The impact of anaerobic digestate and wood ash amendments on the indigenous ^{14}C-phenanthrene catabolism in soil

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in the environment. They are of concern due to their low biodegradability, environmental persistence, and inherent toxic effects on humans. However, PAHs may be degraded in soil under appropriate environmental conditions. This study investigated the impact of nutrient-rich anaerobic digestate (AD) and/or wood-ash (WA) on indigenous catabolism of 9-^{14}C-phenanthrene in soil over a 90-d incubation. The mineralisation kinetics were positively influenced by the amendments, with shorter lag times, faster rates, and higher extents of mineralisation observed in amended soils compared to the unamended soil incubations. Lower amounts of AD (0.173 g, 1.73 g, and 17.3 g kg^{-1} soil), WA (0.0094 g, 0.094 g, and 0.94 g kg^{-1} soil), and their combination (AD + WA) showed significantly ($P < 0.05$) shorter lag times, faster rates, and higher extents of mineralisation when compared to larger amounts of AD (173 g kg^{-1} soil), WA (9.4 g kg^{-1} soil), and their combination (AD + WA). The cumulative extents of mineralisation in soils amended with the lower amendment amounts ranged from 52 – 90 % across the 90-d incubation while it ranged from 12 – 49 % in soils amended with the higher AD and/or WA amounts. The negative impact of high amendment amount was higher under WA addition with 9.4 g WA kg^{-1} soil adversely impacting mineralisation kinetics both singly and in combination with different amounts of AD. These findings demonstrate that optimised application of AD and/or WA can enhance PAH microbial degradation efficiency, but also highlight that excessive amendment application may hinder biodegradation, emphasising the importance of optimising amendment amount in sustainable soil amendment strategies.

Keywords: Digestate, Mineralisation, Organic amendments, Polycyclic aromatic hydrocarbons, Soil microflora, Wood ash

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of hydrophobic organic contaminants (HOCs), which are found in the environment mostly because of anthropogenic industrial processes (Macleod and Semple, 2002; Okere and Semple 2012; Lang et al., 2016; Lukić et al., 2016). The physicochemical properties of PAHs result in reduced biodegradability and increased persistence as their molecular size increases (Riding et al., 2013; Naseri et al., 2014; Yu et al., 2018). Alongside their persistent nature, PAHs exhibit toxicity and possess genotoxic and carcinogenic potential (Macleod and Semple, 2002; Lukić et al., 2016; Baldwin et al., 2020). The presence of PAHs in soil is an indication of environmental contamination, and a long-term soil exposure promotes ageing which fosters sequestration to soil matrices thereby reducing their susceptibility to degradation (Stokes et al., 2006; Riding et al., 2013; Umeh et al., 2017). However, microbial degradation can be employed to remediate PAH-contaminated soils to reduce the risk to human health (Maletić et al., 2013; Naseri et al., 2014).

The biodegradation of organic contaminants in soil can lead to a depletion the available pools of major inorganic nutrients in the soil, especially nitrogen (N) and phosphorus (P), and enrich its carbon (C) content (Margesin and Schinner, 2001; Stroud et al., 2007). The addition of appropriate growth-limiting mineral nutrients can restore the soil's nutrient balance and enhance its indigenous microbial activity (Naseri et al., 2014; Ou et al., 2024). The degradation of organic contaminants in soil has been shown to occur following stimulation of native or indigenous microflora following the addition of N, P, and potassium (K) based mineral fertilizers (Margesin and Schinner, 2001; Aghalibe et al., 2020; Udume et al., 2023). However, repeated addition of inorganic nutrients can encourage soil acidification and accumulation of heavy metal contents as well as soil layer compaction (Savci, 2012; Xu et al., 2014; Massah and Azadegan, 2016), which can adversely affect the soil quality and microbial activity. Currently, a wide variety of low-value organic residues from bioenergy processes are applied to soil as organic fertilizers due to their recyclable and readily available micro- and macro-nutrients (Odlare et al., 2011; Colatorti et al., 2024; Mora-Salguero et al., 2025). The growing industrialization and increased recycling of organic wastes, as renewable energy sources, have led to a huge generation of organic residues with management and disposal challenges (Odlare et al., 2011; Quakernack et al., 2012). However, their addition to soil, as organic fertilizers, has been considered a suitable and sustainable alternative to chemical fertilization (Alburquerque et al., 2012a,b; Fernández-Delgado Juárez et al., 2013; Nabeela et al., 2015; Gómez-Brandón et al., 2016).

