Research article

Stabilization of carbon through co-addition of water treatment residuals with anaerobic digested sludge in a coarse textured soil

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ABSTRACT

Coarse textured soils have low potential to store carbon (C) due to lack of mineral oxides and have low clay content to protect C from biodegradation and leaching. This study evaluated the potential of stabilizing C by adding metal oxyhydroxide-rich water treatment residuals (WTRs) to an aeolian pure sand (<5% clay) topsoil amended with anaerobic digestate (AD) sludge. The AD sludge was applied at 5% (w/w) with aluminum based WTR (Al-WTR) and iron based WTR (Fe-WTR) co-applied at 1:1 and 2:1 WTR:AD (w/w) ratios and incubated at room temperature for 132 days. The cumulative mineralized C was normalized to the total organic C of the treatments. Co-addition with Al-WTR showed to be more effective in stabilizing C through decreased cumulative mineralized C by 48% and 57% in 1Al-WTR:1AD and 2Al-WTR:1AD, respectively, compared to AD sludge sole amendment. Co-application with Al-WTR also decreased permanganate oxidizable C by 37% and dissolved organic C by 51%. Co-application with Fe-WTR did not decrease the concentration of these labile C pools to the same extent, possibly due to the selective use of Fe-WTRs to treat organic-rich raw water. This makes it less effective in stabilizing C in a pure sand relative to Al-WTR due to chemical instability of the Fe-organic complexes. The Al-WTR provides a promising co-amendment to increase C sequestration in pure sands when co-applied with biosolids. The co-amendment approach will not only facilitate C sequestration but also contributes to waste management, aligning to the objectives of a circular economy.

1. Introduction

Carbon (C) stabilization in biosolid amended soils can be variable, either having negative or positive priming effects commonly measured using C mineralization rates (Stumpe et al., 2012). The C mineralization in this context refers to the general function measuring the outcome of aerobic metabolism of microorganisms, typically measured as carbon dioxide (CO_2) emission. The priming effects are influenced by factors such as the biosolid stabilization process, climatic region, incorporation rates and soil type (Torri et al., 2014; Wijesekara et al., 2017). As for soil type, soil texture is an important factor influencing organic C dynamics in soils, with fine-textured soils capable of potentially stabilizing more organic C and reduce C mineralization compared to coarse-textured soils (Bustamante et al., 2010). Low potential to stabilize C in coarse-textured

soils occurs due to enhanced accessibility of soil organic carbon (SOC) to decomposer organisms or catalytic enzymes, facilitated by low clay mineral content that fails to provide physical protection against microbial degradation (Castán et al., 2016). Additionally, the low clay content limits adsorption onto clay mineral surfaces (Shanmugam et al., 2014; Singh et al., 2018; Arunrat et al., 2020; Wang et al., 2021).

High C mineralization rates resulting in an increase of CO_2 emission to the atmosphere can be mitigated by increasing C storage capacity in the soil (Bustamante et al., 2010; Wijesekara et al., 2017). Carbon stabilization in soils is one of the recognized sustainable strategies to overcome anthropogenic climate change (Saidy et al., 2012; Bolan et al., 2012; Lim and Choi, 2014; Wijesekara et al., 2017; Wang et al., 2021; Bhattacharyya et al., 2022). This is achieved through three predominant mechanisms; (i) bio-chemical recalcitrance through processes such as

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the Maillard reaction, (ii) formation of organo-mineral complexes through chemical interactions with minerals and metal ions and (iii) physical protection owing to occlusion within soil aggregates (Torri et al., 2014; Singh et al., 2018; Jeewani et al., 2021; Moore et al., 2023). The formation of organo-mineral complexes through interaction with clay minerals and metal ions is the most important mechanism of SOC stabilization in soils (Torri et al., 2014; Singh et al., 2018; Jeewani et al., 2021). Thus, C mineralization rates in organic waste amended soils enriched with synthetic clay minerals are being explored (Bolan et al., 2012; Saidy et al., 2012; Dodor et al., 2019; Singh et al., 2018).

Biosolids are occasionally co-applied with water treatments residuals (WTRs) to ameliorate physicochemical properties in coarse textured soils (Ippolito et al., 2009; Mahdy et al., 2009, 2012; Elkhatib and Moharem, 2015; Ribeiro et al., 2022). Water treatment residuals are sludges from potable water treatment processing. They are porous in nature with highly reactive surfaces of aluminum (Al) and iron (Fe) hydroxides inherited from the coagulation-flocculation process used to remove suspended and colloidal particulates from drinking water. The common coagulants used are Al (e.g $Al_2(SO_4)_3 \bullet nH_2O$) and Fe (e.g $FeCl_3 \bullet 6H_2O$, $FeSO_4 \bullet 7H_2O$) salts resulting in Al-based WTRs or Fe-based WTRs, respectively (Titshall and Hughes, 2005; Hsu and Hseu, 2011; Turner et al., 2019; Salehin et al., 2020). X-ray diffraction (XRD) analysis of WTRs shows that they are amorphous in nature but contain traces of quartz, feldspar, calcite, illite/smectite, albite and kaolinite minerals (Turner et al., 2019). These minerals share similar characteristics with predominant minerals found in soil environments that contribute to SOC stabilization; which include phyllosilicates, metal oxides, hydroxides, oxyhydroxides and short-range ordered aluminosilicates (Saidy et al., 2012; Singh et al., 2018). The mesoporous nature of the WTRs has made it an efficient adsorbent re-used in numerous applications such as in adsorbing organic compounds and heavy metals (Dias et al., 2021; Sharma and Ahammed, 2023), phosphorus removal in wastewater treatment (Muisa et al., 2020; Nguyen et al., 2022) and total organic C removal in animal farm wastewater (Kang et al., 2022).

The WTRs potential in reducing bio-accessible organic compounds such as dissolved organic carbon (DOC) has been tested in removal of natural organic matter (NOM) during surface water treatment. Khedher et al. (2022) reused Al-WTR with Al based coagulant ($Al_2(SO_4)_3$). The reuse of WTR at 3 g/L dose removed approximately 70% DOC from the surface water and reduced the addition of ($Al_2(SO_4)_3$) coagulant by approximately 50%. Albrektienė et al. (2019) tested Fe-WTR from groundwater treatment to treat Neris river water that can be used as drinking water. The most effective removal of organic compounds (55.51%) was when Fe-WTR was added as 0.3 g/L together with 8 mL 0.95% H_2SO_4. The NOM removal is attributed to a combination of charge neutralization, adsorption, and complexation with coagulant metal ions into insoluble particulate aggregates (Matilainen et al., 2010). In addition, chemical coagulation during the water purification process has been shown to enhance removal of NOM from raw water with the hydrophobic (HOC) fraction efficiently removed more than the hydrophilic (HIC) fraction (Matilainen et al., 2005, 2010). There are limited studies on the interaction of WTRs with bio-accessible organic compounds in terrestrial environments, particularly in relation to the co-application of WTRs with biosolids. Thus, the aim of this study was to investigate the capacity of Al-/Fe-WTR in stabilizing bio-accessible organic C in a pure sand soil amended with biosolids. It was hypothesized that co-addition of Al-/Fe-WTR with biosolids will reduce the short-term net C mineralization and reduce labile C pools. Changes in permanganate oxidizable C (POXC) and DOC were monitored as labile C pools. The DOC leachates were fractionated into HIC and HOC fractions using Liquid Chromatography-Organic C Detection-Organic Nitrogen Detector (LC-OCD-OND) to explore the most affected fractions during the incubation period. Findings of this study will shed light on the potential of WTRs in stabilizing C in coarse textured soils amended with biosolids. The benefits of WTRs co-amendment on nutrient provision for plant growth (Elkhatib and Moharem, 2015; Ribeiro et al., 2022) have

been established. Retention of toxic inorganic elements in pure sands amended with WTRs has also been investigated (Clarke et al., 2019). This study assessed the potential benefits of co-amendment in terms of mitigating the high C mineralization rates and losses of labile C through leaching common in pure sands.

