

RESEARCH ARTICLE OPEN ACCESS

Effect of *Nigella sativa* Residue and Its Biochar on Soil Microbial Activity, Carbon Sequestration and Nitrogen Cycling Across Different Soil Types

Asuman Büyükkılıç Yanardağ 

Soil Science and Plant Nutrition Department, Malatya Turgut Ozal University, Malatya, Turkey

Correspondence: Asuman Büyükkılıç Yanardağ (asuman.yanardag@ozal.edu.tr)**Received:** 23 February 2025 | **Revised:** 14 May 2025 | **Accepted:** 23 May 2025**Funding:** This work was supported by the Scientific and Technological Research Council of Türkiye (TÜBİTAK).**Keywords:** Alfisol | biochar | carbon sequestration | Entisol | Mollisol | *Nigella sativa* | residue | soil improvement

ABSTRACT

This study aimed to assess the effects of black cumin (*Nigella sativa*) residue and its biochar on soil microbial activity, carbon sequestration and nitrogen cycling in three soil types: Alfisol, Entisol and Mollisol. A 120-day incubation experiment was conducted to evaluate changes in soluble carbon (SC), microbial biomass carbon (MBC), soluble nitrogen (SN), microbial biomass nitrogen (MBN), CO₂ emissions, carbon use efficiency (CUE) and metabolic coefficient (qCO₂), calculated to understand microbial functionality and carbon retention. The results revealed that biochar (BC) enhanced long-term carbon stabilisation, while black cumin residue increased microbial activity and short-term nutrient turnover. In the BC2 treatment, SC remained stable until day 120 (419.2 mg/kg), suggesting sustained carbon availability. The highest MBC (528 mg kg⁻¹) and lowest CO₂ emissions were also recorded under biochar treatments, reflecting improved microbial efficiency. Significant interactions ($p < 0.001$) were observed between soil type, treatment and sampling time. These findings suggest that the combined use of biochar and plant residues can enhance both immediate microbial responses and long-term soil health, offering a sustainable strategy for soil fertility improvement and carbon management across different soil systems.

1 | Introduction

Global climate change and unsustainable agricultural practices have significantly intensified the urgency for sustainable soil management strategies. Deforestation, over-cultivation and mismanagement of organic resources have led to soil degradation, depleting soil organic matter (SOM), disrupting critical biogeochemical cycles and reducing the soil's capacity to retain carbon (C) and nitrogen (N). These processes exacerbate greenhouse gas emissions and reduce soil fertility, posing a serious threat to global food security (Bekchanova et al. 2024). Climate change further exacerbates these challenges by accelerating organic matter decomposition and altering nutrient cycles

through erratic precipitation patterns and rising temperatures (Büyükkılıç Yanardağ et al. 2020).

To mitigate these challenges, researchers are exploring innovative soil amendments that enhance SOM restoration and nutrient cycling to address these challenges. Organic amendments, such as biochar and plant residues, have emerged as promising solutions to improve soil health, increase carbon sequestration and reduce nitrogen losses. Biochar, a carbon-rich material produced through the pyrolysis of organic biomass under limited oxygen conditions, is recognised for its stability, porosity and nutrient retention properties, making it an effective long-term carbon sink (Bolan et al. 2022). Studies indicate

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2025 The Author(s). *Soil Use and Management* published by John Wiley & Sons Ltd on behalf of British Society of Soil Science.

that biochar enhances soil microbial communities and fosters beneficial plant–root interactions (Deng et al. 2023, 2025). Conversely, plant-derived residues, like those from *black cumin*, provide labile organic matter that stimulates rapid microbial activity and nutrient cycling (Salehi et al. 2017). While black cumin is traditionally valued for its health benefits, including antioxidant and anti-inflammatory properties (Yanardağ et al. 2015), its potential role in soil health management remains underexplored.

Plant-derived residues, like those from black cumin, provide labile organic matter that stimulates rapid microbial activity and nutrient cycling (Salehi et al. 2017). Their residues influence the carbon and nitrogen cycles, which are central to ecosystem stability and soil fertility. Carbon drives microbial metabolism, while nitrogen supports plant growth and enzymatic activities. The interaction between these elements is reflected in key soil metrics, including the carbon-to-nitrogen (C:N) ratio, microbial carbon use efficiency (CUE) and metabolic quotient (qCO_2). Black cumin is traditionally valued for its health benefits, including antioxidant and anti-inflammatory properties (Gholamnezhad et al. 2015), and its by-products are increasingly available due to the growth of cold-press oil industries across arid and semi-arid regions. Compared to other oilseed residues, such as those from sunflower or canola, black cumin residue contains higher levels of phenolic compounds and lignin, which may affect microbial community dynamics and carbon stability differently (Topcagic et al. 2017; Zribi et al. 2019). Moreover, its potential allelopathic effects and anti-pathogenic properties may suppress harmful microbes while stimulating beneficial microbial processes (Ahmad et al. 2020). Despite these promising attributes, its role in soil nutrient cycling and carbon sequestration remains underexplored. This study, therefore, focuses on the dual use of black cumin residue and biochar derived from the same feedstock, aiming to fill a gap in the literature regarding oilseed residue valorisation for soil health improvement.