Anaerobic digestate (AD), a slurried organic bioenergy residue (Köster et al., 2014), and wood-ash (WA), a biomass combustion inorganic residue have gained more attention as soil conditioners or renewable fertilizers (Fernández-Delgado Juárez et al., 2013; García-Sánchez et al., 2015a). AD is known to be rich in organic matter (due to its residual C) and nutrients (Whelan et al., 2010; Möller and Müller, 2012; Insam et al., 2015), especially N and P (Garcia-Lopez et al., 2023), which are essential for microbial growth and activity (Alburquerque et al., 2012a; Köster et al., 2014; García-Sánchez et al., 2015a; Tiwary et al., 2015). However, the amount of AD to be added to soil should match the soil's N deficiency (Fernández-Delgado Juarez et al., 2013; Gómez-Brandón et al., 2016) to optimise its benefits as well as mitigate soil acidification, nitrate leaching, and emission of nitrous oxide (Insam et al., 2015; Monlau et al., 2016; Tampio et al., 2016; Nicholson et al., 2017). Also, due to the high alkalinity of AD, there is a propensity of N loss as volatilized ammonia (NH_3-N) within a short time of AD addition to soil which can impact microbial activity (Alburquerque et al., 2012a; García-Sánchez et al., 2015a).

Similarly, WA contains a significant amount of macro-nutrients (calcium, magnesium, sodium, and potassium) as well as other inorganic elements (zinc, copper, lead, nickel, and arsenic) (Fernández-Delgado Juarez et al., 2013; García-Sánchez et al., 2015a; Maschowski et al., 2016; Ivezic et al., 2021). Studies have demonstrated that WA can be employed to correct soil acidity to a desired soil pH (Fernández-Delgado Juarez et al., 2013; García-Sánchez et al., 2015a), ameliorate soil physical, chemical, and biological properties (Ivezic et al., 2021; Blonska et al., 2023; de Oliveira et al., 2023), and immobilize heavy metals in soil (García-Sánchez et al., 2015b). However, the high pH (8 – 13) of WA, coupled with its negligible amount or complete absence of N and C, has limited its use as a soil ameliorant (Demeyer et al., 2001; Fernández-Delgado Juarez et al., 2013; Köster et al., 2014; García-Sánchez et al., 2015a,b). This is due to the impact of pH, N, and C on soil microbial population, community composition, and activity. The effects of WA are influenced by factors such as the amendment amount and soil type (Fernández-Delgado Juarez et al., 2013). However, excessive application of WA can disrupt the soil's physicochemical and biological properties (Perucci et al., 2006; Perucci et al., 2008; Insam et al., 2009; An and Park, 2021). A suitable amendment amount for WA has remained a challenge in its application and has been identified as a major constraint to its use as a soil bio-fertilizer (Ferreiro et al., 2011; Fernández-Delgado Juárez et al., 2013).

Previous studies have shown the impact of AD and WA in ameliorating soil characteristics (Pitman 2006; Blonska et al., 2023; Holatko et al., 2023), optimizing soil nutrients (Badagliacca et al., 2020; Glowacka et al., 2020), and stimulating soil microbial growth and activities (Bougnom et al., 2012; De Corato et al., 2023). Therefore, due to the proven positive effects of AD and WA on soil microbial activity (Odlare et al., 2011; Bang-Andreasen et al., 2020; Zhao et al., 2022), they could equally enhance microbial degradation of PAHs. Additionally, because of the complementary nutrients (especially N, P, and K) of AD and WA, their addition to soil as a mixture could produce a potentially valuable amendment with more positive impacts on soil health and fertility (Bougnom et al., 2012). WA enhances the effect of digestate on soil properties, making it a suitable nutrient booster when applied with organic fertilisers (Ibeto et al., 2022). It typically contains variable amounts of P and K (Kuba et al., 2008; Ivezic et al., 2021) but negligible N due to ammonia volatilisation during combustion (Perucci et al., 2006; Whelan et al., 2010; Möller and Müller, 2012; Köster et al., 2014). In contrast, AD provides considerable N, P and degradable organic matter (Alburquerque et al., 2012a; Fernández-Bayo et al., 2017). Studies have shown that AD + WA mixtures improve soil fertility, increase NH_4^+, total C and P (Fernández-Delgado Juárez et al., 2013), and enhance soil macronutrient availability (Kuba et al., 2008). Similarly, co-application of straw and WA improved microbial biomass carbon and enzymatic activity more than either amendment alone (Zhao et al., 2022), while AD + WA combinations enhanced plant growth and photosynthesis (Zusevica et al., 2023), reflecting improved soil health that may support greater microbial activity and PAH biodegradation.