2. Materials and methods

2.1. Sample collection and preparation

The pure sand soil was collected in February 2020 from Jacobsdal Farm, Kuilsrivier, in the Western Cape Province of South Africa (−33.967350 S,18.717388 E). This farm was selected as the soils are representative of the soils (locally known as Cape Flats sands) that surround the major waste treatment plants around the Cape Town Metropol. These soils are derived from highly leached aeolian marine deposits and are classified as Psamments. The soils are slightly acidic with pH (H_2O) of 5.6 (Clarke et al., 2019). The field where the soil was collected was fallow at the time of sampling and occurs on a west facing, lower footslope. Topsoils (0–30 cm) were collected from multiple locations within the field to generate a composite sample. The collected soil was thoroughly mixed, air dried at room temperature and passed through a 2 mm sieve. The soil was stored in 20 L clean containers at room temperature until analysis and experimental setup.

The anaerobic digestate (AD) was collected during the 2019 summer season from a wastewater treatment plant (WWTP) located in the City of Cape Town, in the Western Cape province of South Africa. The plant receives 80% domestic wastewater and 20% industrial wastewater with average sludge production of 182 484 m^3 per year. The sludge is further treated using anaerobic mesophilic digestion operating at 36–38 °C and the resultant digestate is pumped into the site's lagoons at 1–2% total solids (TS). In the lagoon, the digestate is allowed to dry, and later transferred to stockpiles within the wastewater treatment plant. The sample used in this study was collected in the stockpiles where the digestate was allowed to dry for a period of 4–6 months and at approximately 30% TS. After collection from the stockpiles, a 500 g sample was contained in a Ziploc plastic bag and refrigerated at 4–8 °C for microbial analysis. A bulk sample was allowed to air dry by spreading on the ground in an isolated area to constant weight. Studies conducted by Sanchez-Monedero (2004); Bustamante et al. (2010), Costan et al. (2016), Bolan et al. (2012) and Badza et al. (2021) show that particle size is not frequently determined on biosolids or organic substrates. Hence, after air drying, the sample was only crushed to uniform particle size (2 mm) and stored in a 20 L container at room temperature until analysis and experiment setup.

The WTRs were collected from two different potable water treatment plants (WTPs) and were assigned the names Fe-WTR and Al-WTR. Dewatered Fe-WTR was collected in 2018 from the Faure WTP which its water sources are Theewaterskloof and Palmiet reservoirs. Faure uses ferric coagulant ($Fe_2(OH)_3$, hydrated lime ($Ca(OH)_2$), calcitic lime, activated C and additional polyelectrolyte (higher molecular weight polyacrylamide monomers) followed by centrifugation. The ferric coagulant is a preferred at Faure WTP as it is more effective in treating water with high concentrations of humic acids such as that from the Palmiet water source (Steytler, 2021). The Al-WTR was sourced from Blackheath WTP in 2019 which uses the Theewaterskloof as a water source. Blackheath uses aluminum sulphate ($Al_2(SO_4)_3.16 H_2O$) as a coagulant with additional dose of lime and activated C. Following the treatment process, the alum sludge is disposed in a lagoon for further drying so the sludge has gone through a process of aging in the pond prior collection unlike the freshly generated Fe-WTR sludge. The Al-WTR was collected from the lagoon while Fe-WTR was collected from the receiving tanks immediately after centrifugation. Both WTR samples were also allowed to air dry to constant weight, passed through a 2 mm sieve and stored in 20 L containers at room temperature prior analysis.

2.2. Sample analysis

The samples were divided into three sub-samples to allow for analysis of selected properties (Table S1). The AD sludge was further screened for land application suitability following the South African Water Research Commission guidelines (Herselman and Moodley, 2008). Total elemental concentrations were extracted using aqua regia (USEPA Method 3051a). The extracts were analyzed using inductively coupled plasma atomic emission spectroscopy (Thermo ICap 6200 ICP–AES) at the Central Analytical Facility (CAF) in Stellenbosch University. The sludge sample was also subjected to microbial analysis using the plate count method for feacal coliforms (Germida and De Freitas, 2008). The digestate trace element concentrations were all below the minimum threshold limits (Table S1). The faecal coliforms were 1.99 x 10^4 cfu/g-dry weight, qualifying the digestate to be Class B sludge since it exceeds the threshold limit of 1.00 x 10^3 cfu/g-dry weight in Class A (Herselman and Moodley, 2008). Data for helminth eggs was obtained from the WWTP analytical services report, and on average the eggs are normally <10 ova/g of digestate.

2.3. Soil amendment incubation

The incubations were designed such that C dynamics in the pure sand amended with AD sludge alone were compared to the effect of incorporating either Al-WTR or Fe-WTR. The AD sludge as an amendment alone was applied at 5% (w/w) on a dry basis. The Al-WTR or Fe-WTR was incorporated in the AD sludge-amended sand to obtain amendment ratios of 1WTR:1AD and 2WTR:1AD at 5% (w/w) and 10% (w/w) on dry basis. The AD sludge alone or co-amendments ratios with Al/Fe-WTR were added in a 100 g sand on dry basis. The 5% application rate as a base was used to allow detectable changes in C dynamics and to explore mechanisms which may not be detectable under lower application rates of biosolids in soils. In addition, increasing loading rates of WTRs in the co-amendment ratios was intended to assess if effects are stronger with increasing gradients. The amendments with the WTRs alone at 5% (w/w) on dry basis were additional treatments used to evaluate the response of the soil C dynamics with only WTR added in the soil. The sand alone served as a control. The treatments' nomenclature is given in Table 1.

The application rates in Table 1 were added in a 100 g pure sand on dry basis weighed into a 1 L air-tight glass jar. The 1 L air-tight glass jars were all new and rinsed three times with deionized water before use. All treatments were replicated three times and laid out in a completely randomized design. The soil was thoroughly mixed with the amendment materials, moistened to 60% water holding capacity (WHC) and stored in the dark at room temperature for an incubation period of 132 days. Carbon mineralization rate was measured at 10 intervals while all other parameters were measured at the initial and final stages of the incubation period.

Table 1
Treatment combinations of the AD sludge and Al-/Fe-WTRs.