The C:N ratio is a fundamental indicator of organic matter decomposition, nitrogen mineralisation, and microbial activity in soil. High C:N ratios ($> 30:1$) indicate slow decomposition and nitrogen immobilisation, which can limit plant-available nitrogen (Manzoni et al. 2012). Conversely, low C:N ratios ($< 10:1$) suggest rapid decomposition and nitrogen release but can lead to increased nitrogen leaching and greenhouse gas emissions (Zhou et al. 2017). A balanced C:N ratio is crucial for optimising microbial efficiency and sustaining long-term soil fertility.

CUE represents the proportion of organic carbon assimilated into microbial biomass versus that lost as CO_2 through respiration (Geyer et al. 2016). Higher CUE values indicate efficient microbial metabolism, enhancing carbon retention and stabilising SOM. Biochar amendments have been reported to improve CUE by increasing microbial habitat stability and reducing carbon losses (Giagnoni and Renella 2022).

The qCO_2 metric measures microbial respiration per unit of microbial biomass carbon, providing an indicator of microbial stress and efficiency (Anderson and Domsch 1990; Büyükkılıç Yanardağ et al. 2020). High qCO_2 values suggest microbial

communities under stress, leading to inefficient carbon utilisation and increased CO_2 emissions. In contrast, lower qCO_2 values indicate optimal microbial function with reduced energy loss (Anderson and Domsch 1995). The addition of biochar has been shown to decrease qCO_2 by improving microbial habitat conditions and enhancing carbon sequestration (Blagodatskaya and Kuzyakov 2013).

Biochar has demonstrated the ability to stabilise SOM and reduce greenhouse gas emissions through its impact on microbial community dynamics and nutrient retention (Li et al. 2018). Its porous structure enhances water retention and provides habitats for soil microorganisms, thereby improving soil structure and fertility (Ding et al. 2016). Meanwhile, black cumin residues offer readily available carbon and nitrogen, promoting short-term microbial proliferation and enhancing enzymatic activities (Yanardağ et al. 2017). The biochemical cycles driven by these amendments not only improve nutrient availability but also mitigate the risks of long-term carbon and nitrogen depletion.

Despite the growing body of literature on biochar applications, limited research has examined the combined use of biochar and plant-derived residues in addressing short- and long-term soil fertility. Black cumin-derived amendments present a unique approach by combining the immediate nutrient availability of residues with the stabilisation potential of biochar. Despite its biochemical richness, the agricultural use of black cumin residue has received limited attention in scientific literature. This is likely due to the plant's primary cultivation for pharmaceutical purposes, with most studies focusing on the therapeutic properties of its seeds and oils. Consequently, the remaining biomass is often undervalued or treated as waste. Furthermore, its cultivation is geographically concentrated in arid and semi-arid regions, limiting its exposure within global agronomic research networks, which traditionally focus on widely distributed cereal or oilseed crops. This study evaluates the effects of black cumin residue and biochar on soil properties, including SC, SN, MBC, MBN, CO_2 emissions, CUE and qCO_2 , over a 120-day period in Alfisols, Entisols and Mollisols. Alfisols, characterised by moderate fertility and clay-rich subsoil, are responsive to management interventions that enhance nutrient retention. Being younger and less developed, Entisols often lack organic matter, making them reliant on amendments for fertility improvement. Mollisols, naturally high in SOM, provide a unique context to evaluate the synergistic effects of amendments.

This study hypothesises that the combined application of black cumin residue and biochar will enhance soil carbon sequestration and improve nitrogen dynamics across different soil types. Specifically, biochar is expected to provide long-term stabilisation of organic matter, while black cumin residue will supply labile nutrients that stimulate microbial activity and nutrient cycling. This study aims to assess the impact of black cumin residue and biochar on soil SC and SN levels across Alfisols, Entisols and Mollisols; (to) evaluate changes in MBC and MBN in response to the amendments; (to) monitor CO_2 emissions to determine the effects on soil respiration and carbon turnover; and (to) analyse CUE and qCO_2 to understand microbial efficiency and metabolic responses. By exploring these dynamics,

this study aims to provide soil-specific recommendations for integrating black cumin derived amendments into sustainable agricultural practices.

2 | Materials and Methods

2.1 | Study Area and Climate Conditions

The study was conducted in the Yeşilyurt district of Malatya province, Eastern Turkey, a region with diverse topography comprising mountains, plateaus and fertile plains. The area represents a transitional zone between the continental precipitation regime of Southeastern Anatolia and the Mediterranean maritime precipitation regime. The annual mean precipitation (MAP) and temperature (MAT) are 383.6 mm and 13.7°C, respectively. Elevation varies between 1500 and 2000 m, influencing soil development and agricultural potential.