These studies indicate that AD and/or WA can enhance soil microbial activity which could in turn promote the biodegradation of PAHs and other HOCs in soil. However, the impact of AD and/or WA on the indigenous microbial degradation of PAHs in soil has been underexplored. In particular, while AD has been widely studied for its role in stimulating soil microbial activity through nutrient and carbon inputs, and WA for its capacity to modify soil physicochemical properties, there remains a critical gap in knowledge regarding how their combined application influences PAH mineralisation under varying amendment amounts. Additionally, most of the AD and WA studies have focused on their impact on plants and soil carbon dynamics. Therefore, this study investigated the mineralisation of phenanthrene (a model PAH) by indigenous soil microbes as influenced by AD and/or WA amendment. To date, this is the first study to examine the effect of AD and WA on phenanthrene mineralisation kinetics in soil using proportionately increasing single AD and WA amounts, and a varying mixture of AD and WA at proportionately increasing combinations. This study is significant as it provides insights on a cost-effective, sustainable (bio)remediation approach because AD and WA demonstrate potential as sustainable soil amendments for enhancing the (bio)remediation of phenanthrene in soil thereby reducing the associated environmental health risks.

2. Materials and Methods

2.1 Bioenergy residues and chemicals

Pasteurized anaerobic digestate (AD) was obtained as a slurry-like material from an anaerobic digestion plant in the United Kingdom (UK). It was produced from anaerobically digested household food wastes. After collection, it was stored in a dark room at 4^{o}C. The wood ash (WA) – fly-ash – was collected from a biomass power plant in the UK, using timber and bark as feedstocks. Fly-ash was preferred due to its higher concentrations of elements (Sharma and Kalra, 2006) and low alkalinity (Noyce et al., 2016) compared to the bottom ash. The AD and WA characteristics are shown in Table 1. Chemicals used in this study include non-radiolabelled phenanthrene (>96%, HPLC grade) and 9-^{14}C-labelled phenanthrene (55.7 mCi mmol^{-1}; >99% purity); both were supplied by Sigma–Aldrich, UK. Gold Star multipurpose liquid scintillation cocktail and acetone (>98%) were supplied by Meridian, UK.

2.2 Soil sampling and bulk characterization

The soil used for this experiment was collected from a pasture field at Myerscough College, Lancashire, UK. The soil is known to be a Dystric Cambisol soil with a clayey-loam texture. The soil is rich in organic matter and consists of clay (19.5% ± 0.7), silt (20.0% ± 0.9) and sand (60.4% ± 1.2); the sand consists of 0.12% ± 0.01 coarse particles, 6.9% ± 0.1 medium particles and 53.3% ± 0.6 fine particles (Couling et al., 2010). Other physicochemical properties of the soil are presented in Table 1. The soil has no known history of exposure to PAHs. After collection, the soil was sieved using a ≤2 mm sieve for the removal of plant debris and stones, as well as for homogeneity before storage at 4^{o}C in the dark. The soil's moisture content (32.1%) was determined by oven drying at 105^{o}C for 24 h (Rousk et al., 2009).

2.3 Soil spiking with ^{12}C-phenanthrene and amendment using AD and/or WA

Soil (220.5 g; w/w) for each treatment condition (amended, control, and blank), was spiked with ^{12}C-phenanthrene (100 mg kg^{-1}; d/w) at field moisture content (32.1%), homogenized, and vented in a fume cupboard for 2 h to evaporate carrier solvent (acetone). The different amendment conditions were prepared as shown in Table 2. Each soil treatment condition was divided into three replicates (n = 3; 73.5 g each). The mass of AD and WA used as soil amendments in this study were derived from information provided to farmers/land owners for the application of the bioenergy residues to soil through

the British agricultural practice recommendation of N-to-P (3:1) reference dose for soil amendment (AHDB, 2017) and wheat plantation (DEFRA, 2017). All amended soils were incubated at 20 ± 2^{o}C and sampled at defined intervals of 1 d, 15 d, 30 d, 60 d, and 90 d for respirometric assay.

2.4 Respirometric measurement of the 9-^{14}C-phenanthrene mineralisation in the amended soil

The mineralisation of 9-^{14}C-phenanthrene was measured in the soil treatment conditions using a respirometric assay (Reid et al., 2001). At defined sampling intervals (1 d, 15 d, 30 d, 60 d, and 90 d), soil (14.0 g; w/w) was sampled from each microcosm into a modified pre-cleaned 250 ml Schott bottle (Reid et al., 2001). Sterilized deionized water (30 ml) was added to the Schott bottles to form a slurry. Each respirometer (except blank) was spiked with 9-^{14}C-phenanthrene (50 Bq g^{-1} soil). A glass vial (7 ml) containing fresh 1 M NaOH (2 ml) was suspended (attached to the lid) inside each respirometer. The blank soil was set up to monitor the background ^{14}C-activity. All the respirometers were incubated at 20 ± 2^{o}C on a flat-bed shaker (SANYO Gallenkamp) at 100 RPM for 14 d. The $^{14}CO_2$ produced from the mineralisation of the ^{14}C-phenanthrene was trapped in the 1 M $NaOH_{(aq)}$ and sampled at 2, 4, 8, 12, 24 h, and henceforth every 24 h for 14 d (336 h). The sampled $^{14}CO_2$ traps were quantified by LSC (Canberra Packard Tri-Carb 2250CA). Respirometric data was used to calculate the changes in the lag times as well as rates and extents of the mineralisation.

