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Efficacy of bioadmendments in reducing the influence of salinity on the bioremediation of oil-contaminated soil

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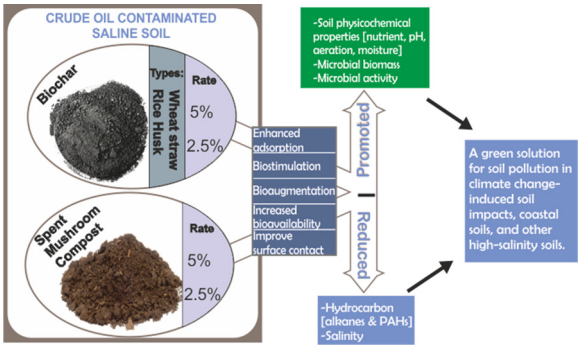
HIGHLIGHTS

- Biochar and spent mushroom compost enhance hydrocarbons degradation.
- Salinity effects on soil microbial community mitigated by bioamendments.
- Biochar promotes microbial activity in non-saline soils.
- Synergistic positive effect of Biochar and SMC for sustainable saline soil remediation.
- Shifts in microbial community structure indicate treatment effectiveness.

GRAPHICAL ABSTRACT



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ABSTRACT

This study aimed to investigate the potential of three bioamendments (rice husk biochar, wheat straw biochar, and spent mushroom compost) to enhance microbial degradation of crude oil in saline soil. A soil microcosm experiment was conducted, comparing the response of soil microorganisms to crude oil under saline (1 % NaCl) and non-saline conditions. The soils were amended with different bioamendments at varying concentrations (2.5 % or 5 %), and degradation rates were monitored over a 120-day period at 20 °C. The results showed that the bioamendments significantly influenced the degradation of total petroleum hydrocarbons (TPH) in both non-saline and saline soils by 67 % and 18 % respectively. Non-saline soils exhibited approximately four times higher TPH biodegradation compared to saline soils. Among the bioamendments, rice husk biochar and spent mushroom compost had the greatest impact on biodegradation in saline soil, while wheat straw and rice husk biochar combined with spent mushroom compost showed the most significant effects in non-saline soil. The study also revealed that the bioamendments facilitated changes in the microbial community structure, particularly in the treatments with rice husk biochar and wheat straw biochar. Actinomycetes and fungi were found to be more tolerant to soil salinity, especially in the treatments with rice husk biochar and wheat straw biochar. Additionally, the production of CO_2, indicating microbial activity, was highest (56 % and 60 %) in the treatments combining rice husk biochar or wheat straw biochar with spent mushroom compost in non-saline soil, while in saline soil rice husk biochar treatment (50 %) was the highest. Overall, this research demonstrates that the application of bioamendments, particularly rice husk biochar and wheat straw biochar combined with spent mushroom compost, can effectively enhance the biodegradation of crude oil in saline soil. These findings highlight the potential of such bioamendments as green and sustainable solutions for soil pollution, especially in the context of climate change-induced impacts on high-salinity soils, including coastal soils.

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1. Introduction

As the global energy transition progresses, the dominance of oil and gas in the world's energy mix persists, with coal, crude oil, and natural gas accounting for a significant portion of the EU's energy production in 2020. Consequently, crude oil exploration continues, resulting in potential soil contamination and the need for soil clean-up in affected environments (Wu et al., 2022).

Bioremediation, despite its limitations such as site specificity and longer treatment time, holds great promise as a low-cost and environmentally safe approach for remediating petroleum-contaminated soils (Vasilyeva et al., 2022). However, climate change poses additional challenges to bioremediation processes. Rising temperatures and increased evapotranspiration can lead to soil salinity, particularly in coastal areas. This salinity, combined with crude oil spills, can alter microbial function and impact the effectiveness of bioremediation (Khamidov et al., 2022). Salinity has a significant influence on the soil microbial community responsible for driving bioremediation processes. It increases osmotic stress and the accumulation of toxic ions, affecting the microbial degradation of contaminants (Yan et al., 2015). Furthermore, salinity can alter the physicochemical properties of contaminants, limiting their bioavailability and the ability of bioremediation organisms to utilise them as substrates (Kumar et al., 2022). Nutrient cycling and availability can also be disrupted by high salinity, further hindering effective bioremediation (Mazhar et al., 2022). To address these challenges, a bioremediation strategy that promotes petroleum hydrocarbon remediation by reducing the effect of salinity on soil microbial degraders is required. Biochar and spent mushroom compost have shown potential for use in saline soil bioremediation due to their contaminant adsorption properties and ability to enhance soil microbial activity (Alhujaily et al., 2018; Guo et al., 2020).

Biochar, a carbon-rich product produced by the thermal decomposition of organic material possesses a number of remarkable properties including high specific surface area, microporosity, and hydrophobicity, which has been exploited for various applications including environmental remediation (Guo et al., 2015; Oliveira et al., 2017; Guo et al., 2020). Spent mushroom compost (SMC), is a by-product of mushroom production, which contain high organic matter, diverse groups of microorganisms and extracellular enzymes important for the biotransformation of contaminants (Gouma et al., 2014). These two materials are product of agricultural waste industry, hence their sustainability, efficiency, and cost-effectiveness (Ahmad et al., 2014; Cipullo et al., 2019). The use of biochar and SMC may be a strategy for an opportunity to overcome soil nutrient limitation, increase sorption/decrease bioavailability of the chemicals (e.g., alkanes and PAHs, higher sodium ion concentration), and increase surface contact of contaminants with the soil microbial community.

Previous studies have demonstrated the efficacy of biochar and spent mushroom compost in petroleum-contaminated soil remediation. For example, the use of maise straw biochar yielded 60 % TPH degradation after 4 months (Wang et al., 2020). Biochar (5 % *w*/w) in combination with nutrients (C:N:P:K), and biosurfactant (Rhamnolipid) removed 23 % TPH after 110 days of landfarming (Okoro et al., 2017). The content of TPH was reduced below 5 g/kg by 2 % pine chips biochar in forest grey soil after two warm seasons (Vasilyeva et al., 2022). Petroleum hydrocarbons degradation was accelerated (54.2 %) after 10 weeks of incubation with microbial consortium immobilised on biochar (Li et al., 2019). Woodchip Biochar improves the TPH phytoremediation of white clover plant by 68 % after 62 days (Yousaf et al., 2022). Similarly, spent mushroom compost has been investigated for its ability to improve soil nutrient content and reduce petroleum hydrocarbon contamination (García-Delgado et al., 2013; Asemoloye et al., 2017; Mohammadi-Sichani et al., 2017, 2019).

However, the exact mechanisms underlying the adsorptive effects of biochar and spent mushroom compost on petroleum soil contamination risk reduction are not fully understood, and the effectiveness can vary based on application rate, feedstock type, and production conditions. Therefore, it is crucial to investigate their effects on soil microbial communities during petroleum hydrocarbon remediation in saline soils. In this study, we hypothesize that rice husk biochar, wheat straw biochar, and spent mushroom compost will enhance petroleum hydrocarbon reduction and increase the abundance and activity of microbial communities in both non-saline and saline soils. By assessing the fate and behaviour of hydrocarbons (alkanes and PAHs) and studying the influence of these amendments on microbial communities, we aim to shed light on their potential for effective remediation in saline soil environments.

2. Materials and methods

2.1. Sample collection: Soil, biochar and spent mushroom compost

Subsurface soil from soil heaps was collected from a construction site (52°04′03.2″N 0°37′40.1″W) at Cranfield University, United Kingdom and was air dried, homogenised, and sieved (2 mm). Rice Husk Biochar and Wheat Straw Biochar were produced in a pilot-scale rotary kiln pyrolysis unit with a nominal peak temperature of 550 °C, a pH of 9.94, and a total carbon content of 68.26 wt% (d.b) (UK Biochar Research Centre, UK). Both are biochars that have been thoroughly characterised (UK Biochar Research Centre, 2014). Spent mushroom compost was produced following white button mushroom *Agaricus bisporus* production (Littleport Mushrooms LLP-Gs Fresh Ltd., UK).

The crude oil was obtained from Shell Gas Direct Ltd., London, UK. It is a Crude Oil Sweet with <0.5 % Sulphur. It is a raw petroleum extracted in its natural state from the ground and containing predominantly aliphatic, alicyclic, and aromatic hydrocarbons, as well as small amounts of nitrogen, oxygen, and sulphur compounds, specifically, n-hexane 0–5 %, toluene 0–4 %, cyclohexane 0–3 %, benzene 0–2 %, ethylbenzene 0–1 %, cumene 0–1 %, naphthalene 0–0.5 %, hydrogen sulfide 0–0.01 %. Sodium chloride (NaCl) (Sigma-Aldrich) was used in the study because it is the most abundant salt in most saline soils and sea water (Singh et al., 2021).