2.2 | Soil Collection and Characterisation

Surface soil samples (0–20 cm) were collected from Entisol (38°14'50.92" N 38°16'16.40" E), Mollisol (38°11'12.4" N 38°17'44.1" E) and Alfisol (38°11'25.2" N 38°18'53.8" E) sites. Samples were air-dried, sieved (< 2 mm) and analysed for physical and chemical properties, including pH (1:2.5 w/v soil:water), electrical conductivity (EC), soil organic carbon (SOC) using the Walkley-Black method, total nitrogen (TN) via Kjeldahl digestion and carbonate content using the Scheibler calcimeter method. Soil texture was determined using the hydrometer method (Bouyoucos 1962).

The main characteristics of the soils used in the experiment show significant differences in their chemical and physical properties. The soil pH values ranged from 7.15 in Mollisol, indicating a slightly acidic condition, to 7.76 in Alfisol, reflecting a near-neutral environment. The electrical conductivity (EC) values varied, with Mollisol having the highest EC (221.33 $\mu\text{S}/\text{cm}$), suggesting a greater ion exchange capacity, while Entisol exhibited the lowest EC (99.60 $\mu\text{S}/\text{cm}$), indicating limited nutrient mobility. Soil organic carbon (SOC) content was notably highest in Mollisol (10.18%), followed by Alfisol (2.16%) and Entisol (1.92%), correlating with their respective organic matter (OM) contents of 17.55%, 3.72% and 3.31%. The total nitrogen (TN) content was highest in Mollisol (0.51%), moderate in Alfisol (0.12%) and lowest in Entisol (0.05%). The C/N ratio was substantially higher in Entisol (40.00), which could limit nitrogen availability due to slower organic matter decomposition, whereas Alfisol (18.78) and Mollisol (19.81) exhibited balanced ratios conducive to microbial activity and plant nutrition. The clay content was highest in Alfisol (42.42%), indicating a higher water retention capacity, while Entisol had the lowest clay percentage (26.42%), making it more prone to drainage and erosion. The silt fraction was dominant in Alfisol (47.42%) and Entisol (43.42%), supporting moderate water retention and aeration properties. Sand content was highest in Entisol (30.16%), reinforcing its rapid drainage tendency, while Alfisol (10.16%) had the least sand content, ensuring better structural stability. The calcium carbonate (CaCO_3) content was highest in Entisol

(34.38%), suggesting alkaline conditions and potential nutrient fixation issues, whereas Alfisol (0.85%) and Mollisol (0.17%) had minimal carbonate presence, which is favourable for plant growth.

2.3 | Soil Amendments and Experimental Design

Five treatment groups were established: (Ahmad et al. 2020) Control (unamended soil), (Anderson and Domsch 1990) low-dose black cumin residue (BCP1, 5 g kg^{-1}), (Anderson and Domsch 1995) high-dose black cumin residue (BCP2, 10 g kg^{-1}), (Atkinson 2018) low-dose black cumin biochar (BC1, 5 g kg^{-1}), and (Bajgai et al. 2015) high-dose black cumin biochar (BC2, 10 g kg^{-1}). The black cumin residues were obtained as by-products of oil extraction, while biochar was produced via pyrolysis at 450°C in a muffle furnace.

Soil samples (300 g dry weight) were thoroughly mixed with amendments and incubated at 25°C under controlled moisture (55% of water holding capacity) for 120 days.

2.4 | Soil Chemical and Microbial Analyses

Soluble carbon (SC) and soluble nitrogen (SN): Extracted using 0.5 M K_2SO_4 and analysed via spectrophotometry (Vance et al. 1987). Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN): Determined using the fumigation-extraction method (Vance et al. 1987; Joergensen and Brookes 1990). Microbial respiration and CO_2 evolution: Measured using NaOH trapping followed by titration with HCl (Anderson and Domsch 1990).

Metabolic Quotient (qCO_2): Calculated as $\text{CO}_2\text{-C (mg C kg}^{-1} \text{ soil h}^{-1}) / \text{MBC (mg C kg}^{-1} \text{ soil)}$, where $\text{CO}_2\text{-C}$ is microbial respiration ($\text{mg C kg}^{-1} \text{ soil h}^{-1}$), and MBC is microbial biomass carbon ($\text{mg C kg}^{-1} \text{ soil}$) according to Anderson and Domsch (1995). Microbial Efficiency (ME): Calculated for carbon (MBC/SC) and nitrogen (MBN/SN) (Wardle 1992). Carbon use efficiency (CUE): Calculated to reflect the proportion of carbon retained in microbial biomass versus lost via respiration (Manzoni et al. 2012). Total carbon storage (TCS): Estimated as providing insights into the soil's carbon sequestration potential (Bekchanova et al. 2024).