2.5 Statistical analysis

Blank-corrected data were plotted with SigmaPlot 10.0. The effects of soil amendments with the AD, WA, and AD + WA on the mineralisation of 9-^{14}C-phenanthrene were analyzed using a one-way analysis of variance (ANOVA) at a 95% confidence level ($P < 0.05$). Tukey's HSD and LSD post-hoc tests were performed to compare the means within and across the different amended soils. Data was analyzed using SPSS (IBM SPSS version 27). The mineralisation kinetics were calculated using MS Excel as lag times (time it took to mineralise 5% of spiked activity), maximum rates (highest mineralised activity in an hour), and extents of mineralisation (cumulative $^{14}CO_2$ evolution in 14 d).

3. Results.

3.1. Mineralisation of 9-^{14}C-phenanthrene in AD-amended soils

The mineralisation of ^{14}C-phenanthrene in soils amended with increasing amounts of AD (0.17, 1.73, 17.31, and 173.1 g kg^{-1} soil) was monitored over a 90 d incubation period (Figure 1; Table 3). Lag times did not differ significantly ($p > 0.05$) between amended soils and the control from day 1 to 60, except in soil amended with 173.1 g kg^{-1} AD, which exhibited significantly longer ($p < 0.05$) lag times. Specifically, the 173.1 g kg^{-1} treatment showed prolonged lag times at days 1, 15, and 60 compared to other treatments. By day 90, however, lag times in all AD-amended soils were shorter than in the control, with the 1.73, 17.31, and 173.1 g kg^{-1} treatments showing significantly ($p < 0.05$) shorter lag times. At the onset of incubation (1 d), amendment with 173.1 g kg^{-1} AD resulted in a significantly longer ($p < 0.05$) lag time (149.8 ± 8.2 h), which decreased progressively with increasing soil–PAH contact time ($p < 0.05$). In general, higher AD application amounts (particularly 173.1 g kg^{-1}) led to slower mineralisation rates throughout the 90 d, with rates at days 1, 15, and 60 significantly lower ($p < 0.05$) than those observed in other treatments. Conversely, by day 90, the control exhibited a significantly ($p < 0.05$) slower mineralisation rate than all amended soils. The extent of ^{14}C-phenanthrene mineralisation decreased with increasing AD amount, with the 173.1 g kg^{-1} treatment showing significantly ($p < 0.05$) lower mineralisation throughout the incubation period (Table 3; Figure 1). Overall, soils amended with lower AD amounts (0.17 g and 1.73 g kg^{-1}) achieved significantly higher ($p < 0.05$) extents of mineralisation compared to the higher amounts (17.31 g and 173.1 g kg^{-1}) and the unamended control.

3.2 Mineralisation of 9-^{14}C-phenanthrene in soils amended with WA

The effects of increasing WA additions (0.0094, 0.094, 0.940, and 9.400 g kg^{-1} soil) on the mineralisation of ^{14}C-phenanthrene were monitored over 90 d (Figure 2; Table 4). Lag times at 1 d were significantly longer ($p < 0.05$) than at later contact times. Soils amended with 9.4 g kg^{-1} WA exhibited the longest lag times across 1 – 90 d, which were significantly longer ($p < 0.05$) from 1 – 60 d. In contrast, lag times did not differ significantly ($p > 0.05$) between the control and lower WA treatments (0.0094, 0.094, and 0.940 g kg^{-1}) over the same period. The 0.0094 g kg^{-1} treatment generally showed the shortest lag times from 1 – 60 d. Lower WA amounts (0.0094 – 0.940 g kg^{-1}) supported higher mineralisation rates ($p < 0.05$) than the 9.40 g kg^{-1} treatment from 1 – 60 d. Soils treated with 0.094 g kg^{-1} WA showed the fastest rates overall, while the 0.0094 and 0.094 g kg^{-1} treatments exceeded the control. The control, however, exhibited significantly faster ($p < 0.05$) rates than the 9.40 g kg^{-1} treatment during 1 – 60 d. By day 90, all WA-amended soils showed faster mineralisation rates than the control. The extent of mineralisation was consistently higher in soils amended with lower WA amounts (0.0094 – 0.940 g kg^{-1}) compared to the 9.40 g kg^{-1} treatment throughout 1 – 90 d, with significant differences ($p < 0.05$) at 1, 15, 60, and 90 d (Figure 2; Table 4). The 9.40 g kg^{-1} treatment recorded significantly lower ($p < 0.05$) mineralisation extents than the control, while lower WA amendments achieved higher extents than the control.