2.2. Microcosms experimental design

The sieved soil was spiked with crude oil at 87579 mg/kg (10 % *w*/w spike). Half of the soil was then spiked with 1 % NaCl (*w*/w) to provide a salt stress. From the spiked soil, 250 g was then placed in pots (8X10cm), and then amended with either rice husk biochar (RHB), wheat straw biochar (WSB), or spent mushroom compost (SMC) at rates of 2.5 % or 5 % with the following conditions: soil +5 % RHB, Soil +5 % WSB, Soil +5 % SMC, Soil +2.5 % RHB + 2.5 % SMC, Soil +2.5 % WSB + 2.5 % SMC, and Soil (unamended). All conditions were done in triplicate, The 5 % biochar to soil ratio used in this work was chosen because it is frequently reported as the most effective application rate for reducing mobile contaminant concentrations in contaminated soils (Wang et al., 2017; Novak et al., 2018; Cipullo et al., 2019). All the microcosms were manually mixed to ensure homogeneity and incubated in 20 °C constant temperature room for the 120 days. The soil moisture was adjusted twice a week by adding deionised water equivalent of the microcosms' weight loss within the range of the soil moisture content at the onset of the experiment. Soil was sampled for chemical, and microbiological analyses after 10, 60 and 120 days.

2.3. Physico-chemical characterisations

Air-dried soil samples were analysed based on BS EN 13654–2:2001 and BS 7755 Section 3.8:1995 for Total nitrogen (TN) (0.001 mg), Total Carbon (TC) (0.001 mg) and Total Organic Carbon (TOC: following the removal of carbonates with 4 mol/L hydrochloric acid dropwise until visible reaction stops) with Vario EL 3 Element Analyzer (Elementar Analysensysteme GmbH, DE). Total phosphorous was determined by extracting with acid mixture (6 mL 11.65 mol/L hydrochloric and 2 mL 15.8 mol/L nitric) and determining the phosphorus content of the extract (ISO 11047, 1998) using a NexION ® 350 D ICP-MS (Perkin Elmer). Available phosphorous (AP) (5 g) was extracted from the soil with a 0.5 mol/L

sodium hydrogen carbonate solution at pH 8.5, the extract was then analysed by spectrometry (ISO 11263, 1994). Soil pH was determined according to ISO 10390 (2005) using a soil:water ratio of 1:5 (Jenway 3540 pH Meter, Keison Products, UK). The organic content of the soil (%) was calculated using loss of ignition (LOI): (BS EN 13039, 2000). The particle size distribution was determined using the sieving and sedimentation method according to BS ISO 11277:2009, and the following soil texture classes were calculated using a soil texture calculator (Natural England Technical Information Note TIN037, 2008) and eventual sieving using 0.6 mm, 0.212 mm, and 0.063 mm sieves. Drying at 105 °C was used to determine gravimetric soil moisture and dry matter (%). (ISO 11465, 1993).

2.4. Chemical analyses – Total hydrocarbon

Total petroleum hydrocarbons, comprising both aliphatic and aromatic components, were determined using a variant of the method given by Risdon et al. (2008). Soil extraction was done by taking 2.5 g of soil and combining it with 15 mL volume of 1:1 dichloromethane:hexane. The samples were thereafter sonicated for 20 min at room temperature (Ultrasonic Bath, U2500H, Ultrawave (UW), UK) then shaken for 16 h at 150 r p m (Multi-Reax Shaker, Heidolph Instruments GmbH & CO. KG). On the second day, samples were sonicated for 20 min (room temperature) before centrifuging (2000 g for 10 min) (Thermo ScientificTM, SorvallTM ST 40 Centrifuge Series). Following that, the supernatant was transferred to 6 mL SPE DSC-Si silica tubes for cleaning. A 0.5 mL sample was taken from the 10 mL and combined with 0.5 mL of internal standards, which included a deuterated alkane mix ($C10^{d22}$, $C19^{d40}$ and $C30^{d62}$) and deuterated polycyclic aromatic hydrocarbons (PAH) mix (naphthalene^{d8}, anthracene^{d10}, chrysene^{d12} and perylene^{d12}).

Gas chromatography-mass spectrometry (GC–MS) was used to identify and measure the concentration of petroleum hydrocarbons using an Agilent gas chromatograph connected to a Turbomass Gold mass spectrometer operating in positive ion mode at 70 eV. As described in Cipullo et al. (2019), a split-less injection was used with a sample volume of 1 μL. For a total run time of 38 min, the oven temperature was raised from 60 °C to 220 °C at a rate of 20 °C per minute, then to 310 °C at a rate of 6 °C per minute, and kept at this temperature for 15 min. For the quantitative measurement of the target aliphatic and aromatic hydrocarbons, the mass spectrometer was run in full scan mode (*m/z* range 50–500). Quantification for each compound was carried out by integrating the peak at a particular m/z. External multi-level calibrations were performed using alkane (C8-C40) and PAH (EPA 525 PAH Mix A) standard solutions (Sigma Aldrich, Dorset, UK) with concentrations ranging from 1 to 5 μg/mL. Blank controls were analysed every 18 samples for quality control.

2.5. Microbiological analysis

2.5.1. Respiration

MicroResp™ colorimetric microplate-based respiration system for measuring CO_2 evolved from soil which water or carbon substrates have been added is based on Campbell et al. (2003a). This method gives responses of microbial decomposition and conversion of the substrates, and the activity are reflected by measuring CO_2 production after 6 h. The method in brief: The detection plates – microplate plates with purified agar and indicator solution (cresol red, KCl, $NaHCO_3$) are added in a 1:2 ratio – were prepared and stored in sealed desiccator prior to use to avoid absorbing CO_2 from the environment. In the deep well plates, 0.32 g of soil samples and 93.6 mg/mL substrates solution were added into it. Detection plate were read at 570 nm (Microplate readers, SpectraMax® Plus384, Molecular Devices) and assembled onto the deep well plate with the MicroResp™ seal, secured in metal clamp and incubate at 25 °C for 6 h and re-reading the Detection plate at 570 nm. Substrates (alanine, citric acid, glucose, gamma-aminobutyric acid, α-ketoglutaric acid, malic acid) were selected considering the Creamer et al. (2016); lignin was added as a complex carbon source based on availability in the lab. The soils microbe's utilisation of these substrates was assessed as cumulative CO_2 production in a MicroResp™ system (Campbell et al., 2003b). The basal respiration rate was calculated using the CO_2 generated by the wells in which water other than substrates were added.

2.5.2. Phospholipid fatty acid analysis (PLFA)

Using Phospholipid fatty acid (PLFA) analysis based on Frostegard et al. (1993), the microbial community structure was examined. In brief, from the freeze dried (Christ Alpha 1–2 LD plus −80 °C Freeze Dryer) soil samples, solid-phase soil extraction using 10 g of each sample was performed using Bligh and Dyer solution (chloroform, methanol, and citrate buffer in 1:2:0.8 by volume). The extract was further derivatised by mild alkaline methanolysis. By using a GC-FID (Agilent Technologies 6890 N) equipped with an HP-5 (Agilent Technologies) fused silica capillary column (30 m length, 0.32 mm ID, 0.25 m film), fatty acid methyl esters were analysed. GC conditions were as described by Pawlett et al. (2013). The target responses of all discovered PLFA peaks were sum up to determine the relative abundance of each unique PLFA, which was reported as a percentage (mol%).

2.6. Data analysis

Data analysis was done on each amendment at the 10-, 60-, and 120-day time points. This include ANOVA test (Repeated measures), which was used to determine the significance and relationship between soil amendment [rice husk biochar (RHB), wheat straw biochar (WSB), spent mushroom compost (SMC), RHB + SMC, WSB + SMC, or un-amended] and incubation time on the alkanes, PAHS, and microbial PLFA profiles and respirations datasets. Principal Component Analysis was used for multi-variate datasets, to evaluate the variations between soil amendment and incubation time on microbial community dynamics and respiration profiles from multiple substrates induced respiration. Both Repeated-measures ANOVA and PCA were performed using Statistica® version 14.0.0.15. Pearson correlation was used to establish correlation between the Total petroleum hydrocarbon with PLFA profile and microbial soil activity dataset using SPSS (IBM SPSS Statistics for Windows, Version 21.0).

3. Result and discussion

3.1. Soil sample and physicochemical properties

The initial soil analysis revealed total petroleum hydrocarbon (TPH) concentrations of 5871 mg/kg and 3471 mg/kg, representing 7 % and 4 % recovery in non-saline and saline soils, respectively, at the 10-day baseline of the study. Over the course of 120 days, the study investigated the influence of bioamendments on the fate and behaviour of TPH, as well as the structure and function of the microbial community.