2.5 | Fourier-Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of biochar–residue-amended soils were recorded with a Bruker Alpha II spectrometer equipped with a DTGS detector. Oven-dried (< 40°C) and finely ground (< 75 μm) samples were homogenised (1 mg sample + 100 mg KBr) and pressed into pellets under 7 t. Spectra were acquired in the mid-IR range (4000–400 cm^{-1}) at 4 cm^{-1} resolution, averaging 32 scans. Baseline correction and vector normalisation were performed in OPUS 8.2. Principal absorption bands were assigned according to Kögel-Knabner (2002) and Hagemann et al. (2018) to track carbohydrate (1034 cm^{-1}), aliphatic (2920 cm^{-1}) and aromatic (1620 cm^{-1}) domains.

2.6 | Biochar Production

Black cumin residue was pyrolyzed at 420°C in a laboratory-grade muffle furnace. The pyrolysis process involved a heating rate of 10°C min⁻¹ and a residence time of 2 h. The process was carried out under limited oxygen conditions (oxygen-limited pyrolysis) without the introduction of an external carrier gas such as nitrogen. The resulting biochar was allowed to cool in a desiccator and then ground to pass through a 2 mm sieve before use.

2.7 | Amendment Dosage Rationale

Two application rates were used: 5 g kg⁻¹ (low dose) and 10 g kg⁻¹ (high dose) of either biochar or black cumin residue. These rates were chosen based on benchmarks from related incubation studies involving biochar and crop residues (Lehmann et al. 2011; Liang et al. 2021), and reflect equivalent field application rates ranging from 10 to 20 t ha⁻¹. This allowed for comparison with existing literature while maintaining practical relevance.

2.8 | Statistical Analyses

Two-way ANOVA was performed to assess treatment effects and interactions, followed by Tukey's HSD test ($p < 0.05$). Principal component analysis (PCA) and hierarchical clustering heat map analysis were conducted to visualise soil biochemical relationships. All statistical analyses were performed using R (v4.2) and Python (v3.11) with ggplot2 and seaborn libraries for visualisation. All pairwise statistical comparisons for each parameter across soil types, treatments and time points are provided in Tables S1–S3.

This comprehensive approach ensures a detailed understanding of soil biochemical processes under black cumin-derived amendments and their implications for sustainable soil management.

3 | Results

3.1 | Soil Organic Matter (SOM)

The study assessed the impact of various soil amendments: Black cumin residue, biochar and their combination on soil organic matter (SOM) content across three soil types, Alfisol, Entisol and Mollisol (Table 1). A summary of treatment effects is shown below; full statistical comparisons by soil type, treatment and incubation time are provided in Tables S1–S3.

TABLE 1 | Organic matter levels (%) in Alfisol, Entisol and Mollisol soils.

Soil type	Control	Black cumin seed residue	Biochar	Black cumin biochar
Alfisol	3.1%	3.5%	3.4%	3.7%
Entisol	1.2%	1.6%	1.5%	1.9%
Mollisol	6.0%	6.4%	6.5%	6.9%

The control group exhibited a SOM content of 3.1%. The application of black cumin residue increased SOM to 3.5%, biochar to 3.4%, and the combination of black cumin residue and biochar to 3.7% in Alfisol. The control group had a SOM content of 1.2%. Black cumin residue application raised it to 1.6%, biochar to 1.5% and the combined treatment to 1.9% in Entisol. The control group's SOM was 6.0%. With black cumin residue, it increased to 6.4%; biochar application resulted in 6.5%; and the combination treatment elevated it to 6.9% in Mollisol.

3.2 | FTIR Spectral Analysis Across Soil Types

The FTIR spectra for Alfisol, Entisol and Mollisol show distinct patterns in organic matter composition influenced by the application of black cumin residue, biochar and their combination