3.3. Mineralisation of 9-^{14}C-phenanthrene in soils amended with a mixture of increasing amounts of AD and WA

The effects of combined additions of proportionately increasing AD and WA on the mineralisation of ^{14}C-phenanthrene were monitored over 90 d (Figure 3; Table 5). Soils amended with the highest combination (173.1 g kg^{-1} AD + 9.40 g kg^{-1} WA) exhibited significantly longer ($p < 0.05$) lag times at days 1, 15, 60, and 90 compared to all other treatments (Table 5). No significant differences ($p > 0.05$) in lag times were observed between the other amended soils and the control throughout the incubation period, except for this highest combined dose. Consistent with the lag time trend, soils treated with 173.1 g kg^{-1} AD + 9.40 g kg^{-1} WA showed generally slower mineralisation rates than other treatment conditions (Table 5). All other AD + WA amended soils exhibited faster mineralisation rates than the control, whereas the 173.1 g kg^{-1} AD + 9.40 g kg^{-1} treatment remained significantly slower ($p < 0.05$). The extent of ^{14}C-phenanthrene mineralisation was significantly lower ($p < 0.05$) in soils amended with 173.1 g kg^{-1} AD + 9.40 g kg^{-1} WA compared to other treatments throughout the 90 d incubation (Table 5; Figure 3). No significant differences ($p > 0.05$) were observed between the control and the other AD + WA combinations. However, all mixed amendment treatments (except the highest amount) generally resulted in higher mineralisation extents than the control (Figure 3; Table 5).

3.4. Mineralisation of 9-^{14}C-phenanthrene soils amended with mixtures of proportionately increasing amounts AD and a single amount of WA.

The effects of proportionately increasing additions of AD (0.1731, 1.731, 17.31, and 173.1 g kg^{-1} soil) combined with a single amount of WA (0.94 g kg^{-1} soil) on the mineralisation of ^{14}C-phenanthrene in ^{12}C-phenanthrene-exposed soils were examined at defined soil–PAH contact times of 1, 15, 30, 60, and 90 days (Figure 4; Supplementary Table S1). Shorter lag times were observed in soils amended with 0.1731 g AD + 0.94 g WA after 1 d and 60 d, 1.731 g AD + 0.94 g WA after 60 d, and 17.31 g AD + 0.94 g WA after 60 d and 90 d compared to the control. In contrast, soils amended with 173.1 g AD + 0.94 g WA exhibited longer lag times than all other treatments, although these progressively decreased with increasing soil–PAH contact time. By 90 d, lag times in this treatment (173.1 g AD + 0.94 g WA) became shorter than in the control ($p > 0.05$). Maximum mineralisation rates were significantly higher ($p < 0.05$) than the control after 60 d and higher ($p > 0.05$) after 90 d in soils amended with 17.31 g AD + 0.94 g WA. Similarly, mineralisation rates exceeded the control ($p > 0.05$) after 30 d in the 0.1731 g AD + 0.94 g WA treatment and after 90 d in the 173.1 g AD + 0.94 g WA treatment. Cumulative extents of mineralisation were significantly higher ($p < 0.05$) than the control after 60 d in soils amended with 0.1731 g AD + 0.94 g WA, 1.731 g AD + 0.94 g WA, and 17.31 g AD + 0.94 g WA. After 90 d, mineralisation extents remained higher than the control in all AD + WA treatments, with significant differences ($p < 0.05$) observed for 173.1 g AD + 0.94 g WA and non-significant ($p > 0.05$) but higher values for the 0.1731 g AD, 1.731 g AD, and 17.31 g AD + 0.94 g WA treatments (Figure 4; Supplementary Table S1).

3.5. Mineralisation of 9-^{14}C-phenanthrene soils amended with mixtures of proportionately increasing amounts of WA and a single amount of AD.