Treatment no.	Treatment name	Substrate	Application rate
1	Sand	Sand only	No amendment
2	Al-WTR 5	Sand and Al-WTR	5%
3	Fe-WTR 5	Sand and Fe-WTR	5%
4	AD 5	Sand and AD sludge	5%
5	1Al-WTR:1AD	Sand, Al-WTR and AD sludge	5% WTR and 5% AD sludge
6	2Al-WTR:1AD	Sand, Al-WTR and AD sludge	10% WTR and 5% AD sludge
7	1Fe-WTR:1AD	Sand, Fe-WTR and AD sludge	5% WTR and 5% AD sludge
8	2Fe-WTR:1AD	Sand, Fe-WTR and AD sludge	10% WTR and 5% AD sludge

2.4. Soil C assays

2.4.1. Carbon mineralization

2.4.1.1. Cumulative CO_2–C mineralization. One batch of the prepared microcosm sets consisting of all treatments was used to determine C mineralization rate using the closed chamber system with alkali CO_2 traps (Hopkins, 2006). The alkali trap system quantifies microbe-mediated soil respiration expressed as CO_2–C emitted into the chamber atmosphere. In summary, one vial with 10 mL of 1M NaOH and one with 10 mL distilled water were placed inside each 1 L glass chamber. Empty jars with only the two vials were included as blanks. After inserting the vials, the jars were sealed with airtight lids and stored in the dark at ambient temperature. The CO_2 emission was measured at day 3, 7, 14, 21, 28, 35, 42, 72, 102 and 132. Incorporation of sludge in soils provides labile C that produces a large initial CO_2–C emitted due to microbial respiration. This respiratory phase is called the "C dominated phase" (Torri et al., 2014). Sampling intervals were designed with this background information such that there are short periods between sampling intervals at the initial stage until day 42. Beyond day 30, respiration rates become steady and almost equivalent to the soil without amendment (Torri et al., 2014), hence period between sampling intervals after day 42 were longer relative to initial stage of incubation. Studies with similar intervals were conducted by Sánchez-Monedero et al. (2004), Bustamante et al. (2010), Zornoza et al. (2013) and Shahbaz et al. (2017). For each interval, the vials were removed from the chamber and the NaOH was back titrated with 0.5 M HCl, after removing the dissolved CO_2 and carbonates by precipitation with the addition of 2 mL of $BaCl_2$. A new 10 mL 1 M NaOH solution was added to the vial and returned to the jars and the 10 mL water was changed each time the NaOH was titrated. Before sealing, the jars were allowed to stand open for regular aeration. The CO_2 in the traps was calculated using Equation (1).

$$CO_2 - C \ emitted \left(\frac{ug}{g}\right) = \frac{MWC \ (Vb - Vc) \ x \ M}{DW \ x \ t \ x \ 2} x \ 1000 \ x \ 24hr \Big/ day \quad (1)$$

where MWC = molecular weight of C (12 g mol^{-1}), Vb is the volume for the blank titration, Vc = volume for the sample titration, M = concentration of the HCl (0.5 M), DW is the dry weight of the soils and t is the incubation period in hours. A factor of 2 was included in the formula since two OH$^-$ are consumed per CO_2 precipitated (Pell et al., 2006; Bolan et al., 2012).

2.4.2. Total organic C (TOC) analysis

The total inorganic carbon (TIC) in the pure Al and Fe WTR materials was extremely low (<0.5%) while the AD had a TIC of 2.2% (Table S1). Thus, when diluted to 5% with the soils the TIC concentration would be negligible (0.03% for 5% AD treated samples), therefore total carbon (TC) was used to represent TOC for the incubation samples. The pH measured during the incubation decreased to pH values less than 7 (Table S2) suggesting that possible formation of biogenic carbonates was also insignificant. Air-dried incubation samples, were ground into homogenous powders using a ball mill, and analyzed for TC using the dry combustion method in an Elementar analyzer (Elementar, Vario Macro Cube).

2.4.3. Permanganate oxidizable C (POXC)

Permanganate oxidizable C (POXC) is the active soil organic C pool used to indicate biologically active C (Culman et al., 2012). It was measured using the method developed by Weil et al. (2003). In summary, 1 g of air-dried sample crushed using mortar and pestle to ensure homogeneity of the sample was reacted with 20 mL of 0.02 M $KMnO_4$ by mechanically shaking the suspension for 2 min at 2000 rpm. After shaking, the sample was centrifuged for 5 min at 3000 rpm to separate the solid particles from the solution. A 0.2 mL aliquot was pipetted into a

clean volumetric flask and diluted with deionized water. Absorbance was measured at 550 nm using the UV–Vis spectrophotometer. Sample absorbances were compared to a standard curve (0–0.02 M KMnO$_4$) also diluted with deionized water. Active POXC (mg/kg) was calculated using Equation (2).

$$POXC \left(\frac{mg}{kg}\right) = [0.02\,M - (a + b * abs)] * \left(9000\,mg\frac{C}{mol}\right)$$
$$* \frac{0.02\,L}{0.001\,kg - soil} \tag{2}$$

where 0.02 M is the initial solution concentration, a is the intercept and b is the slope of the standard curve, 9000 is the mg C (0.75 mol) oxidized by 1 mol of MnO$_4^-$ changing from Mn^{7+} to Mn^{4+}, 0.02 L is the volume of KMnO$_4$ solution reacted and 0.001 is the mass of soil (kg).

Since all substrates used added C to the soil, it was also important to normalize the proportion of POXC relative to the TOC content and express it as POXC: TOC (%) calculated using Equation (3).

$$POXC : TOC\,(\%) = \frac{POXC}{TOC}\,x\,100 \tag{3}$$

2.4.4. Dissolved organic C extraction and fractionation

The DOC is defined as a range of organic molecules of varying sizes, composition and structures that can pass through 0.45 μm membrane filters (Ramesh et al., 2019). It was extracted from a moist sample equivalent to 4 g-oven dry mass with 40 mL 18.2 M Ohm deionized water by mechanically shaking for 16 h at 200 rpm at room temperature. After shaking, the sample was centrifuged at 1000 rpm (g) for 10 min to reduce clogging during filtering. The supernatant was filtered using 0.45 μm pore size Polyethersulfone (PES) syringe filters and stored at 0–4 °C in pre-combusted glass TOC vials until analysis on the next-generation Model 9 liquid chromatography-organic C detection-organic-nitrogen detection system (LC-OCD-OND) (DOC Labor, Karlsruhe, Germany; Huber et al. (2011)). At the inlet of the organic C detection (OCD), the solution was acidified at a flow rate of 0.2 mL min^{-1} (gravity-driven) to convert carbonates to carbonic acid. The column was bypassed with a restricted flow (flow rate 0.1 mL min^{-1}, back pressure-driven) to obtain DOC concentration (Pereira et al., 2024).