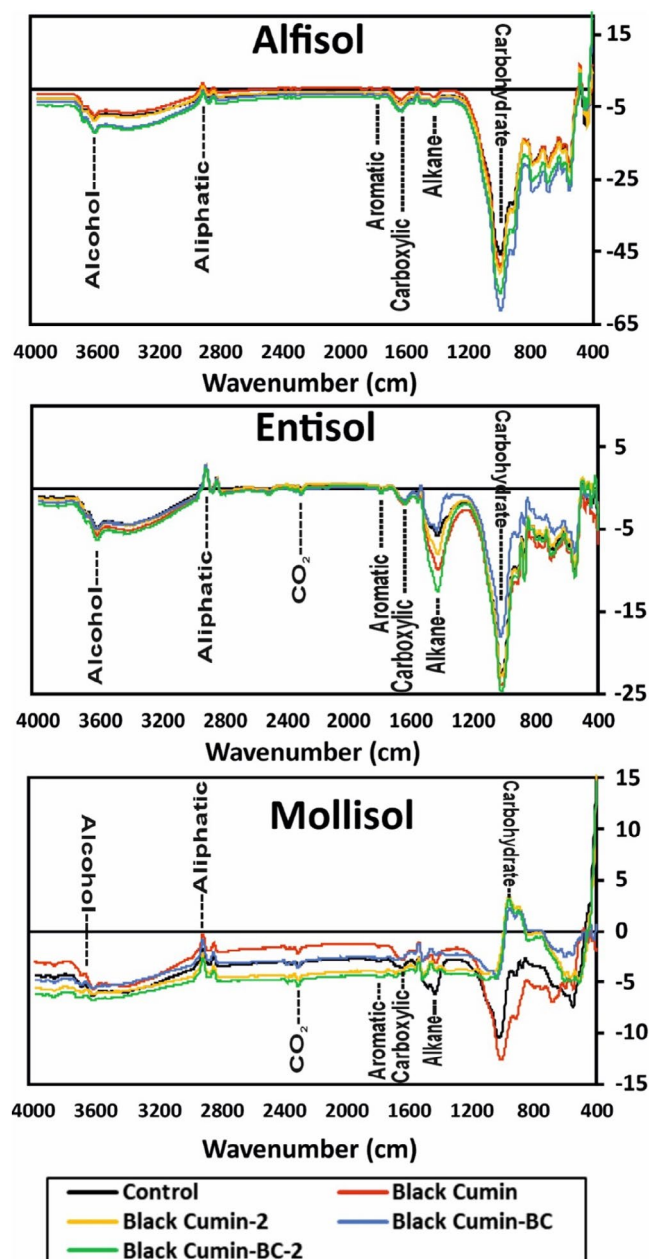


FIGURE 1 | FTIR spectra of Alfisol, Entisol and Mollisol soils after 120-day incubation with black cumin residue and biochar treatments.

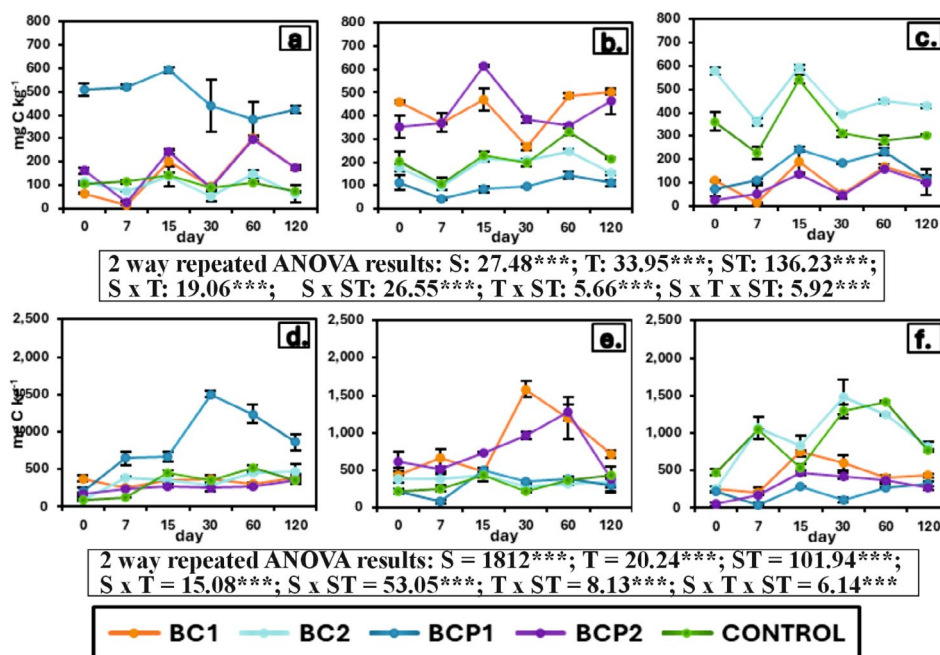


FIGURE 2 | Evolution of soluble C (a: Alfisol, b: Entisol and c: Mollisol) and microbial biomass C (d: Alfisol, e: Entisol and f: Mollisol) incubated for 120 days with black cumin residue, its biochar and control. Error bars denote standard deviation ($n=3$). F values and significance of the two-way repeated measures ANOVA are shown in each graph. S = soil type, ST = sampling time, and T = treatment. Significant at *** $p < 0.001$. BC1 = black cumin biochar dose 1, BC2 = black cumin biochar dose 2, and BCP1 = black cumin residue dose 1, BCP2 = black cumin residue dose 2.

(Figure 1). The control samples (black lines) serve as the baseline reference, highlighting changes induced by different amendments.

In Alfisol, spectral variations indicate moderate changes in organic functional groups across treatments. The most prominent peaks appear around 1600 and 1200 cm⁻¹, corresponding to C=O stretching in carboxyl groups and C-O stretching in polysaccharides. The application of black cumin residue (red and yellow lines) caused a slight increase in peak intensity, indicating the presence of fresh organic compounds. The biochar treatments (blue and green lines) exhibited more stable spectral patterns, suggesting higher carbon stability and slower decomposition compared to the black cumin residue alone.