The mineralisation of ^{14}C-phenanthrene in ^{12}C-phenanthrene-exposed soils amended with mixtures of proportionately increasing WA amounts (0.0094, 0.094, 0.940, and 9.400 g kg^{-1} soil) and a fixed AD amount (17.31 g kg^{-1} soil) was monitored at defined soil – PAH contact times of 1, 15, 30, 60, and 90 days (Figure 5; Supplementary Table S2). Lag times were significantly shorter ($p < 0.05$) than the control in soils amended with 17.31 g AD + 0.0094 g WA after 15 d and 90 d, and with 17.31 g AD + 0.094 g WA after 90 d. Shorter, though not statistically significant ($p > 0.05$), lag times were also observed for 17.31 g AD + 0.0094 g WA after 30 d and 60 d; 17.31 g AD + 0.094 g WA after 15, 30, and 60 days; and 17.31 g AD + 0.94 g WA after 60 d and 90 d. Mineralisation rates were significantly higher ($p < 0.05$) than the control in soils amended with 17.31 g AD + 0.0094 g WA after 15, 30, and 60 days; 17.31 g AD + 0.094 g WA after 15 d and 30 d; and 17.31 g AD + 0.94 g WA after 30 d and 60 d. Similarly, higher but non-significant ($p > 0.05$) mineralisation rates were observed after 15 d and 90 d in soils amended with 17.31 g AD + 0.94 g WA and after 60 d with 17.31 g AD + 0.094 g WA. The cumulative extent of mineralisation across amended soils was higher than the control at several time points. Although differences were not statistically significant ($p > 0.05$), soils amended with 17.31 g AD + 0.94 g WA showed higher extents after 15 d, while 17.31 g AD + 0.0094 g WA exhibited higher extents after 30, 60, and 90 days. Similarly, 17.31 g AD + 0.094 g WA resulted in higher mineralisation extents after 30 d (Figure 5; Supplementary Table S2).

4. Discussion

4.1 Impact of AD amendment on 9-^{14}C-phenanthrene mineralisation in soil.

The enhanced mineralisation kinetics from the addition of proportionately increasing amounts of anaerobic digestate (AD), were indicative of the potential of AD in stimulating the indigenous microbial community in the soil (Gielnik et al., 2019; Ibeto et al., 2020). These observations imply that the indigenous microbes in soil can adapt to the addition of the AD or utilize the nutrients in the AD for proliferation. Longer lag times, slower rates, and reduced extents of mineralisation were observed in soil amended with higher amount of AD (173.1 g kg^{-1} soil). This may result from the excess carbon and nutrients from AD (Alburquerque et al., 2012a; Fernández-Delgado Juárez et al., 2013; Koszel and Lorencowicz, 2015; Garcia-Lopez et al., 2023), readily available to indigenous soil microbes compared to phenanthrene. This abundance of carbon can shift microbial preference toward more accessible sources, thereby hindering the microbial degradation of the target contaminant. However, Suproniene et al. (2022) noted that AD amendment might not significantly alter the diversity of the soil prokaryotic community, suggesting that other factors and soil properties should be considered in these scenarios. One possible situation beside substrate competition could be sorption of phenanthrene to AD-related organic matter reducing bioaccessibility and possible mineralisation.

The addition of AD (0.17 g, 1.73 g, and 17.31 g kg^{-1} soil) generally showed faster rates and higher extents of mineralisation especially at 1.73 g kg^{-1} soil, when compared to unamended soil. An increase in phenanthrene mineralisation indicates enhanced soil microbial activity, and amendments that boost soil microbial activity can promote PAH mineralisation. AD demonstrates this potential, as its addition to soil has been shown to significantly enhance microbial activity, including structure, biomass, abundance, and physiological diversity, both in the short and long term (García-Sánchez et al., 2015a; Garbini et al., 2022; De Corato et al., 2023). The application of sewage sludge digestate to petroleum hydrocarbon-contaminated soil enhanced microbial gene concentrations and achieved 74% degradation of petroleum hydrocarbons within two months (Gielnik et al., 2021). However, studies have shown that while digestate generally increases microbial abundance (Garbini et al., 2022), its effect on soil microbial activity varies depending on soil properties and the type of digestate feedstock used (Doyeni et al., 2021).

As the soil-PAH contact time increased (from 15 – 90 d), shorter lag times, faster rates, and higher extents of mineralisation were observed compared to 1 d. These depicts improved biological activity as the interactions of the soil and AD nutrients increased. The delayed effect on the incubation onset suggests that the soil microorganisms were adapting to the phenanthrene and AD, and/or withstanding any possible unfavorable effect(s), as the soil has no previous history of PAH contamination. This may occur either through delayed microbial synthesis of the enzymes required for phenanthrene degradation, or through microbial turnover and competition, whereby non-phenanthrene-degrading microbes are outcompeted or eliminated, allowing PAH-degrading populations to proliferate and drive increased mineralisation over time. This delayed effect aligns with previous findings that spiking soil with phenanthrene adversely impacts soil nutrients and microbes (Ibeto et al., 2020), and also supports the notion that a specific period is necessary for the activation of microbial catabolic functions in soil indigenous PAH-degrading bacteria (Semple et al., 2001).