For DOC fractionation, a 1 mL aliquot was injected onto a size exclusion column (2 mL min^{-1}; HW50S, Tosoh, Japan) with a phosphate buffer (potassium dihydrogen phosphate 1.2 g L^{-1} plus 2g L^{-1} di-sodium hydrogen phosphate x 2 H$_2$O, pH 6.58) and separated into five compound-group specific HIC DOC fractions: (i) biopolymers (BP) (high molecular weight, largely non-UV absorbing extracellular polymers); (ii) humic substances (HS) (higher molecular weight, UV absorbing); (iii) building blocks (BB) (lower molecular weight, UV absorbing humics); (iv) low molecular weight neutrals (LMWN) (hydro- or amphi-philic, non-UV absorbing); and (v) low molecular weight acids (LMWA). All peaks were identified and quantified with bespoke software (Labview, 2013) provided by DOC Labor normalized to International Humic Substances Society Humic Acid and Fulvic Acid (IHSS HA and FA) standards. The hydrophobic DOC fraction (HOC) is calculated as the difference between the total DOC measured via the bypass and the sum of DOC measured from HIC. In addition, the LC-OCD-OND utilizes UV absorbance at 254 nm which provides information on the aromaticity (SUVA-HS) and the molecular weight (MW) of the humic substances. It also provides information on dissolved organic bound nitrogen (DON) content with DON being a subset of DOC which includes HS-DON and BP-DON (Huber et al., 2011).

The DOC proportion relative to TOC content was calculated using Equation (4).

$$DOC : TOC\,(\%) = \frac{DOC}{TOC}\,x\,100 \tag{4}$$

2.4.5. Carbon loss estimation

The pure sand used in this study lacks clays and sesquioxides (Clarke et al., 2019). As a result, poses high risk of C losses not only through C mineralization but DOC leaching with potential of contaminating groundwater sources. This background was used to derive a new variable which was termed "potential C loss" and calculated as the sum DOC and cumulative CO$_2$–C relative to the TOC (Equation (5)). The cumulative CO$_2$–C data was also normalized to TOC to determine the mineralized CO$_2$–C relative to the TOC content (Equation (6)).

$$Potential\,C\,loss\,(\%) = \frac{DOC + cumulatove\,CO_2 - C}{TOC} \tag{5}$$

$$Mineralized\,CO_2 - C\,(\%) = \frac{Cumulative\,CO_2 - C}{TOC}\,x\,100 \tag{6}$$

2.5. Data analysis

Full factorial analysis of variance (ANOVA) was performed to compare the treatments at a significance level of $P < 0.05$. Normal distribution was assessed before ANOVA and where assumptions were violated, the data was first transformed using the log transformation. Where transformation failed, the P-value was obtained by Wilcoxon non-parametric test using John's Macintosh Project (JMP) version 16.0 statistical software. Pearson's correlation coefficients were done to measure the linear association between the variables using the Multivariate method in the same JMP software.

3. Results

3.1. Carbon mineralization

The cumulative CO$_2$–C emitted during the 132-day incubation period rapidly increased for the first 42 days and showed a steady increase thereafter (Fig. 1). The sand alone had the lowest mineralized C followed by the Al-WTR 5 and Fe-WTR 5 treatments, respectively. Addition of AD sludge at AD 5 and with co-addition of both Al-WTR and Fe-WTR at 1:1 and 2:1 (WTR:AD) ratios significantly increased CO$_2$–C emitted during the incubation period. A significant mineralization increase ($P < 0.05$) was most apparent in Fe-WTR co-application with 1Fe-WTR:1AD and 2Fe-WTR:1AD treatments being 21% and 29% more, respectively, than AD 5 treatment (Fig. 1). The CO$_2$–C cumulative emission rate in 1Al-WTR:1AD was not significantly different to AD 5 and a slight decrease was observed in 1Al-WTR:1AD CO$_2$–C emitted after 63 days of the incubation period (Fig. 1 and Fig. S1).

3.2. Effect of AD sludge and WTRs co-amendments on labile C pools

3.2.1. Permanganate oxidizable C (POXC)

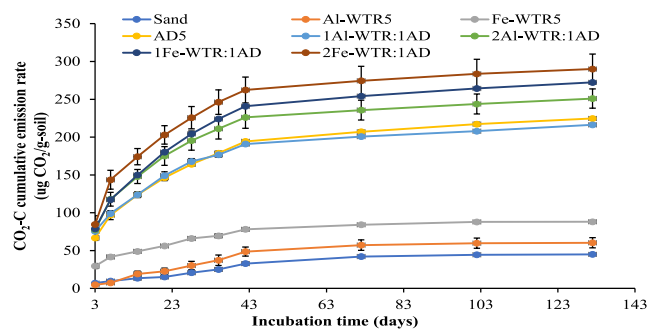The sand initially had significantly ($P < 0.05$) lower POXC

Fig. 1. Cumulative CO$_2$–C mineralization as a result of adding Al-WTR and Fe-WTR in a pure sand amended with AD sludge. Error bars indicate standard deviation of the mean from three replicates.

concentrations which increased with addition of all the amendment treatments (Fig. S2). The AD 5 treatment increased initial POXC concentrations by 121% and co-addition with Al-WTR and Fe-WTR at 1:1 and 2:1 ratio also enhanced POXC concentrations in comparison to the sand control, but they were not significantly different to the AD 5 treatment (Fig. S2). At the end of the incubation, 1Fe-WTR:1AD and 2Fe-WTR:1AD were significantly higher than AD 5, with a 32% and 27% increase in POXC, respectively, whereas 1Al-WTR:1AD and 2Al-WTR:1AD treatments significantly decreased POXC concentrations by 37% and 24%, respectively, when all compared to AD 5. Interferences by reduced Fe and Mn species in the POXC analysis (Fan et al., 2013) were ruled out by testing for changes in diethylenetriaminepentaacetic acid (DPTA) exchangeable Fe (Fig. S3).

The POXC:TOC ratio, at the final stage of incubation, was the highest in the sand alone and AD 5. Co-addition of AD sludge with Fe-WTR significantly decreased the POXC:TOC from 5% to 4% (1Fe-WTR:1AD) and to 3% (2Fe-WTR:1AD) when compared to AD 5 treatment while Al-WTR significantly decreased POXC:TOC from 5% to 3% and 2% in 1Al-WTR:1AD and 2Al-WTR:1AD, respectively (Fig. 2A).

3.2.2. Dissolved organic C (DOC)

Addition of AD sludge in the sand significantly increased the ($P <$ 0.05) DOC concentration by 232% relative to the amount of DOC in the sand alone (Fig. S4). Co-addition of Al-WTR resulted in an immediate decrease in DOC concentrations with a 24% significant decrease in 1Al-WTR:1AD when compared to the AD 5 treatment. In contrast, Fe-WTR co-addition significantly increased DOC by 16% and 26% in 1Fe-WTR:1AD and 2Fe-WTR:1AD, respectively (Fig. S4).

After the incubation period, the DOC concentrations significantly decreased in all the treatments. The percentage change between initial and final stage of incubation was calculated and the decrease in DOC over time followed the order 2Al-WTR:1AD > 1Al-WTR:1AD > AD5 > 1Fe-WTR:1AD > 2Fe-WTR:1AD (Table S3). The DOC:TOC % followed the order AD 5 > sand > 1Fe-WTR:1AD > 2Fe-WTR:AD > Fe-WTR 5 > 2Al-WTR:1AD > 1Al-WTR:1AD > Al-WTR 5 (Fig. 2B).