The distribution of different TPH fractions showed higher levels of lower molecular weight alkanes, with decane (C10) being the least abundant (Fig. 1a&b). The aliphatic compounds constituted a significant portion of the TPH, accounting for 96 % in both non-saline and saline soils. This distribution pattern is consistent with recent crude oil spill sites where paraffins (alkanes) are the predominant hydrocarbon constituents (Truskewycz et al., 2019). The aromatics, on the other hand, exhibited a more even distribution of fractions (Fig. 1c and d), with higher occurrences of specific compounds such as phenanthrene, chrysene, and indeno[1,2,3-cd] pyrene in the non-saline soils, and benzo[k] fluoranthene, benzo[b] fluoranthene, and chrysene in the saline soil. This observation reflects the composition of the crude oil spike used in the study, as well as the characteristics typically observed in recent oil spill incidents.

Table 1 presents the properties of the non-saline and saline soils, including moisture content (29 % and 30 %), organic matter (93 % and 92 %), and pH (8.5 and 8.2), respectively. These soil property levels align with those reported to support optimal soil function (Griffiths et al., 2018).

Although the recorded CNP ratio of 100:2:0.04 does not reflect the typical nutrient proportion found in agricultural soils for optimal soil function, it has been observed that low nutrient levels, as indicated by a low CNP ratio, can lead to an increase in PAH microbial degraders (Singh et al.,

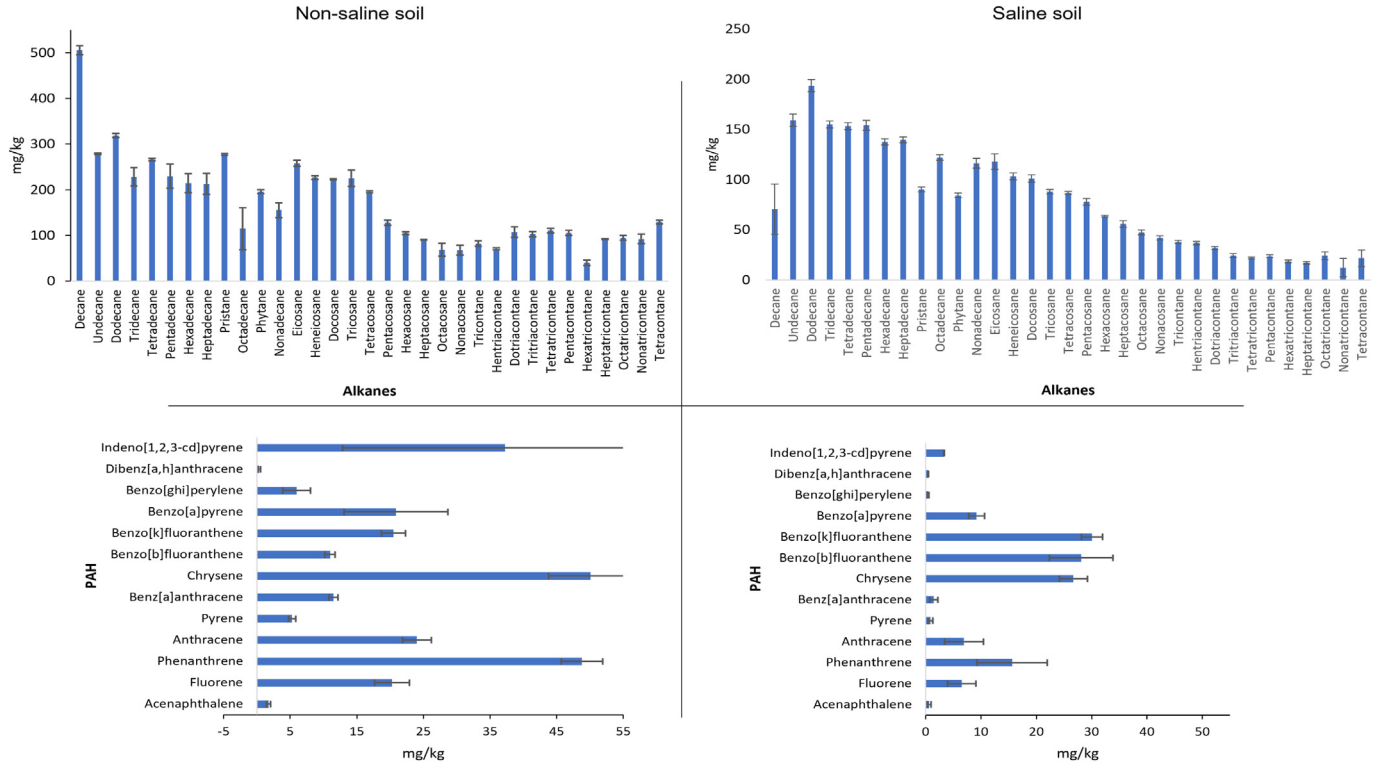

Fig. 1. Total petroleum hydrocarbons (TPH) profile of the non-saline and saline soils arranged in order of increasing carbon number and molecular weight.

2014). This may be attributed to an increase in microbial activity due to environmental stress induced by the presence of contaminants.

Furthermore, the available inorganic phosphorus, which is known to promote microbial activities according to Zheng et al. (2019), appeared to be reduced in these soils, with values of 6.19 mg/kg in non-saline soil and 5.63 mg/kg in saline soil. Soils with pH levels above 8.0 are typically associated with low soil phosphorus content (Griffiths et al., 2018) because phosphorus becomes complexed and less bioavailable at higher pH levels.

The high organic matter content in the soil, primarily attributed to carbon, provides certain advantages such as enhancing water-holding capacity, cation exchange capacity, and improving the structural stability of clay soils by promoting particle consolidation into aggregates (FAO, 2022). This condition creates a favourable environment for improved microbial activities involved in hydrocarbon degradation processes.

3.2. Fate of hydrocarbons – Alkanes and PAHs

The impact of the bioadmendment on crude oil contamination in the non-saline and saline soils was assessed, focusing on aliphatic (alkanes) and aromatic (PAHs) hydrocarbons (Figs. 2 and 3). The initial concentrations of aliphatic hydrocarbons (alkanes) were 5612.9 ± 308 mg/kg in non-saline soil and 3340.8 ± 105 mg/kg in saline soil, and after 120 days of incubation, there was an average degradation of 73 % and 19 % in the respective treatments compared to the control. The most effective treatments were RHB-SMC (15 %) in non-saline soil and SMC (16 %) in saline soil. Although the degradation of alkanes induced by the bioadmendment did not show a significant difference ($p = 0.3331$) between non-saline and saline soils, the presence of salinity in the saline soils had a notable impact on alkane degradation. Salinity can affect soil pH, ion exchange capacity, soil organic matter, and microbial biomass abundance, all of which influence the ability of the microbial community to degrade hydrocarbons in the soil (Zhang et al., 2019). Previous studies have reported higher levels of alkane degradation under saline conditions, including the use of halophilic bacteria strains and indigenous halotolerant hydrocarbon degraders (SadrAzodi et al., 2019; Akbari et al., 2021). Therefore, the observed alkane degradation in this study is likely attributed to the selection of halotolerant strains that are capable of alkane degradation.

However, the impact of salinity on the degradation of PAHs (Fig. 3) was less pronounced, especially in the two biochar treatments (RHB and WSB). After 120 days of incubation, the average degradation of PAHs in the non-saline and saline soil treatments was 61 % and 39 %, respectively, compared to the control. The most effective treatments were WSB-SMC (27 %) in non-saline soil and RHB (49 %) in saline soil, with the higher percentage attributed to the two biochar treatments. Biochars can stabilize ions and reduce their bioavailability through enhanced sorption, which may explain their capacity to mitigate the effects of salinity. This is achieved through biochar's electrostatic attraction, ion exchange capacity, and surface complexation (Guo et al., 2020). Previous studies have shown higher PAH degradation rates under saline conditions when using biochar-immobilised bacteria or combining biochar with halotolerant microbial degraders (Song et al., 2021; Cui et al., 2023). These approaches address the reduced microbial activity caused by salinity-induced osmotic stress and toxic ions in the soil (Yan et al., 2015). Bioaugmentation with halotolerant microbial degraders has been employed in other studies to tackle this issue (Ebadi et al., 2017; Qu et al., 2022; Wang et al., 2022).

Table 1
Physiochemical characteristics of the crude oil spiked non-saline and saline soil.

Characteristics	Analysis	Non-saline soil	Saline soil
Elements	Total C (%)	6.11	6.39
	Total N (%)	0.11	0.14
	Total P (%)	0.07	0.07
	C:N:P	100:2:0.04	100:2:0.04
	C:N	54.54	44.64
	Total Organic C (%)	4	4
	Total P (mg/kg)	24.77	22.54
	Available phosphorus (mg/kg)	6.19	5.63
Physical properties	Dry matter content (%)	71	70
	Water content (%)	29	30
Chemical properties	pH	8.5	8.2
	Loss on ignition (%)	93	92

N: nitrogen, C: carbon, P: phosphorus.