In Entisol, spectral changes were more pronounced, reflecting its lower initial organic matter content. The most significant differences were observed between control and biochar treatments, where biochar-amended soils showed higher absorbance in the 1400–1600 cm⁻¹ region, indicative of enhanced aromatic and carboxyl functional groups. The combined black cumin residue and biochar treatment (blue and green lines) led to a more uniform spectral pattern, suggesting that biochar enhanced carbon retention by adsorbing labile organic compounds from the black cumin residue.

In Mollisol, which initially contained higher organic matter, spectral changes were less dramatic. However, around 2800–3000 cm⁻¹, noticeable shifts were observed, corresponding to C-H stretching from aliphatic compounds. The black cumin residue treatments (red and yellow lines) increased peak intensities in this region, indicating higher microbial activity and decomposition of plant-derived inputs. In contrast, biochar treatments (blue and green lines) led to flattened spectral curves, reflecting

a more recalcitrant carbon structure with reduced microbial degradation.

3.3 | Soluble C and Microbial Biomass C (MBC) Response to Soil Amendments

The application of black cumin residue, biochar and their combination significantly influenced soluble carbon (SC) levels across the three different soil types: Alfisol, Entisol and Mollisol (Figure 2). The control groups exhibited the lowest SC values in all soil types, while the amended treatments led to notable increases. The results indicate distinct SC accumulation and retention patterns, depending on the amendment type and soil characteristics.

In Alfisol, SC levels in the control group remained relatively low throughout the incubation period. The application of black cumin residue alone resulted in a moderate increase in SC, likely due to the rapid decomposition of organic residues. Biochar treatments (BC1 and BC2) initially showed a greater SC increase, with BC2 reaching the highest SC levels on Day 0 (179.2 mg/kg). However, SC levels gradually declined over time. The combined treatment of biochar and black cumin residue (BCP + BC) led to the most sustained SC levels, suggesting a balance between immediate organic matter availability and long-term carbon stabilisation.

In Entisol, a soil type characterised by low organic matter content, SC levels were initially low across all treatments. The addition of black cumin residue significantly increased SC, reflecting the input of easily decomposable organic material. Biochar applications (BC1 and BC2) had a marked effect, particularly at higher doses, where SC levels remained elevated longer than in Alfisol. The combined treatment produced the highest SC levels

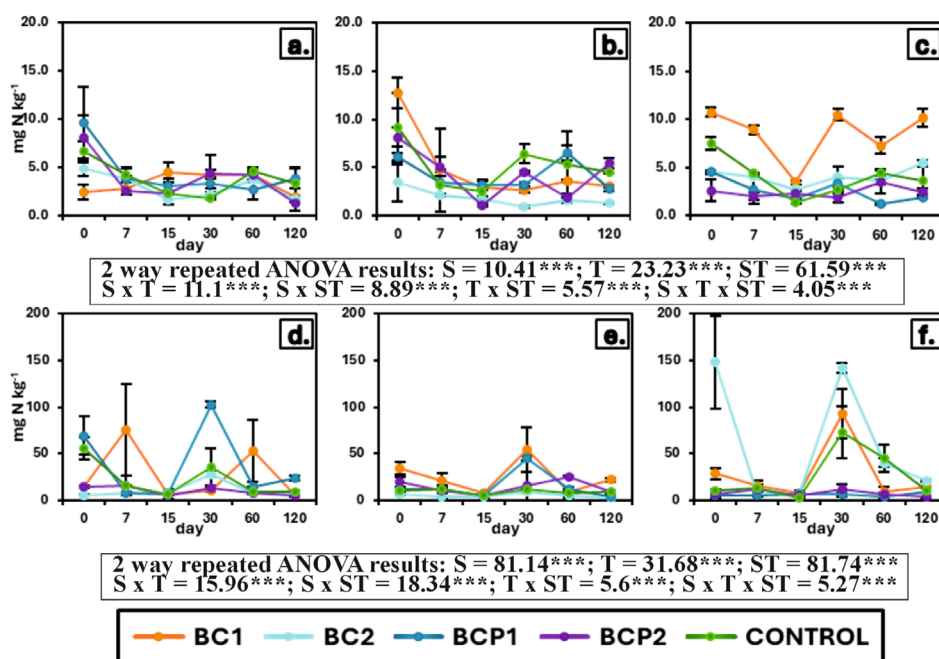


FIGURE 3 | Evolution of soluble N (a: Alfisol, b: Entisol and c: Mollisol), and microbial biomass N (d: Alfisol, e: Entisol, and f: Mollisol) incubated for 120 days with black cumin residue, its biochar, and control. Error bars denote standard deviation ($n=3$). F values and significance of the two-way repeated measures ANOVA are shown in each graph. S=soil type, ST=sampling time, and T=treatment. Significant at *** $p<0.001$; ns=not significant ($p>0.05$). BC1=black cumin biochar dose 1, BC2=black cumin biochar dose 2, BCP1=black cumin residue dose 1, and BCP2=black cumin residue dose 2.

over time, indicating that biochar helped retain soluble carbon derived from plant residues, preventing its rapid loss.