The nature of the DOC extracted from the samples was largely HOC as shown by its higher concentrations relative to the HIC (Fig. 3). Between HOC and HIC, the HOC decreased more over time than the HIC across all treatments (Table S3). The HIC was further fractionated using the LC-OCD-OND and all treatments had detectable amounts of HS, BP, BB and LMWN. The HIC fractions followed the same trend as the total

DOC with the Al-WTR resulting in a significant decrease over time than Fe-WTR (Fig. S5). The dominating HIC fraction was the HS across all treatments, ranging between 46% and 67%, whereas BB and BP and LMWN were existing in approximately similar proportions generally below 30%. The HS and LWMN proportions increased overtime in all treatments, except in AD 5 which had a slight decrease in HS (Fig. 4). The HS increased by 25%, 19%, 21% and 16% in 1Al-WTR:1AD, 2Al-WTR:1AD, 1Fe-WTR:1AD and 2Fe-WTR:1AD, respectively. The BP and BB concentrations generally decreased in all the treatments over time.

The sand DOC had a significantly higher MW of 481.0 g mol^{-1} at the initial stage of incubation. Addition of AD sludge alone or in combination with the WTRs significantly decreased the apparent MW to values ranging between 359.3 g mol^{-1} to 416.7 g mol^{-1} (Table 2). However, in contrast to the initial decrease of the MW in Al-WTR based treatments, the incubation period resulted in higher MW in Al-WTR based amendments when compared to AD 5 and Fe-WTR amendments. The sand alone remained with the largest MW even after the incubation period (Table 2).

The aromaticity of the HS measured as SUVA-HS followed the same trend as the MW. The sand had significantly higher SUVA-HS values (0.61–0.52 kg/mg*m) and amendment with AD 5 or with both WTRs reduced the SUVA-HS to values in the range 0.43–0.29 kg/mg*m (Table 2). The SUVA-HS in 1Al-WTR:1AD, 2Al-WTR:AD and Al-WTR 5 remained the lowest both at the initial and final stage of the incubation period. The protein content (%) relative to the BP concentration increased in the sand from 40% to a range between 74 and 100%, with co-addition of the AD sludge and both WTRs. The AD 5 treatment had 100% protein even at the initial stage of incubation while other co-amendment treatments yielded BP as 100% protein at the end of the incubation period including the sand alone (Table 2).

3.2.3. Carbon loss estimation

Potential C loss estimation (through DOC leaching and soil respiration) showed that the AD 5, sand alone, 1Fe-WTR:1AD and 2Fe-WTR:1AD treatments have a significantly higher ($P <$ 0.05) potential C loss than Al-WTR based amendments (Fig. 5). Carbon loss estimation was significantly 2–3 times lower in 1Al-WTR:1AD and 2Al-WTR:1AD when compared to AD 5. The 1Al-WTR:1AD and 2Al-WTR:1AD treatments significantly ($P <$ 0.05) decreased mineralizable C relative to the TOC by 48% and 57%, respectively. The Fe-WTR had 26% and 40% lower mineralizable C in 1Fe-WTR:1AD and 2Fe-WTR:1AD treatments,
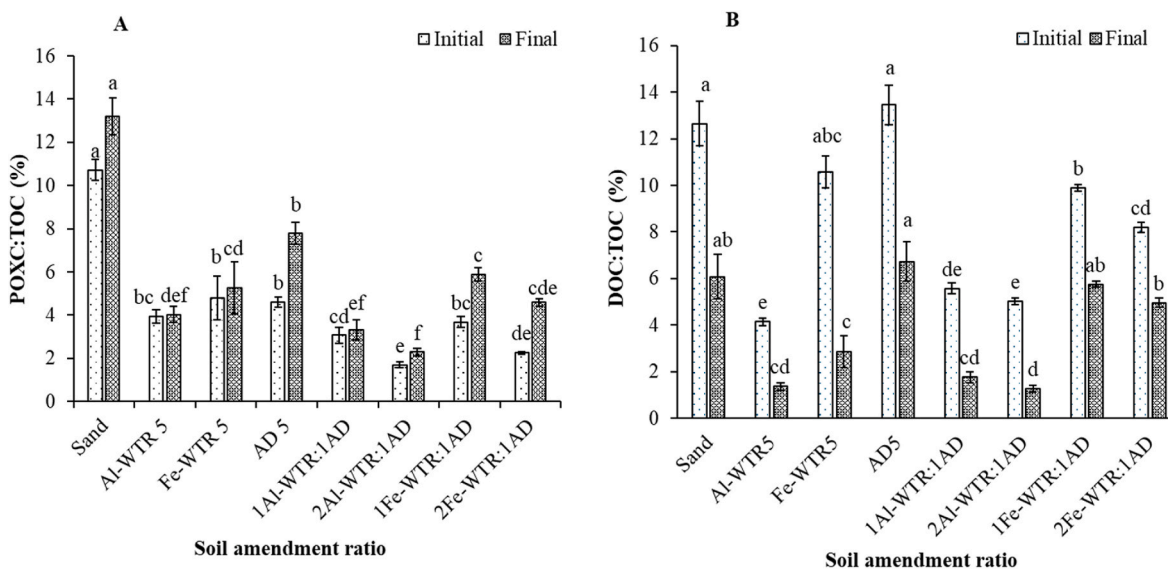

Fig. 2. A) Permanganate oxidizable C (POXC) normalized to TOC content during incubation of a pure sand co-amendment with AD sludge and Al-WTR or Fe-WTR. **B)** DOC concentrations normalized to TOC content. Error bars indicate standard deviation of the mean from three replicates. Bars with the same letter(s) within each incubation stage are not statistically different at $P < 0.05$.