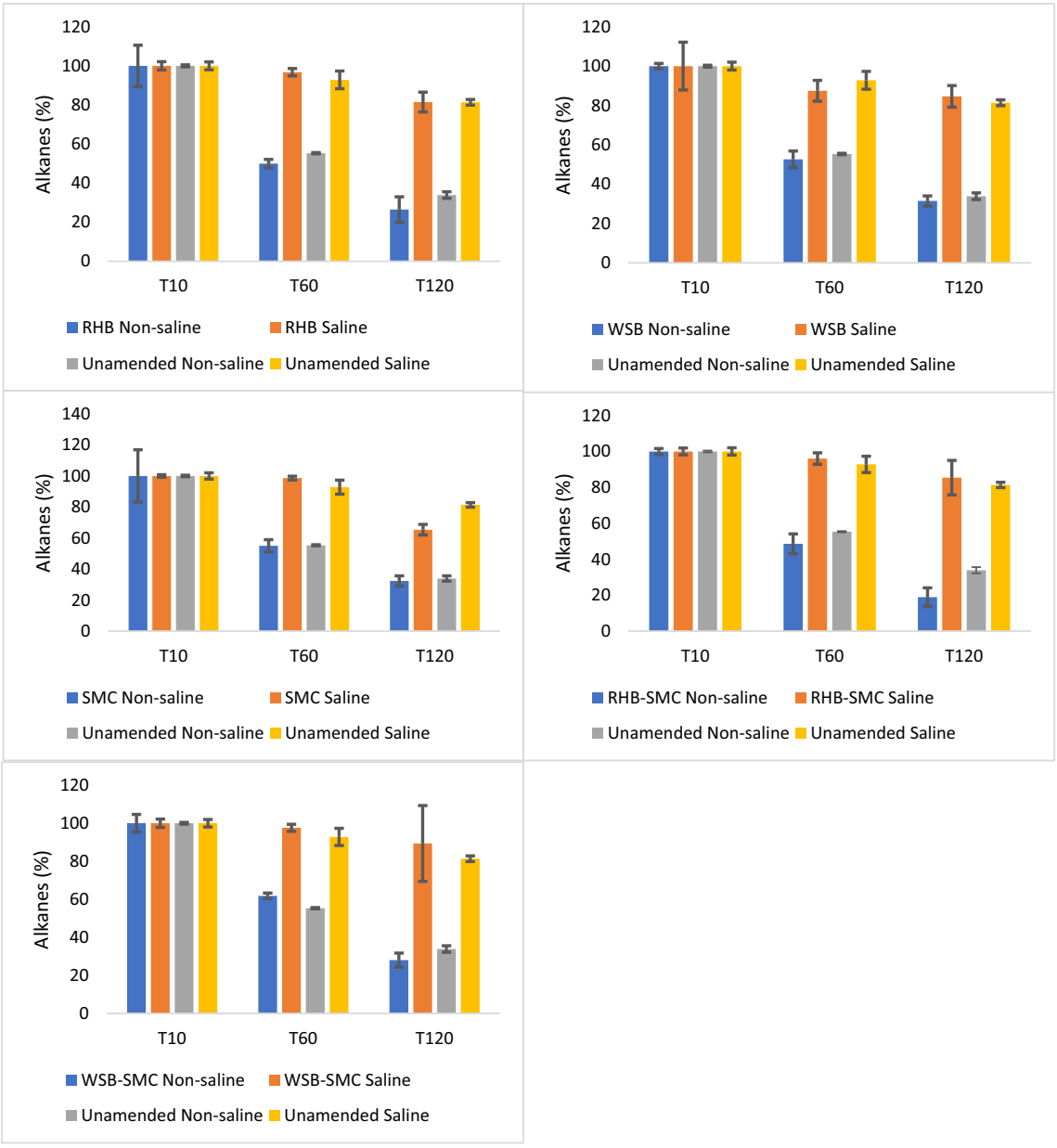

Fig. 2. Percentage degradation of total alkanes over time in the various soil treatments.

It is important to acknowledge that the studies referenced to support higher degradation rates of alkanes and PAHs in saline soils employed various additional factors that contributed to their results. These factors included the presence of low concentrations of hydrocarbons in the soil (Wei et al., 2020; Cui et al., 2023), the isolation and selection of competent halotolerant bacterial strains capable of degradation (SadrAzodi et al., 2019; Guo et al., 2022), the immobilisation of bacteria on biochar (Song et al., 2021; Guo et al., 2022), and the use of a single hydrocarbon contaminant (Cui et al., 2023). These studies required additional material and energy inputs, which may compromise their status as sustainable and environmentally friendly green technologies.

In contrast, our study utilised a combination of biochar and SMC, a low-cost and easily accessible waste material, offering several advantages. Biochar possesses properties such as high surface adsorption, chemical precipitation, and a high ash content, while spent mushroom compost (SMC) is rich in organic matter, enzymes, and a diverse microbial community. These properties facilitate the contact between hydrocarbons and contaminants, reduce the bioavailability of hydrocarbons and sodium chloride ions, thereby mitigating their toxicity to the microbial community, and address nutrient limitations for microbial growth.

By combining biochar and SMC, a synergistic effect is achieved, leading to more efficient remediation of crude oil in soils. The mixed treatments, specifically RHB-SMC and WSB-SMC, demonstrated excellent performance in non-saline soils for both alkanes and PAHs, serving as a compelling example of the effectiveness of this approach.

3.3. The influence of the bioamendments on the microbial community in the soils

3.3.1. Effects on community dynamics

Evidence of the impact of crude oil and treatments was observed from the beginning and throughout the treatment period, as indicated by the noticeable changes in the structure of microbial communities in the treatments (Fig. 4). The influence of crude oil contamination on the microbial community differed between non-saline and saline soils, leading to community shifts at different time points (10–60 days) in each soil type. This observation aligns with the findings of Steven and Martiny (2008), who

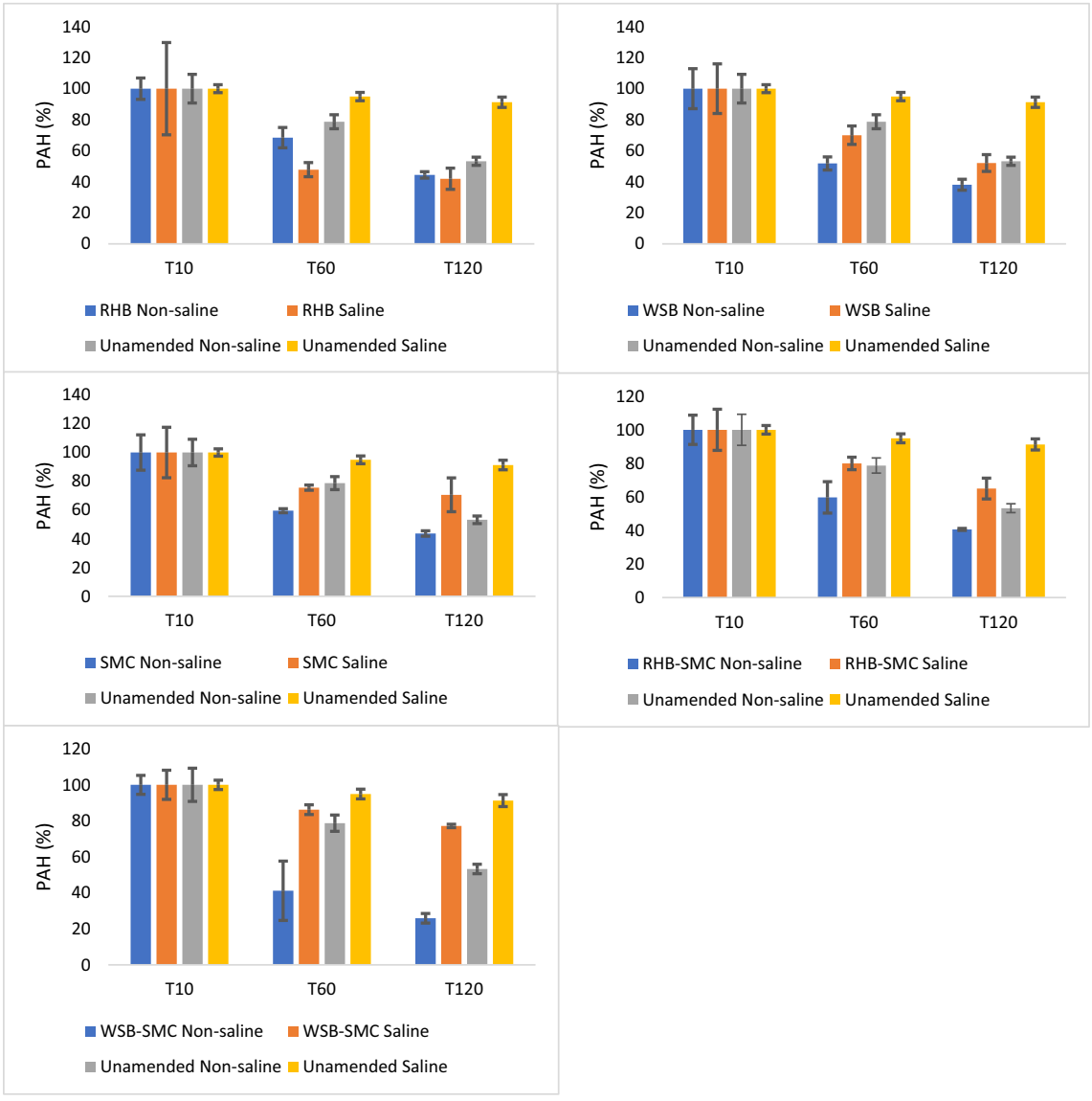

Fig. 3. Percentage degradation of total PAHs over time in the various soil treatments.

highlighted the sensitivity of microbial communities to population disturbances. Such changes in microbial communities are often associated with alterations in ecosystem processes, although in rare cases, functional redundancy may occur where the modified community continues to perform its intended functions. In some instances, relative stability in the communities can be observed, indicating the resistance of the microbial community to ecosystem disturbances (Shade et al., 2012).