In Mollisol, which naturally contains high organic matter, the control group already exhibited relatively high SC levels. However, treatments further enhanced SC content, with biochar applications, particularly BC2, leading to the highest observed SC values. The combination of biochar and black cumin residue showed a more stable SC trend over time, reinforcing the role of biochar in moderating carbon turnover in soils with high organic matter content.

The application of black cumin residue, biochar, and their combination significantly influenced microbial biomass carbon (MBC) across the three soil types: Alfisol, Entisol and Mollisol (Figure 2). The control groups exhibited the lowest MBC levels, whereas the treatments led to significant increases, with varying effects depending on soil type and amendment composition. According to Tukey's HSD test results, microbial biomass carbon (MBC) was significantly higher in the BC2 treatment compared to BCP2 at Day 120 in all three soil types ($p<0.05$). For full ANOVA and post hoc results, see Tables S1–S3.

In Alfisol, MBC levels in the control group remained relatively low. Biochar treatments (BC1, BC2) showed a moderate increase in MBC, with BC1 leading to a greater microbial biomass accumulation than BC2 at early stages. The highest MBC was observed in the combined black cumin residue and biochar treatment, suggesting a synergistic effect between plant residues and biochar in promoting microbial growth.

In Entisol, the control group had the lowest MBC levels among all soil types. The application of black cumin residue significantly enhanced microbial biomass, likely due to its high degradability and immediate nutrient availability. Biochar treatments alone had a positive but lesser impact, indicating that Entisol microbes responded more effectively to labile organic inputs rather than the stable carbon fraction of biochar. The combined treatment exhibited the greatest MBC enhancement, demonstrating that biochar helped retain microbial-derived carbon while black cumin residue was an energy source for microbial proliferation.

In Mollisol, where the control group already exhibited high MBC levels due to its naturally high organic matter, biochar treatments resulted in a less pronounced increase than Alfisol and Entisol. However, the highest MBC was again observed in the combined treatment, indicating that biochar may contribute to microbial habitat formation rather than direct biomass stimulation.

3.4 | Soluble Nitrogen (SN) and Microbial Biomass Nitrogen (MBN) Response to Soil Amendments

The application of black cumin residue, biochar and their combination significantly influenced soluble nitrogen (SN) and microbial biomass nitrogen (MBN) levels across Alfisol, Entisol and Mollisol (Figure 3). The control groups exhibited the lowest SN and MBN levels, while organic amendments led to notable increases, with different effects depending on soil type and amendment composition.