4.2 Impact of WA amendment on 9-^{14}C-phenanthrene mineralisation in soil

The changes in lag times as the soil-PAH contact time progressed did not follow a consistent fixed pattern. However, the lag times were consistently shorter at lower amounts of wood ash (WA) compared to higher amount (9.4 g kg^{-1} soil). Slower rates and lower extents of mineralisation were also observed at 9.4 g kg^{-1} soil. WA addition to soil has been indicated to be maximally beneficial at lower amounts, and possibly toxic at higher amounts (Pitman, 2006; An and Park, 2021; Neimane et al.; 2021). At lower WA amounts, WA may enhance phenanthrene bioavailability by increasing soil pH and ionic strength thereby modifying sorption equilibria within soil organic-mineral matrices and promoting desorption of sorbed phenanthrene into aqueous phase, where it becomes more accessible for microbial uptake and enzymatic degradation. For example, Yu et al. (2016) reported higher phenanthrene extractability in phosphate buffer solution (pH 7), which was attributed to a higher dissolution of soil organic matter producing more dissolved organic matter, which subsequently led to a higher solubility of phenanthrene. Conversely, while phosphorus is an essential microbial nutrient, excessive P input, from high WA application, relative to nitrogen can disrupt nutrient balance, leading to nitrogen limitation, altered microbial community structure, and reduced efficiency of enzymatic pathways involved in phenanthrene mineralisation. These effects may be further compounded by pH-induced microbial stress. Earlier studies have shown that WA indirectly impacts soil microbial processes by altering soil pH and other physicochemical properties (Bougnom and Insam, 2009; García-Sánchez et al., 2015a). However, low-dose WA additions are considered safe for soil microbiota (Cruz-Paredes et al., 2021). Bacteria, which play a crucial role in PAH mineralisation, are particularly sensitive to WA-induced pH changes, often exhibiting reduced activity as soil pH increases, whereas fungi are generally more tolerant and are affected only at higher WA application amounts (Cruz-Paredes et al., 2021). This microbial sensitivity to pH shifts may explain the diminished mineralisation observed with higher WA amendments. Perucci et al. (2006) had earlier reported that the addition of a lower amount of WA (5 t ha^{-1}) increased soil microbial activity, while the addition of a higher amount of WA (20 t ha^{-1}) resulted in reduced microbial activity after four (4) months of treatment.

The increased mineralisation observed with the addition of lower amounts of WA suggests enhanced microbial activity, but it may also reflect improved PAH bioavailability and favourable soil physicochemical conditions. At low amounts, WA can slightly increase soil pH and supply essential minerals (e.g., Ca, K, Mg), enhancing enzyme stability and microbial metabolism. Additionally, moderate alkalinity may promote desorption of phenanthrene from soil particles, increasing its accessibility to degrading microbes. This is supported by studies that have demonstrated improved soil microbial biomass, microbial respiration, and enzyme activity under WA treatment (Fernández-Delgado Juárez et al., 2013; García-Sánchez et al., 2015b; Campos et al., 2018). This is possible through WA enhancing soil properties (Bang-Andreasen et al., 2020; Rocha et al., 2023; de Oliveira et al., 2023) which boosts bacterial abundance and subsequently enhance phenanthrene biodegradation. However, few studies have reported no significant effects on microbial activity in WA-amended soils (Bougnom et al., 2012; García-Sánchez et al., 2015a; Noyce et al., 2016), and a recent study by Joseph et al. (2022) concluded that WA's impact on soil quality is limited and inconsistent in forest soil. The varying effects observed in WA-amended soils might be due to soil type, WA feedstock, WA pre-treatment, WA amendment amount, length of the experiment, parameters of analysis, as well as sampling time. Studies have reported that these factors affect the reported impacts of WA in amended soil (Kuba et al., 2008; Fernando-Jaurez et al., 2013; Gómez-Brandón et al., 2016).