Significant treatment and time effects ($p = 0.0001$; 0.0012) on the microbial community were observed, and these effects were different in non-saline and saline soils. In non-saline soils, an initial increase in community diversity was observed at day 10, followed by a reduction in diversity over time and convergence of the community profile towards day 120 (Fig. 4). Conversely, in saline soils, the soil microbial communities were relatively similar at the onset of the experiment and diverge towards the end of the experiment.

The behaviour of the microbial communities in the saline soil can be attributed to the impact of salinity, which generally leads to reduced microbial activity due to osmotic stress and potential toxicity of ions. Microbes can adapt to such environments by synthesizing osmolytes, which help maintain cell turgor and metabolism (Yan et al., 2015). Additionally, the modifications of soil properties, the influence on the bioavailability of hydrocarbons and sodium chloride, and the provision of nutrients resulting from the application of biochar and spent mushroom compost (SMC) may have contributed to the resolution of these communities.

The reduction in spread and the formation of clusters observed in the microbial communities as the treatment progressed indicate a simplification of the soil complexity, primarily driven by the degradation of hydrocarbons and moderation of salinity through the applied treatments. The use of biochar and SMC successfully influenced the microbial communities, leading to the remediation of contaminants. Previous studies have shown that these materials can impact microbial communities in contaminated soil through three main mechanisms: (1) effects on the soil environment, including improvements in soil structure, water retention, aeration, nutrient availability, pH, and electrical conductivity (Hu et al., 2021); (2) effects on contaminants, such as immobilisation through surface sorption and complex formation by biochar, as well as the provision of microbial shelter, changes in enzyme activity, and facilitation of electron transfer (Zhu et al., 2017); and (3) effects of spent mushroom compost, including sorption of contaminants, provision of enzymes, and serving as a rich source of microorganisms and organic matter to enhance microbial activity in the soil (Dabrowska et al., 2021; Sun et al., 2021).

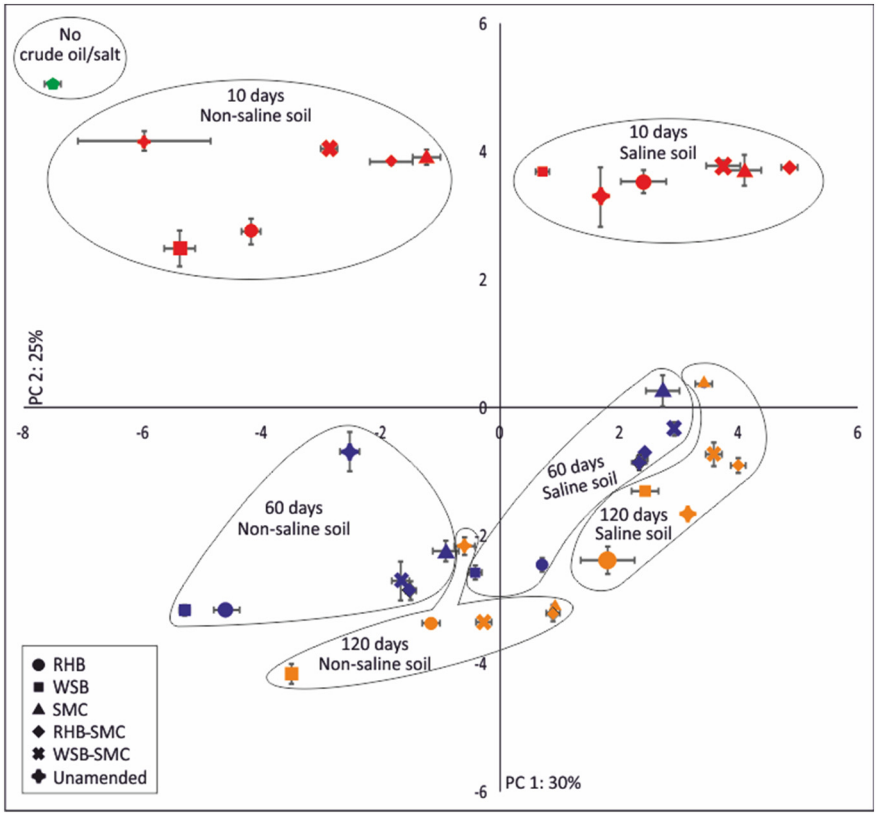

Fig. 4. Changes in the composition of the microbial communities based on PLFA in both non-saline and saline soils over the course of incubation and different treatments. The error bars represent the standard error for each treatment's replicates. RHB: rice husk biochar; WSB: Wheat straw biochar; SMC: Spent mushroom compost.

Moreover, the clustering of some saline soils with non-saline soils at 60 and 120 days indicates the effectiveness of the treatments in mitigating the impact of soil salinity, particularly in the case of biochar treatments (RHB and WSB) in saline soils. Biochar's ability to improve soil characteristics, such as enhancing cation exchange capacity, water holding capacity, and air porosity (de Vasconcelos, 2020), may have influenced the salinity in the soil by altering the distribution of salt ions. Previous studies have demonstrated that adding 0.4 % biochar to soil can reduce salt ion concentration by 26 % (Xiao and Meng, 2020), and a 5 % biochar application can alleviate salt stress and oxidative damage (Huang et al., 2019).

Overall, the use of bioamendments to mitigate the effects of salinity, induce microbial adaptation, and restore ecological function has shown a certain level of effectiveness. It is well-established that microbial communities in saline soils undergo changes due to a combination of factors such as salt tolerance, alterations in soil pH and structure, and resource competition (Gamalero et al., 2020). The structure and interactions within microbial communities are modified to enhance the adaptability of microorganisms to salinity stress (Wang et al., 2019). Previous reports have also highlighted the influence of biochar on reducing soil electrical conductivity (EC) values by 27 %, as well as decreasing the concentrations of Na^+ and Cl^- by 13 % and 15 %, respectively. And high-throughput sequencing analysis has revealed that organic amendments significantly enhance the richness of soil bacterial communities and increase the relative abundances of beneficial salt-tolerant bacterial genera like Flavobacterium, Bacillus, and Arthrobacter by 32 %, 39 %, and 36 %, respectively (Mao et al., 2022).

3.3.2. Effects on the microbial groups' relative abundance

The introduction of salt and crude oil into the soil environment had an impact on the microbial community composition, leading to changes in the microbial population (Fig. 5). In both non-saline and saline soils, the abundance of Gram-positive bacteria increased by a relatively smaller percentage (0.5 % and 3 % respectively), with no significant difference ($p = 0.4413$) between the Gram-positive bacteria groups in the two soil types.

The abundance of Gram-negative bacteria steadily decreased to an average of 19 % and 21 % in non-saline and saline soils, respectively. The biochar treatments (RHB and WSB) showed the highest abundance in both soil types. Salinity posed a limitation for these microbial groups, and there was a significant difference ($p = 0.0001$) between the Gram-negative groups in non-saline and saline soils. Although Gram-negative bacteria are associated with petroleum hydrocarbon degradation, their numbers may decline once the Gram-negative catabolic process is completed, allowing other groups to dominate (Xu et al., 2018).

The actinomycetes microbial group was more abundant in saline soil treatments. However, the increase in abundance over time was greater in non-saline soils, particularly in RHB (5 %), SMC (30 %), RHB-SMC (46 %), and WSB-SMC (8 %). Only the WSB treatment showed an increase (16 %) in saline soil. There was a significant difference ($p = 0.0001$) between non-saline and saline soils, with salinity promoting a 57 % average increase in Actinomycetes growth compared to non-saline soil. The highest abundance was observed in saline soils amended with RHB and WSB, indicating that biochar promoted the growth of actinomycetes more than other treatments.

