment. This study has demonstrated the important role of biochar amendment and supplementary leaching process in improving unweathered BRS' rhizosphere biochemical properties. The amendment of acidic and alkaline biochars to BRS reduced N loss (NH_3 volatilisation) and increased N retention capacity (TN and

DON) in ryegrass rhizosphere. Sodium was the dominant exchangeable cation of BRS and the cumulative concentration of leached cations were in the order of $\text{Na}^+ > \text{Ca}^{2+} > \text{K}^+ > \text{Mg}^{2+}$. Supplementary leaching process successfully reduced BRS salinity by ca. 62% in biochar-amended treatments, while the pH values remained unchanged (ca. 8). These changes have resulted in a more favourable environment for ryegrass above- and below-ground growth and consequently improved BRS rehabilitation performance. Therefore, the application of organic amendments (such as biochar) to fresh BRS, followed by optimum supplementary leaching process prove to be an efficient rhizosphere management strategy for establishment of a sustainable ecosystem on residue storage areas as part of progressive closure. These findings would also provide an excellent baseline information for assessing the effect of organic amendments on reducing the potential environ-

mental risks associated with alkaline wastes' disposal strategies.

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Appendix

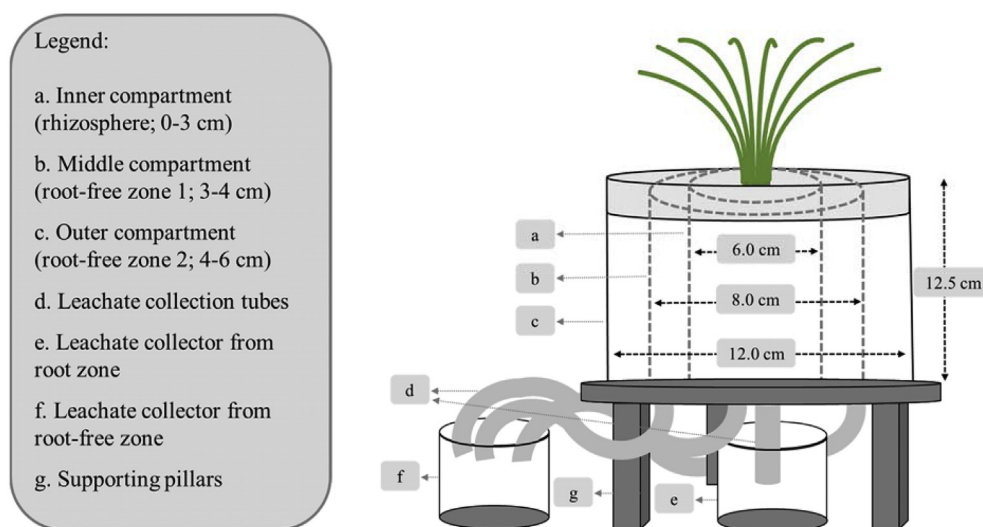


Fig. A.1. Schematic presentation of the pots (rhizobox).

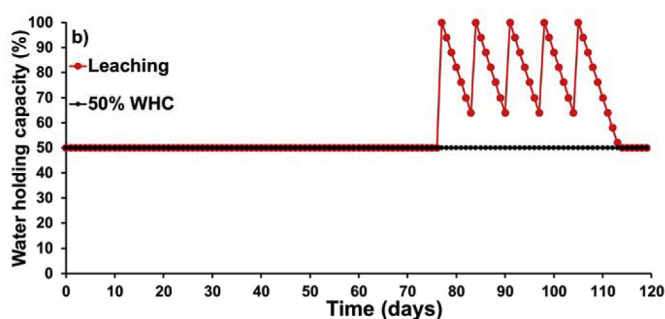


Fig. A.2. Treatments moisture fluctuation during the study period. WHC = Water holding capacity.

Table A.1

Selected initial properties of BRS and applied biochars

	Production temperature (°C)	Moisture (%)	BET ($\text{m}^2 \text{g}^{-1}$)	Bulk density (g cm^{-3})	pH (1:5)	EC ($\mu\text{S cm}^{-1}$)	Total C (%)	Total N (%)	$\text{NH}_4\text{-N}$ (mg kg^{-1})	$\text{NO}_3\text{-N}$ (mg kg^{-1})
AC	350–550	4.4	108	0.54	3.1	110	51.7	0.16	19.9	1.1
PC	700	7.5	382	0.27	8.6	315	81.6	0.16	0.11	0.61
BRS	ND	4.5	ND	1.70	11.3	34×10^4	0.07	0.01	ND	ND

BRS = bauxite-processing residue sand; AC = acidic aged biochar; PC = pine biochar; BET = BET surface area; ND = not detectable.

Table A.2The cumulative NH₃-N volatilisation and N₂O-N and net CO₂ emissions

Treatment	NH ₃ -N volatilisation (mg kg ⁻¹)	N ₂ O-N emission (mg kg ⁻¹)	Net CO ₂ emission (mg kg ⁻¹)	N ₂ O-N/NH ₃ -N ratio	NH ₃ -N/Net CO ₂ ratio	N ₂ O-N/Net CO ₂ ratio
Unleached CK	0.36 d*	0.005 d	76.68 bc	0.013 a	5 × 10 ⁻³ b	7 × 10 ⁻⁵ de
DAP	87.30 a	0.073 bc	77.63 bc	0.001 c	1.13 a	0.001 cd
DAP + AC	17.12 c	0.361 a	205.19 a	0.021 a	0.08 b	0.002 bc
DAP + PC	65.61 b	0.144 b	60.36 c	0.002 bc	1.14 a	0.003 ab
Leached CK	0.36 d	0.002 d	-46.57 d	0.006 b	-0.01 b	-5 × 10 ⁻⁵ de
DAP	87.30 a	0.028 c	-63.14 de	0.001 c	-1.40 d	-4.5 × 10 ⁻⁴ e
DAP + AC	17.12 c	0.328 a	90.60 b	0.019 a	0.19 b	0.004 a
DAP + PC	65.61 b	0.093 bc	-80.95 e	0.001 c	-0.83 c	-0.001 e

* Means followed by different letters within a column indicate significant differences between the treatments at P < 0.05. The net cumulative CO₂ emissions were the difference between total CO₂ emissions and plants' CO₂ consumption. CK = Control; DAP = Di-ammonium phosphate; DAP + AC = Di-ammonium phosphate + acidic aged biochar; DAP + PC = Di-ammonium phosphate + pine biochar; WHC = Water holding capacity.

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