4.3 Additions of mixtures of AD and WA to soil and the effect on mineralisation of 9-^{14}C-phenanthrene

Soil amendment with proportionately increasing mixtures of AD and WA enhanced the mineralisation of ^{14}C-phenanthrene in the soil with a trend similar to what was observed under separate additions. The influence of high amendment amounts as observed in the separate additions was also evident in the combined amendment. It is likely that at lower amendment amounts, soil nutrient balance remained largely governed by baseline soil conditions, whereas at the highest AD and WA application, soil nutrient would have shifted towards the intrinsic composition of the amendments, indicating increasing amendment control over soil system nutrient balance thereby altering the microbial response to phenanthrene degradation. Lower amounts of AD and WA mixture generally reduced the lag times and enhanced both the rates and extents of ^{14}C-phenanthrene mineralisation compared to control. These results suggest that lower combined amounts of AD and WA created more favourable conditions for soil microbial activity and phenanthrene biodegradation than higher amounts. At lower amendment levels, the balance of nutrients, pH, and available organic carbon likely supported active microbial populations capable of mineralising phenanthrene. The observation that AD and WA, when applied singly at proportionately increasing amounts, produced similar ^{14}C-phenanthrene mineralisation kinetics to their combined proportionately increasing application, indicates an absence of strong synergistic effects between the two amendments. This suggests that both materials may act through comparable mechanisms, such as improving nutrient supply, altering pH, or stimulating microbial activity, leading to overlapping rather than complementary effects. It is possible that nutrient or carbon availability, rather than amendment type, governed the extent of microbial activity responsible for ^{14}C-phenanthrene mineralisation. Additionally, at higher amounts, substrate competition or excessive nutrient or pH shifts may have constrained microbial efficiency, masking any potential benefits of co-application.

On the other hand, despite the higher mineralisation extents observed in proportionately increasing WA/AD but fixed AD/WA at lower amounts compared to increasing AD + WA mixtures and single amendments, the pattern of the mineralisation kinetics was similar to the other amendment conditions. However, the higher mineralisation extents may indicate that the interaction between AD and WA though not synergistic, could be complementary depending on mixing ratio. Both amendments contribute beneficial properties (e.g., organic matter from AD, pH and nutrients from WA), but at excessive amendment levels, these same properties could become inhibitory as observed in this study. Thus, the optimal biodegradation effect occurs at moderate AD and WA combinations, where the balance of nutrient supply and pH enhancement maximises microbial degradation without causing stress or substrate inhibition. A similar observation was reported by Kuba et al. (2008) where soil amendment with mixtures of organic wastes and lower amounts of WA resulted in higher available macronutrients. Also, in the study by Fernando-Jaurez et al. (2013), the addition of mixtures of AD and lower amounts of WA (0, 1, and 3 t ha^{-1}) increased total C and P as well as NH_4^+ in soils. In Bougnom et al. (2012), soil amendment with mixtures of WA and anaerobic sludge reduced soil biological activities in proportion to the amounts of WA added, and an increase in soil pH corresponded to the amounts of WA applied.

4.4 Real life implication of findings and possible limitations

Anaerobic digestate (AD), widely available as by-product of biogas production, can stimulate microbial activity through the provision of labile carbon and nitrogen, while WA can modify soil physicochemical conditions by increasing pH and supplying mineral nutrients. Together, these amendments can enhance contaminant microbial degradation, offering a dual benefit of waste valorisation and soil (bio)remediation.

The findings of this study have important implications for the remediation of PAH-contaminated soils. The observed enhancement of phenanthrene mineralisation at low to moderate amendment amounts demonstrates that the application of AD and/or WA can effectively stimulate indigenous microbial degradation processes, thereby accelerating the breakdown of PAHs. This highlights the potential of using waste-derived amendments not only as soil conditioners but as functional biostimulation agents in (bio)remediation strategies. Importantly, the observed effect of amendment amount, including the decline in mineralisation at higher amendment amounts, shows the importance of optimising amendment application amount to avoid inhibitory effects associated with possible nutrient imbalance, excessive alkalinity, microbial stress, and preferential substrate utilisation. These results provide practical guidance for field-scale applications, suggesting that controlled, moderate amendment additions can enhance microbial contaminant degradation efficiency, while over-application may compromise remediation outcomes. In addition, this study contribute to the understanding of how organic and mineral amendments can be combined to enhance PAH biodegradation, offering a cost-effective and sustainable remediation approach.

While the AD and /or WA approach holds considerable potential as a low-cost and sustainable remediation approach to enhancing PAH biodegradation in soils, its successful field-scale application requires careful optimisation of amendment amounts and ratios, site-specific assessment, and management of potential environmental risks to ensure effective and safe implementation. Further constraints may arise from the variability in AD and WA composition, which depends on feedstocks and processing conditions, potentially affecting consistency in amendment property and predictability of outcomes. There is also a risk of introducing secondary contaminants such as heavy metals from WA or organic compounds from AD, which may raise environmental and regulatory concerns

5. Conclusions

The findings indicate that high amounts of AD and/or WA inhibit phenanthrene mineralisation in soil. When applying both together, an optimal mixing ratio is crucial to maximise their beneficial effects on mineralisation kinetics. This study provides novel insight that AD and WA could promote phenanthrene mineralisation indicating that amendments that improve soil properties and agronomic benefits can promote phenanthrene mineralisation. Overall, this study demonstrated that AD and/or WA could be beneficial for remediating phenanthrene-contaminated soil, offering a promising approach to reduce the associated risks of phenanthrene and potentially other PAHs.

6. References

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