For the fungal groups, the non-saline and saline soil treatments showed increases of 130 % and 55 % respectively. The SMC, RHB-SMC, and WSB-SMC treatments had the highest abundance in both soil types. Significant differences ($p = 0.00633$) were observed between non-saline and saline soils once again, with salinity not significantly limiting the fungal populations. The presence of a diverse fungal community in the spent mushroom compost contributed to increased fungal occurrence in the SMC and other SMC-biochar treatments.

While this study did not provide detailed information on the specific genera and species of observed hydrocarbon degraders, an increase in

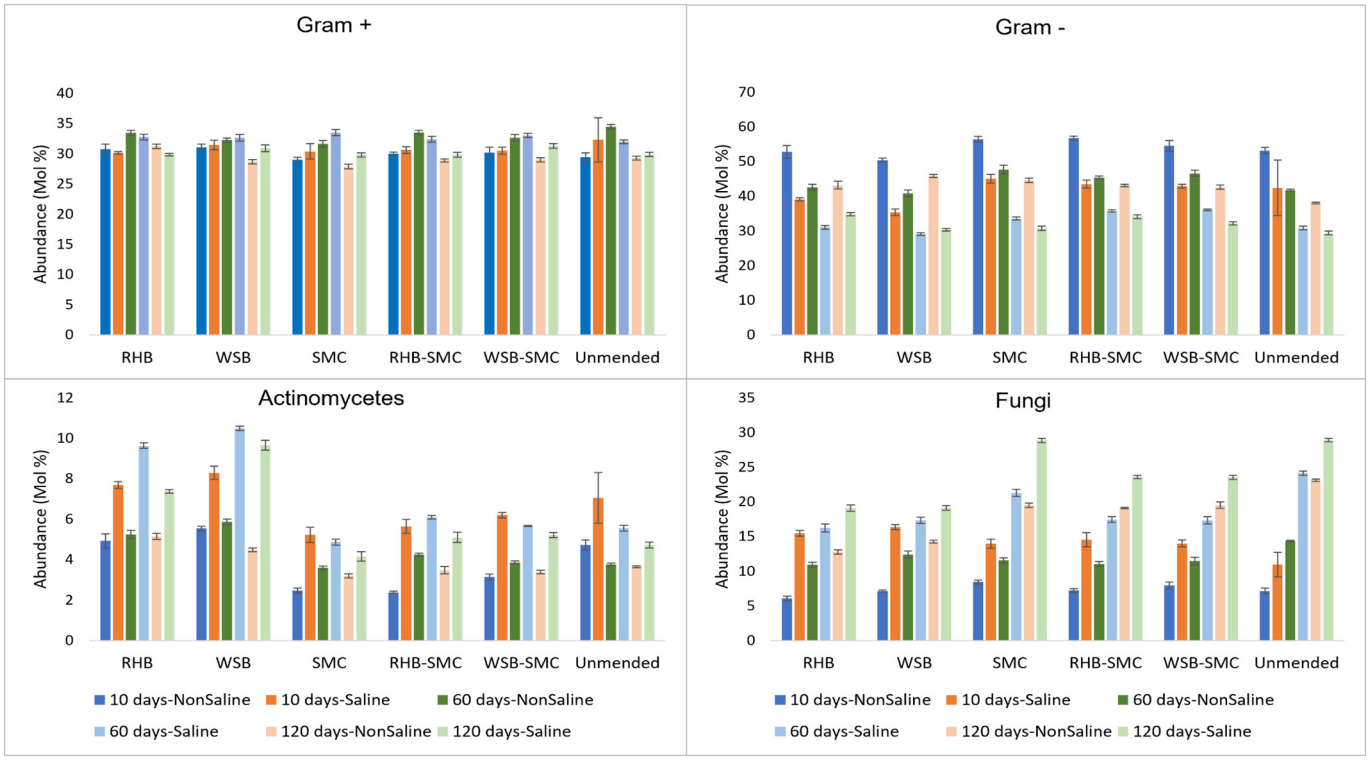

Fig. 5. The influence of bioamendments on the microbial community demonstrated by the relative (Rel.) abundance of microbial groups in all treatments and their changes over 10, 60, and 120 days in the non-saline and saline soils. The error bars represent the standard error for each treatment's replicates.

the abundance of certain microbial groups, such as actinomycetes, Gram-negative bacteria, and fungi, was observed. Previous studies have correlated these groups with hydrocarbons reduction (Al-Hawash et al., 2018; Cipullo et al., 2019; Wai et al., 2020). The bioadmendment treatments in this study facilitated positive changes in the populations of these microbial groups. Actinomycetes and fungi showed higher tolerance to soil salinity, and the addition of rice husk biochar (RHB) and wheat straw biochar (WSB) seemed to enhance their adaptability. The effects of the added substrate outweighed the impact of salinity, as microbial activity, and biomass, particularly fungi, increased significantly after rice straw addition (Wichern et al., 2020). Salinity may either select for halotolerant species or suppress microbial abundance (Akbari et al., 2021). Other studies have also observed an increase in the richness of fungal genera in saline soil amended microcosms (Sajid et al., 2023). The bioamendments in this study appeared to mitigate the effects of salinity, resulting in the success of microbial communities, as evidenced by the increased abundances of fungi, actinomycetes, and Gram-negative bacteria.

3.4. The influence of bioamendments on the non-saline and saline soil microbial activities

3.4.1. Microbial function (basal respiration rate)

Carbon dioxide (CO_2) production and changes in microbial communities are both useful indicators of degradation rates (Chi and Hieu, 2017). The effects of the treatments were observed in both non-saline and saline soils through the measurable increase in CO2 production (Fig. 6), indicating that the characteristics of biochar and spent mushroom compost influenced soil microbial activities in the presence of crude oil contamination and soil salinity. Previous studies have reported that bioamendments, such as biochar and compost substrates, can enhance soil function, including respiration (Yazdanpanah et al., 2016; Cipullo et al., 2019).

In non-saline soil, the amount of CO_2 produced generally decreased over time from 10 to 120 days in most treatments, including RHB, WSB, and SMC, indicating a decline in microbial activity. However, the mixed treatments (RHB-SMC and WSB-SMC) showed 56 % and 60 % more CO_2 production than the control at 60 days, respectively, before declining towards 120 days. This suggests that adding SMC to RHB-SMC and WSB-

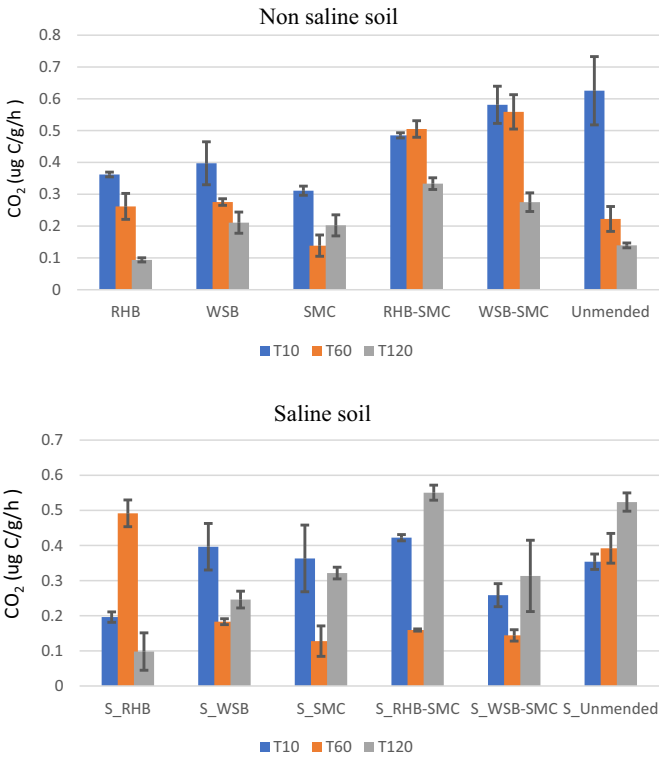

Fig. 6. Soil respiration expressed as CO_2 production (μg CO_2/g soil) for each treatment at day 10, 60, and 120. The error bars represent the standard error for each treatment's replicates.

SMC may have enhanced microbial potential, resulting in increased CO2 production. The decrease in soil respiration in the other treatments could be attributed to a reduction in carbon availability as degradation progressed. Biochar is known for its ability to sequester carbon in soil for extended periods, potentially reducing CO_2 emissions to the atmosphere (Guo et al., 2015) by locking up carbon and preventing its release as CO_2 (Zhu et al., 2017). When biochar is added to soil and there is a net accumulation of carbon, overall CO_2 production may decrease as carbon is sequestered in the biochar-amended soil (Coxa et al., 2017).