Fig. 3. Changes in DOC hydrophilic (HIC) (Fig. 3A) and hydrophobic (HOC) (Fig. 3B) fractions during incubation of a pure sand soil co-amendment with AD sludge and Al-WTR or Fe-WTR. Error bars indicate standard deviation of the mean from three replicates. Bars with the same letter(s) within each incubation stage are not statistically different at $P < 0.05$.
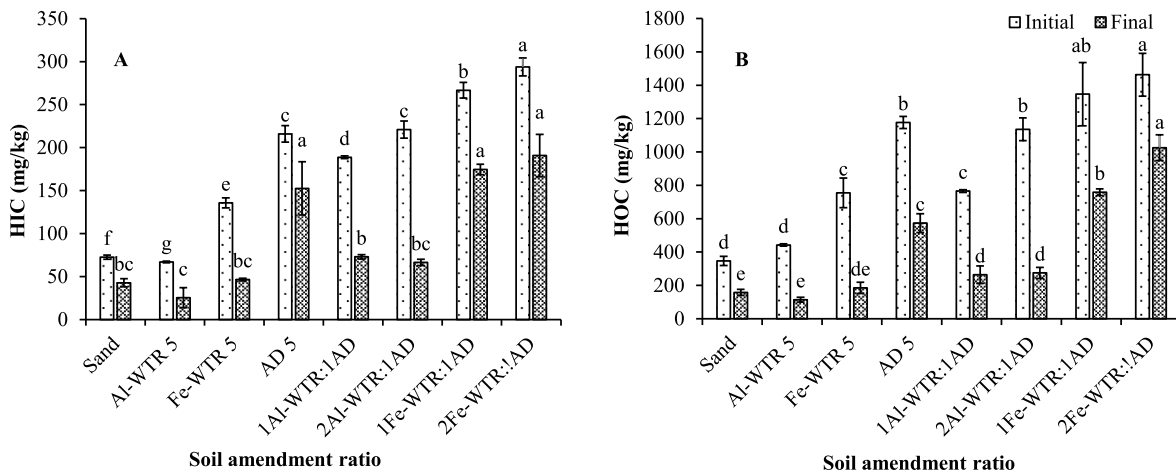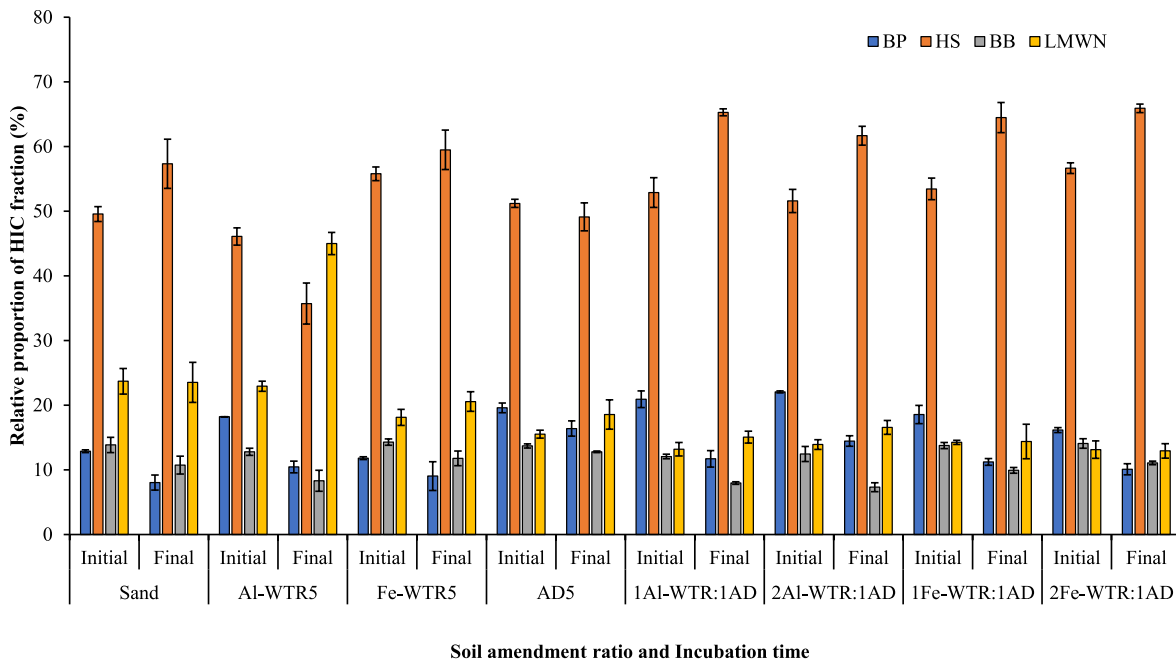

Fig. 4. Relative proportion of the HIC fractions in the pure sand co-amended with AD sludge and Al-/Fe-WTR.

respectively.

A correlation matrix was constructed to aid the interpretation of labile carbon pools (Table 3). Normalized POXC and DOC data from the initial and final stages of the incubation as well at the difference between these measurements were included in the correlation.

4. Discussion

4.1. Carbon mineralization

Soil organic C stabilization is measured as a decrease in C mineralization rates. In this study, all treatments added to the pure sand as sole amendments or co-applied increased C mineralization rates relative to the control sand (Fig. 1 and S1). The increase in C mineralization was most apparent at the initial stages of the incubation and could be due to the preferential degradation of more labile, carbohydrate rich fractions of DOC, known to have weaker sorption to soils minerals relative to

other soluble C fractions. These labile C pools are rapidly decomposed in solution by enzymatic depolymerization (Wan et al., 2019; Dodor et al., 2019; Bustamante et al., 2010). Biosolids are generally rich in labile C and dense in microbial communities, as such, the co-existence of both in a substrate together with competition between different microbial populations translates to high C mineralization rates (Soriano-Disla et al., 2010). Changes in soil humidity and temperature with rewetting of dry soils activates microbial activity. This stimulates metabolic processes that degrade easily biodegradable organic C even in the sand with relatively lower organic C content (Fierer and Schimel, 2002; Jin et al., 2013).

Since both materials used in this study added C to the soil, it was important to establish the C fluxes normalized to TOC, rather than absolute amounts. Soil organic C stability can also be evaluated by looking at the relationship between the outflux rate and the pool size, with low outflux rates indicating stability (Cotrufo and Lavallee, 2022). The significantly lower portion of C mineralized (Fig. 5) and the lower POXC

Table 2

Changes on biopolymer and humic substance characteristics over the incubation period. Values are mean ± standard deviation.