In saline soil, all treatments (except RHB) showed a decrease in CO_2 production at 60 days, followed by an eventual increase at 120 days. At 120 days, the RHB-SMC treatment exhibited the highest CO_2 production, characterised by an initial decline followed by an increase. This pattern could be attributed to the effects of contaminants and their eventual remediation facilitated by the bioamendments. Previous studies have also observed that saline soils inhibit microbial activity, leading to lower CO_2 production, while bioamendments have been shown to increase mineralizable carbon pools and enhance microbial activity by directly supplying carbon or alleviating chemical stress induced by contaminants (Kruger et al., 2020). Biochar has also been found to reduce oxidation and osmotic stress, thereby promoting microbial activity in saline soils (de Vasconcelos, 2020). Furthermore, the application of spent mushroom compost can improve soil physicochemical properties, which in turn benefit soil microbial activities (Gumus and Seker, 2017). Amendments like compost significantly increase microbial metabolism, resulting in an increase in soil CO_2 efflux from saline surface soils in agricultural fields and thermal desorption-treated subsoils from oil-contaminated sites (Kruger et al., 2020).

3.4.2. Microbial activities occasion by multiple induced substrate respiration

Efficient preservation of soil function and microbial decomposition is essential for sustainable remediation of contaminated soil (Kaurin and Lestan, 2018). The substrate-induced respiration (SIR) technique involves measuring the rate of microbial respiration in samples amended with an abundant and readily available nutrient source, typically glucose (Aira and Domínguez, 2010). In our study, we conducted a substrate addition assay using sugars and organic acids, including oxalic acid, malic acid, gamma-aminobutyric acid, alpha-ketoglutaric acid, citric acid, glucose, and hydroxypropyl cyclodextrin, in that order of increasing complexity. CO_2 production resulting from substrate utilisation was calculated and subjected to principal component analysis, and the variations in CO_2 responses of the treatments were plotted on graphs (Fig. 7).

The results revealed changes in soil function (respiration) induced by the bioamendments from 10 to 120 days, as evident from the pattern of substrate utilisation. However, the differences between non-saline and saline soils were not statistically significant ($p = 0.8204$). At 60 and 120 days, the biochar treatments in saline soil, particularly RHB, exhibited distinct responses compared to the other treatments. Moreover, the two biochars, WSB for non-saline soils and RHB for saline soils, appeared to favour most of the microbial activity with the tested substrates. This finding is intriguing considering the distinct performance of biochar treatments in saline soils described in other sections of this study. Based on the characteristics of biochar, such as its potential as a porous habitat for microbial growth and protection from predators, provision of mineral nutrients to microbes, ability to sequester organic and inorganic compounds, and alteration of soil structural, biological, and chemical properties (Dai et al., 2021), it can be inferred that these qualities influence the effects of salinity, which in turn impact microbial activity in these treatments.

3.4.3. Correlation between total petroleum hydrocarbon and microbial community responses

The relationships between total petroleum hydrocarbons (TPH) degradation and microbial indicators, including microbial relative abundance based on PLFA and activity based on respiration, were examined (Figs. 8 and 9). The strength of the correlations between TPH and microbial data provides insights into which treatments promoted hydrocarbon

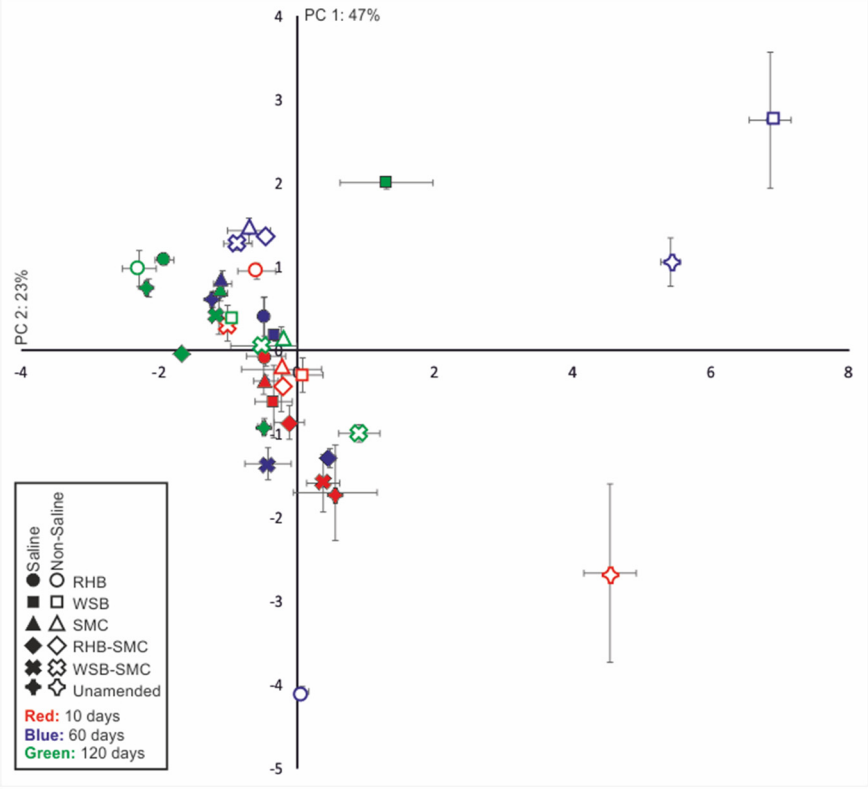

Fig. 7. Soil microbial activity profile in the non-saline and saline soils over time and across treatments (RHB: rice husk biochar; WSB: Wheat straw biochar; SMC: Spent mushroom compost, and RHB-SMC or WSB-SMC: mixture of the two biochars with Spent mushroom compost).

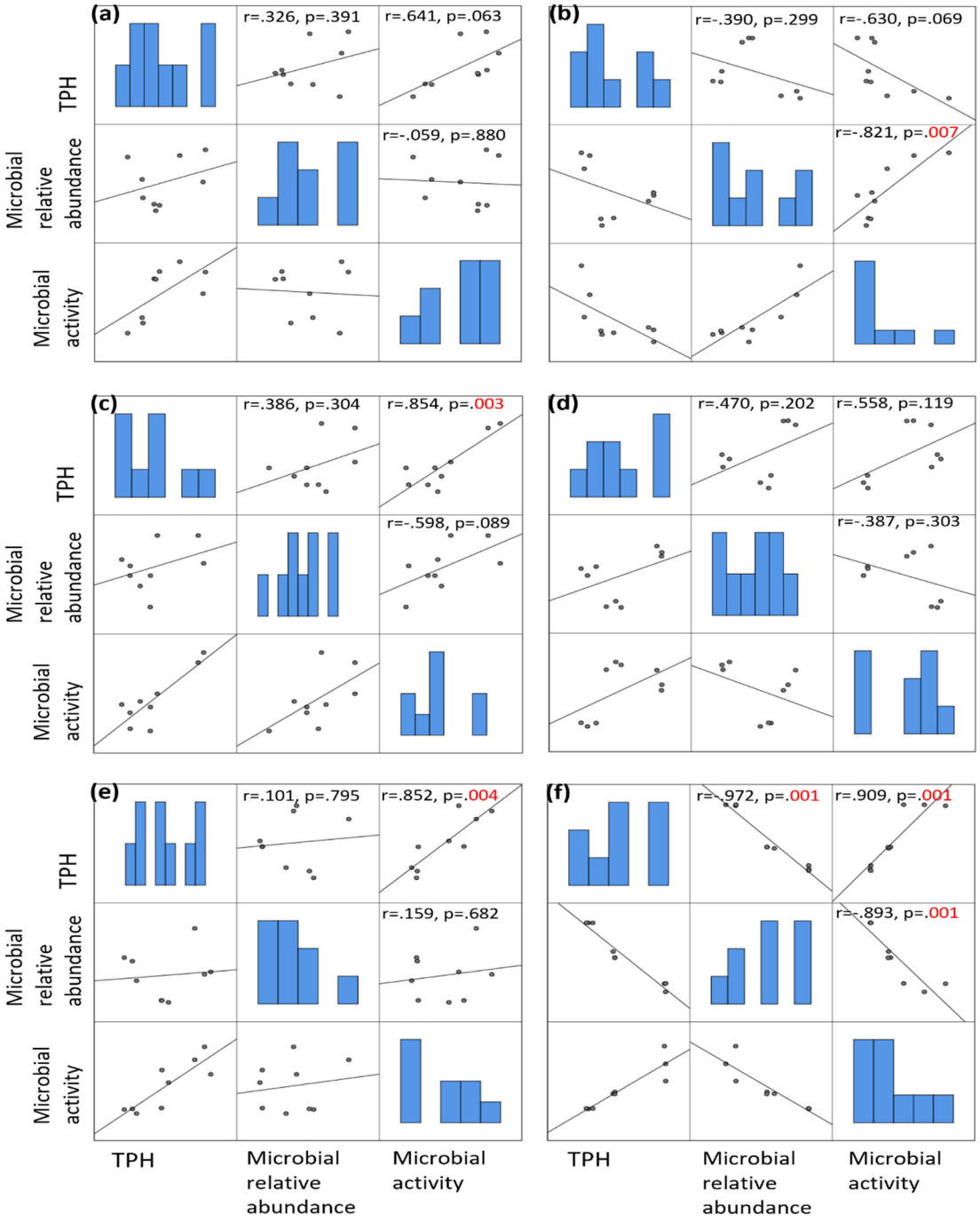

Fig. 8. Correlation between TPH degradation and microbial indicators (PLFA, soil respiration) for the non-saline soil treatments (a: RHB; b: WSB; c: SMC; d: RHB-SMC; e: WSB-SMC; f: unamended).

degradation through microbial abundance and activity in the different soils. A strong negative correlation indicates favourable conditions for hydrocarbon degradation, as observed in other studies (Ławniczak et al., 2020). Conversely, a strong positive correlation between microbial relative abundance and activity suggests high microbial activity, which is typically expected in a healthy soil with a substantial biomass. Conversely, high activity coupled with low biomass may indicate the presence of environmental stress, as reported by Fließbach et al. (1994).

In the non-saline soil treated with WSB (Fig. 8b), a strong negative correlation was observed between TPH levels and microbial relative abundance, as well as microbial activity. This suggests that promoting microbial abundance can lead to enhanced activity, such as the degradation of hydrocarbon substrates in the environment, as supported by previous studies (Chikere et al., 2011; Ławniczak et al., 2020; Pandolfo et al., 2023). Similar positive correlations between microbial relative abundance and activity were observed in WSB and SMC treatments (Fig. 8), indicating that higher biomass can indeed result in increased activity. The statistical significance of these correlations was determined.

In the saline soil treatments (Fig. 9), correlations were observed between TPH levels and microbial relative abundance, as well as microbial activity, but the strength of these correlations varied among the treatments. Strong correlations were observed in RHB, SMC, RHB-SMC, and WSB-SMC treatments, while a low correlation was observed in the WSB treatment, and a moderate correlation was observed in the Unamended

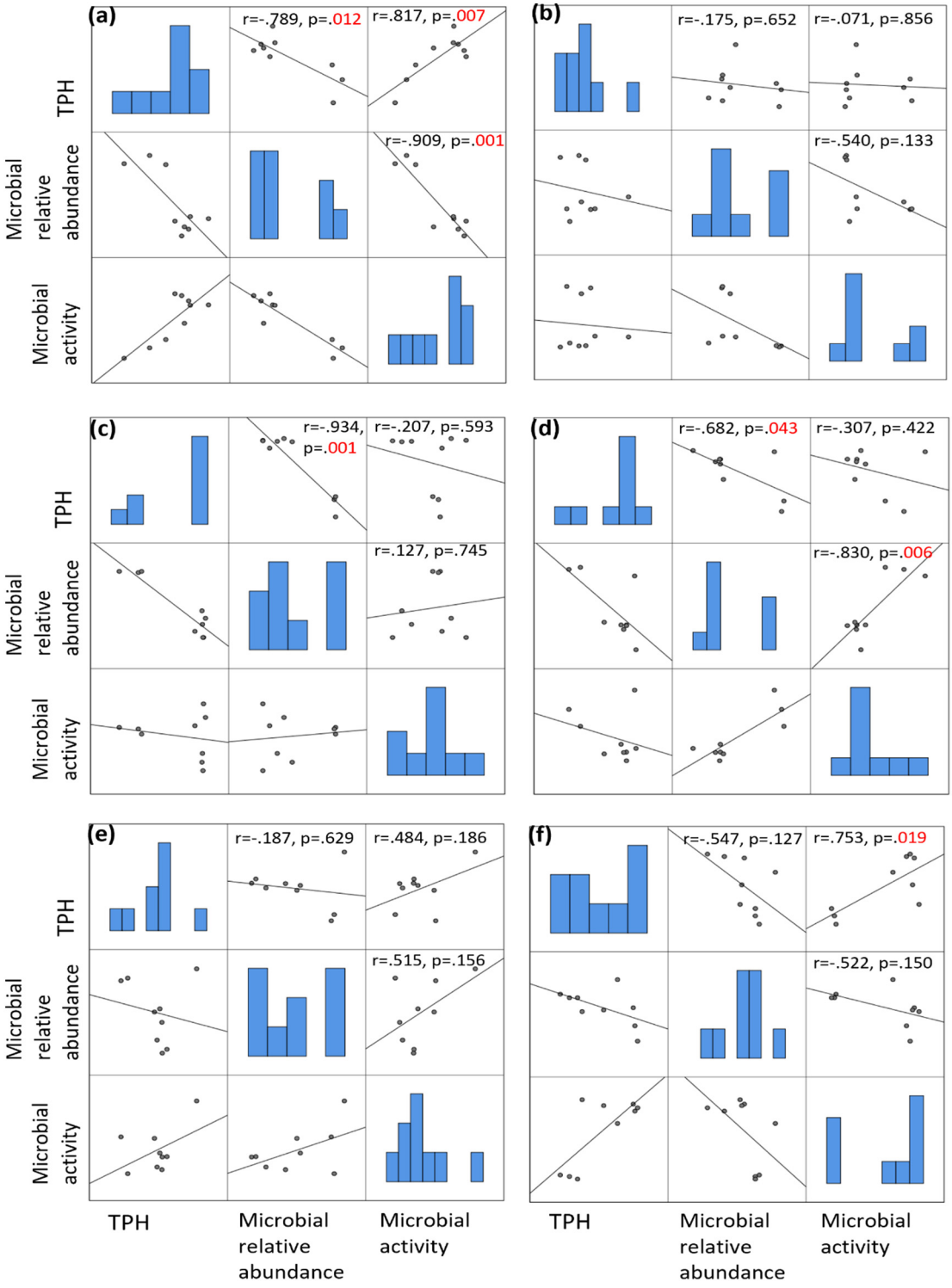

Fig. 9. Correlation between TPH degradation and microbial indicators (PLFA, soil respiration) for the saline soil treatments (a: RHB; b: WSB; c: SMC; d: RHB-SMC; e: WSB-SMC; f: unamended).

treatment. Strong, moderate, and low correlations between microbial relative abundance and microbial activity were observed in RHB-SMC, WSB-SMC, and SMC treatments, respectively.

These findings confirm the efficacy of the remediation strategy by identifying the relationship between microbial populations and hydrocarbon degradation, as well as the varying strengths of these associations. Under similar conditions, biochar, SMC, and a combination of both can be applied at the specified rates and durations to achieve the desired hydrocarbon degradation outcome. This information can assist decision-making in determining remedial measures and serve as a basis for further validation and standardization efforts.

4. Conclusion

This study evaluated the impact of bioamendments on hydrocarbon degradation and microbial communities in both non-saline and saline soils. The results revealed that the bioamendments significantly influenced the degradation of total petroleum hydrocarbons (TPH) in both soil types.

In non-saline soils, the degradation of TPH was four times higher compared to saline soils, with the most significant degradation observed in specific treatments. The RHB-SMC and SMC treatments were particularly effective in degrading alkanes, while the WSB-SMC and RHB treatments showed high degradation rates for polycyclic aromatic hydrocarbons (PAHs) in non-saline and saline soils, respectively. The introduction of biochars (RHB and WSB) and spent mushroom compost (SMC) also had a notable impact on the microbial communities. The bioamendments led to a reduction in the community diversity, indicating a convergence towards a specialised soil microbial community primarily due to hydrocarbons degradation. Moreover, the biochars (RHB and WSB) demonstrated the ability to mitigate the adverse effects of soil salinity on microbial populations. Overall, the bioamendments had a significant positive impact on mitigating the effects of soil salinity on hydrocarbon degradation, as well as on microbial community abundance and function. The RHB and WSB biochars were particularly effective in enhancing these processes, and the addition of SMC further improved their performance. The findings of this study contribute to the knowledge and understanding of sustainable remediation strategies for contaminated soils. The successful application of bioamendments in mitigating the effects of salinity on hydrocarbon degradation and microbial communities demonstrates their potential as environmentally friendly and effective approaches for soil remediation. These findings further highlight the importance of considering bioamendments, specifically biochars and spent mushroom compost, in the design and implementation of soil remediation plans, with the potential to significantly enhance the efficiency and success of such strategies.

CRediT authorship contribution statement

Emmanuel Atai: Conceptualization, Investigation, Resources, Formal analysis, Writing – original draft. **Raphael Butler Jumbo:** Resources, Investigation. **Tamazon Cowley:** Investigation. **Ikeabiama Azuazu:** Resources, Investigation. **Frederic Coulon:** Conceptualization, Supervision, Resources, Writing – review & editing. **Mark Pawlett:** Conceptualization, Supervision, Resources, Writing – review & editing.

Data availability

Data supporting this study are openly available from Cranfield Online Research Data repository at 10.17862/cranfield.rd.23036036.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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