| Treatment		Biopolymer			Humics			
		DON (mg/kg)	N/C (ug/ug)	Protein (%BP)	DON (mg/kg)	N/C (ug/ug)	Aromaticity SUVA-HS (kg/mg*m)	MW (g/mol)
Sand	*Initial*	1.26 ± 0.1c	0.14 ± 0.0c	40.3 ± 1.2d	2.2 ± 0.0d	0.06 ± 0.0bc	0.52 ± 0.0a	481.0 ± 27.7a
	Final	2.6 ± 0.8bc	0.7 ± 0.1 ab	100	1.2 ± 0.1b	0.5 ± 0.0b	0.61 ± 0.0a	540.7 ± 15.3a
Al-WTR5	*Initial*	2.3 ± 1.5c	0.11 ± 0.01c	35 ± 3.6d	2.5 ± 1.5d	0.0 5 ± 0.0cd	0.3 ± 0.0e	359.3 ± 17.0d
	Final	2.3 ± 0.2c	0.9 ± 0.2 ab	100	0.4 ± 0.2b	0.04 ± 0.0b	0.29 ± 0.0e	418 ± 10.5c
FeWTR5	*Initial*	2.2 ± 0.1c	0.14 ± 0.0c	41.3 ± 1.5d	3.0 ± 0.1d	0.04 ± 0.0d	0.43 ± 0.0b	390.3 ± 7.6d
	Final	3.3 ± 1.1 c	0.8 ± 0.2 ab	100	1.2 ± 0.1b	0.04 ± 0.0b	0.38 ± 0.0c	413.3 ± 15.0c
AD5	*Initial*	14.6 ± 0.5a	0.34 ± 0.0a	100a	10.3 ± 0.9a	0.09 ± 0.0a	0.32 ± 0.1de	402.7 ± 9.1bc
	Final	12.2 ± 3.0a	0.5 ± 0.2b	100	7.9 ± 1.9a	0.09 ± 0.0a	0.42 ± 0.0a	427.0 ± 16.1c
1Al-WTR:1AD	*Initial*	9.8 ± 0.9b	0.2 ± 0.0a	74.0 ± 2.6	7.6 ± 0.6bc	0.07 ± 0.0 ab	0.33 ± 0.0d	396.3 ± 17.4bcd
	Final	9.3 ± 1.1 ab	1.1 ± 0.1a	100	2.7 ± 0.1b	0.06 ± 0.0b	0.35 ± 0.0d	466.7 ± 10.6b
2Al-WTR:1AD	*Initial*	12.3 ± 0.8 ab	0.3 ± 0.0b	75.7 ± 2.5bc	7.1 ± 0.3c	0.06 ± 0.0	0.33 ± 0.0cd	385.3 ± 8.1cd
	Final	10.6 ± 1.2a	1.1 ± 0.1a	100	2.1 ± 0.3b	0.05 ± 0.0 b	0.3 ± 0.0d	443.0 ± 6.7bc
1Fe-WTR:1AD	*Initial*	13.5 ± 1.3a	0.3 ± 0b	81.7 ± 2.5b	9.5 ± 0.5 ab	0.07 ± 0.0a	0.40 ± 0.0c	406.3 ± 13.0bc
	Final	11.2 ± 0.7a	0.6 ± 0.0b	100	6.1 ± 0.1a	0.05 ± 0.0b	0.42 ± 0.0b	417.7 ± 7.6c
2Fe-WTR:1AD	*Initial*	11.9 ± 0.6 ab	0.3 ± 0b	75 ± 1.0bc	8.9 ± 0.5abc	0.05 ± 0.9bc	0.4 ± 0.0b	416.7 ± 12.9bc
	Final	10.9 ± 1.1a	0.6 ± 0.2b	100	6.1 ± 0.8a	0.05 ± 0.0b	0.4 ± 0.b	412 ± 8.5c
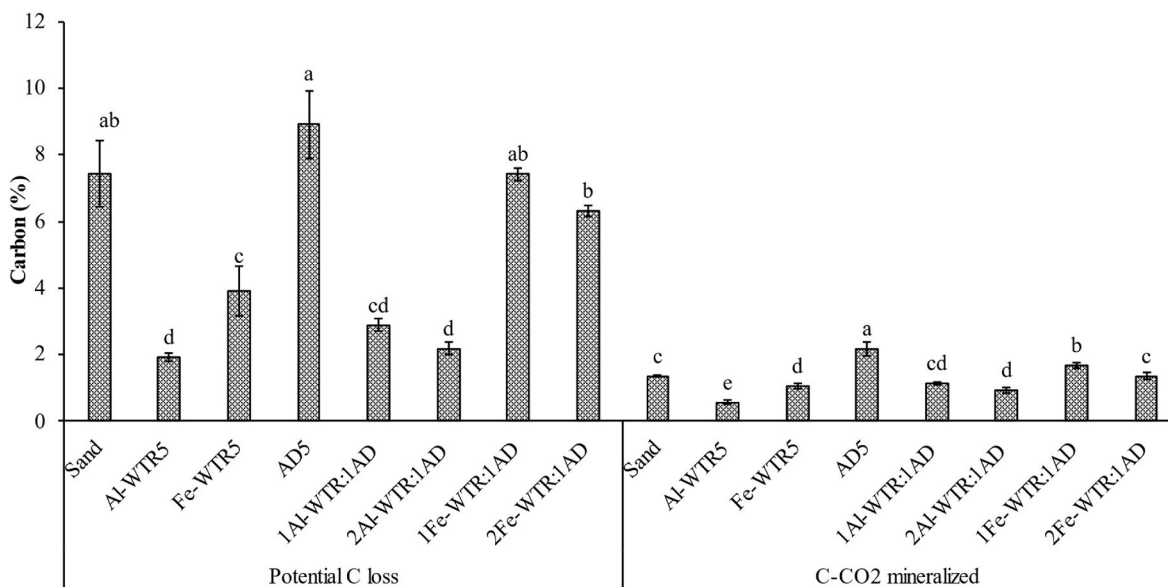
Fig. 5. The proportion of the potentially mineralizable and C mineralized (DOC + cumulative CO_2–C mineralized) and cumulative CO_2–C mineralized normalized to the TOC content of each treatment. Error bars indicate standard deviation of the mean from three replicates. Bars with the same letter(s) are not statistically different at $P < 0.05$.

Table 3

Pearson correlation using multivariate analysis. C mineral = C mineralized; Pot. C loss = potential C lost, f, i and d subscripts represent initial and final stage of incubation, and the difference (f-i) calculated between the incubation stages, respectively.

	TOC	C mineral.	Pot. C loss	POXC:TOC$_i$	POXC:TOC$_f$	POXC:TOC$_d$	DOC:TOC$_i$	DOC:TOC$_f$
C mineral.	−0.165							
Pot. C loss	−0.382	0.902***						
POXC:TOC$_i$	−0.832***	0.162	0.422*					
POXC:TOC$_f$	−0.764***	0.434*	0.674***	0.934***				
POXC:TOC$_d$	−0.231	0.802***	0.878***	0.320	0.637***			
DOC:TOC$_i$	−0.612**	0.774***	0.867***	0.602**	0.760***	0.718***		
DOC:TOC$_f$	−0.419*	0.859***	0.996***	0.467*	0.709***	0.873***	0.864***	
DOC:TOC$_d$	−0.624**	−0.412*	−0.420*	−0.551**	−0.559**	−0.293	−0.814***	−0.421*

* Significant at $P < 0.05$.
** Significant at $P < 0.01$.
*** Significant at $P < 0.001$.

and DOC concentrations with co-addition of the WTRs (Fig. 2) suggest that a greater portion of C was stabilized against mineralization. The decrease in DOC:TOC in all treatments during the incubation could either be as a result of mineralization or stabilization. The weak correlation (r = −0.41, P < 0.05) between the difference (final – initial) in normalized DOC before and after incubation and mineralized C (Table 3) suggests stabilization was more dominant. The increase in POXC:TOC during the incubation in most treatments, is possibly related

to the increase in microbial biomass which has been shown to be positively related to POXC (Bongiorno et al., 2019; Hurisso et al., 2016). This is further supported by the strong positive correlation (r = 0.80, P < 0.001) between difference (final – initial) in POXC:TOC at the start and end of the incubation and mineralized C (Table 3). Between Fe-WTR and Al-WTR, the Al-WTR showed the greatest potential to reduce C loss and had the least labile C pools.

The WTRs contain oxyhydroxides introduced during the coagulation process in water purification. During the coagulation process, added aqueous Al (III) and Fe (III) salts dissociate to their respective trivalent ions forming several highly positive charged soluble complexes which can adsorb onto the surface of negative colloids. The polymers formed as hydrolysis products can remove DOC from solution through bridging or sweep flocculation, while medium or monomer species have a high capacity to remove DOC by complexation, adsorption, charge neutralization or co-precipitation (Matilainen et al., 2005, 2010). These mechanisms are like that of C stabilization reported in studies of soil environments, suggesting that the inherited oxyhydroxides behave similarly to soil minerals (Eusterhues et al., 2005; Wan et al., 2019; Cotrufo and Lavallee, 2022). Adsorption and coprecipitation are the predominant mechanisms of organic matter association with metal oxides (Wan et al., 2019; Lenhardt et al., 2022). The presence of Fe- and Al-oxides can also help to stabilize aggregates in the soil, resulting in occlusion of OM at the clay microstructure level (Eusterhues et al., 2005; Fang et al., 2019; Wiesmeier et al., 2019). Occlusion restricts both physical access of microbes, as well as their extracellular enzymes to the co-precipitated OM (Lenhardt et al., 2023). The Fe- or Al-oxides also increase the specific surface area (SSA) via coating, thus protecting OC from microbial degradation (Wang et al., 2021). Differences in the rate of C mineralization reflect differences in the SSA, with larger SSA suggesting more and stronger interactions (Saidy et al., 2012). The Al-WTR had a larger SSA than Fe-WTR (Table S1) which possibly contributed to the lower C mineralization rate in Al-WTR co-amendments. Soil mineral oxides have affinity to adsorb more HOC relative to HIC via ligand exchange with OH and H bonding (Avneri-Katz et al., 2017). This can explain the higher decrease of HOC concentrations relative to HIC observed in this study (Table S3). The HOC consists of humic substances and is rich in aromatic C, phenolic structures, and conjugated bonds (Matilainen et al., 2010).

Low molecular weight compounds can also be removed from the solution via adsorption onto metal hydroxide surfaces (Matilainen et al., 2010; Lin et al., 2012). For example, the readily available carbohydrates, preferred by microorganisms are expected to be absorbed via H bonding and van der Waals interaction (Avneri-Katz et al., 2017). Co-precipitation of short-range ordered aluminosilicates also allows for substantial sorption of carbohydrates, and the protection of co-precipitated carbohydrates from enzyme depolymerization may contribute to their accumulation in soils (Lenhardt et al., 2022, 2023). The potential adsorption of low molecular weight compounds could mean that HIC fractions were not only decreased by microbial accessibility but may also have been adsorbed by the WTR mineral surfaces.

The greater potential to stabilize C in Al-WTR:AD could also be attributed other factors such as reduced mineralization as a result of a shift in microbial population composition influenced by changes in C/N ratio and vice versa (Jin et al., 2013; Jeewani et al., 2021). However, this measurement was beyond the scope of this study. Bacteria and fungi can also be affected by high metal concentrations (Rajapaksha et al., 2004), however, potential toxicity of metals such as the high Al concentrations in Al-WTR was unlikely since the pH range of the soils were above the pH 4.5 where Al is highly soluble (Table S2) (Li and Johnson, 2016). The pH also plays a crucial role in the sorption of OM on metal oxides by influencing the protonation of functional groups and the surface charge of clays and metal oxides (Kupka and Gruba, 2022; Wang and Kuzyakov, 2024). This phenomenon may account for the significant reduction in DOC observed in the pure sand solely treated with Al-WTR, which had an average pH value of 5.6. Co-application of WTRs and AD sludge also

decreased pH values from an average value of 6.9 to 6.6, however, pH effects in co-amendments treatments may have not been pronounced. According to Wang and Kuzyakov et al. (2024), pH values in the range 6–8 generally favour positive priming effects.

Ferric-based coagulants are known to be more effective in removing NOM than Al-based coagulants (Matilainen et al., 2005). Even in terrestrial environments, iron oxides play an important role in preserving organic C (Silva et al., 2015; Wan et al., 2019). The lower potential of Fe-WTR to stabilize C in this study can be attributed to at least two possible factors. Firstly, the knowledge that the Faure WTP treats water with high concentrations of humic acids resulting in a possibility that the Fe-WTR was already saturated with organic C at the time of collection from the WTP. Substrates with surfaces saturated with organic C have a lower potential to stabilize C from external inputs (Li and Evanylo, 2013). Secondly, ferrihydrite, defined as a poorly crystalline Fe oxide, can sequester a large amount of OM via sorption or co-precipitation. However, since it is thermodynamically unstable, transformation into more crystalline Fe oxides, such as hematite and goethite can happen and may affect the fate of OM associated with ferrihydrite. As a result, the transformation of ferrihydrite to hematite reduces the stability of OM on Fe oxides and causes the release of portions of mineral-associated OM back into solution (Liu et al., 2022). This phenomenon could explain the significantly higher amounts of DOC and POXC that were released into the solution with co-addition of Fe-WTR.

4.2. Implications of WTR and AD sludge co-addition to improve soil C storage in coarse textured soils

Understanding the mechanisms underlying SOC stability are important to predict the contribution of soil management systems to climate change (Ahn et al., 2009; Wan et al., 2019). This study shows the potential of Al-WTR co-amendment in C stabilization (defined as the low C outputs relative to the input amounts) when AD sludge is used as an amendment in a pure sand soil. The predominant mechanism of C stabilization was likely coagulation-adsorption and coprecipitation resulting in mineral-bound organic C. The stabilization of C through these mechanisms protects the labile C pools from microbial decay, suggesting accumulation and long-term soil C storage with the co-amendment of Al-WTR. Therefore, findings of this study broaden the scope of benefits with co-amendment of biosolids with WTR. Co-amendment will not only improve productivity of sands thus meeting the objectives of the Sustainable Development Goals of the United Nations (SGDs) such as addressing the end of hunger through sustainable agriculture (SGD 2). But also inform the potential buildup of soil C levels in a pure sand soil thus working towards the net zero goals and presents a strategy to combat climate change (SGD 13). In addition, adoption of the co-amendment of both waste streams will meet the aim of circular economy to reduce waste in the environment.

5. Conclusions

Addition of AD sludge, Al-WTR or Fe-WTR as sole amendments or co-applied increased C mineralization rates in the pure sand soil. At the end of 132-day incubation period, the cumulative CO_2–C emitted relative to the TOC was significantly decreased by co-amendment of AD with WTRs while the AD sludge alone had significantly higher CO_2–C emitted. The decrease was apparent in Al-WTR co-amendments translating to lower potential C losses when compared to sand amended with AD sludge alone or co-applied with Fe-WTR. Despite the lower mineralized C relative to TOC in Al-WTR, this treatment also had significantly lower DOC and POXC concentration compared to AD sludge, suggesting potential stabilization in the soil likely through adsorption and coprecipitation which result in mineral-bound organic C. The Fe-WTR had lower potential to stabilize C or reduce mineralization rates due to possible saturation of binding sites during the water treatment process. There is a need to explore Fe-WTRs from other WTP possibly treating a

variety of water sources of differing organic matter composition. Field experiments should be carried out to explore the full capacity of Al-WTRs in stabilizing C in the soil under natural conditions in the long term. Evidence of C storage and adoption of the practice at large scale will contribute towards the net zero agenda and circular economy through waste management.

CRediT authorship contribution statement

Noxolo S. Lukashe: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Wendy Stone:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization. **Ryan Pereira:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Sara Trojahn:** Investigation, Formal analysis. **Ailsa G. Hardie:** Writing – review & editing, Supervision, Methodology. **Karen L. Johnson:** Writing – review & editing, Investigation. **Catherine E. Clarke:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Wendy Stone reports financial support was provided by Water Research Commission. NS Lukashe reports financial support was provided by National Research Foundation. W Stone reports financial support was provided by DSI BIOGRIP. 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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2024.121904.

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