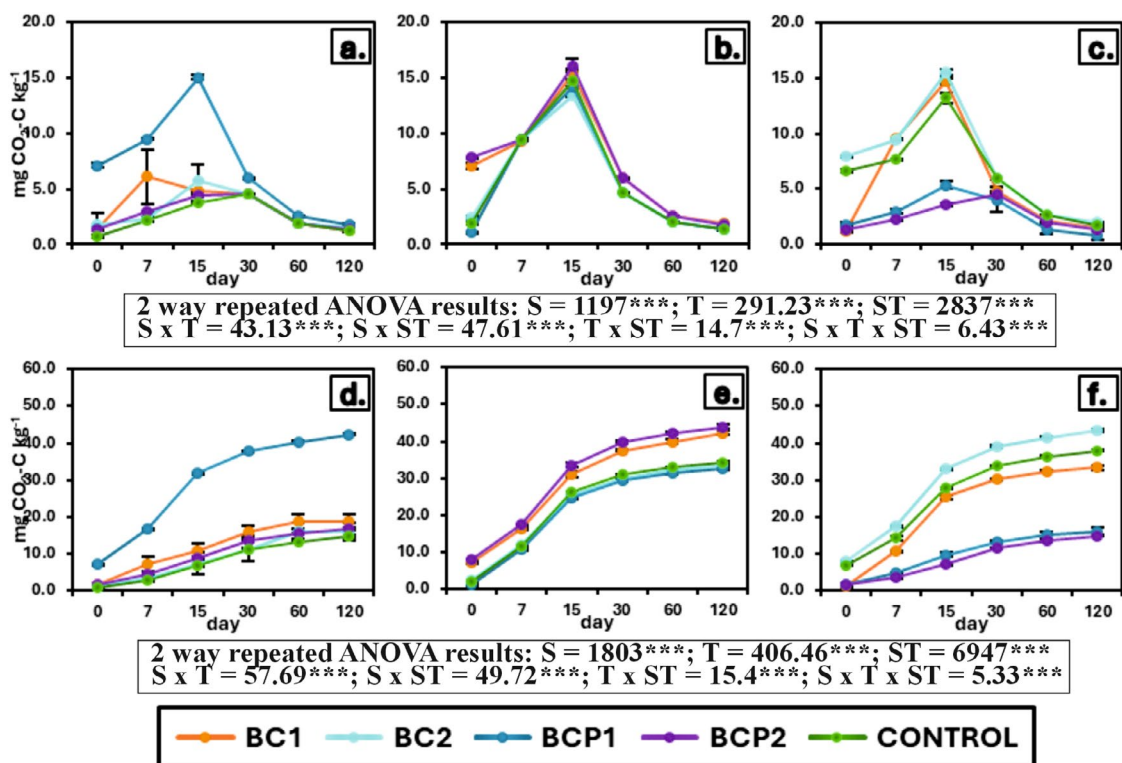


FIGURE 4 | Evolution of CO₂ emission (a: Alfisol, b: Entisol and c: Mollisol) and Microbial Respiration (qCO₂) (d: Alfisol, e: Entisol, and f: Mollisol) incubated for 120 days with black cumin residue, its biochar, and control. Error bars denote standard deviation ($n=3$). F values and significance of the two-way repeated measures ANOVA are shown in each graph. S = soil type, ST = sampling time, and T = treatment. Significant at *** $p < 0.001$. BC1 = black cumin biochar dose 1, BC2 = black cumin biochar dose 2, BCP1 = black cumin residue dose 1, and BCP2 = black cumin residue dose 2.

In Alfisol, SN levels were relatively low in the control treatment but increased substantially following amendments. The application of black cumin residue alone led to a rapid increase in SN, suggesting the immediate mineralisation of organic nitrogen from plant residues. Biochar applications (BC1, BC2) had a stabilising effect, with BC2 showing the highest SN levels at Day 0 (11.43 mg/kg). The combination of biochar and black cumin residue resulted in a sustained increase in SN, indicating improved nitrogen retention and reduced leaching. MBN levels followed a similar trend, with the highest values observed in the combined treatment, confirming that biochar helped retain nitrogen within microbial biomass.

In Entisol, which initially exhibited low SN and MBN levels due to its poor organic matter content, amendments significantly increased both parameters. The application of black cumin residue alone resulted in an immediate rise in SN, whereas biochar treatments led to a more gradual and sustained increase. The combined treatment showed the highest SN and MBN levels, suggesting that biochar enhanced microbial nitrogen assimilation and reduced nitrogen loss.

In Mollisol, which naturally contains high organic matter and nitrogen, the control group already had higher SN and MBN values compared to Alfisol and Entisol. However, amendments further enhanced nitrogen availability. Biochar treatments alone resulted in moderate SN increases, while the combined treatment led to the highest SN and MBN levels, indicating that

biochar contributed to nitrogen stabilisation and reduced microbial nitrogen turnover.

3.5 | CO₂ and Cumulative CO₂ Emissions (qCO₂) Response to Soil Amendments

The application of black cumin residue, biochar and their combination significantly influenced CO₂ emissions and cumulative CO₂ emission (qCO₂) across Alfisol, Entisol and Mollisol (Figure 4). The control groups exhibited the lowest CO₂ release. In contrast, organic amendments led to respiration patterns and carbon turnover variations.

In Alfisol, CO₂ emissions were moderate in the control group but increased significantly following amendments. The highest CO₂ emissions were observed in the black cumin residue treatments, indicating rapid microbial activity and organic matter decomposition. Biochar treatments resulted in lower but sustained CO₂ emissions, suggesting a more stable carbon retention effect. The combined treatment exhibited moderate CO₂ emissions initially but maintained a more gradual and prolonged release over time. The qCO₂ values followed a similar pattern, with biochar reducing microbial stress and enhancing carbon use efficiency.

In Entisol, which initially had low microbial activity, CO₂ emissions were significantly enhanced by all treatments.

The black cumin residue application led to an early surge in CO₂ emissions, indicating a rapid mineralisation process. In contrast, biochar applications led to lower but prolonged CO₂ emissions, confirming its role in stabilising microbial respiration. The combined treatment resulted in a more balanced CO₂ release pattern, with the lowest qCO₂ values, indicating efficient microbial metabolism and reduced respiratory carbon losses.

In Mollisol, the control group already exhibited relatively high CO₂ emissions due to its naturally high organic matter content. However, biochar treatments lowered overall respiration rates, suggesting better carbon stabilisation and reduced microbial stress. The combination of biochar and black cumin residue maintained moderate CO₂ emissions while optimising microbial efficiency, reflected by the lower qCO₂ values.

The application of black cumin residue, biochar and their combination significantly influenced respiration rate (Resp_R) and microbial respiration (qCO₂) across Alfisol, Entisol and Mollisol (Figure 4). The control groups exhibited the lowest respiration activity. In contrast, the treatments made noticeable changes depending on amendment type and soil characteristics.

In Alfisol, the control group maintained a relatively stable and low respiration rate throughout the incubation period. The application of black cumin residue alone resulted in a sharp increase in respiration rate, indicating rapid microbial metabolism due to readily degradable organic matter availability. Biochar applications (BC1 and BC2) exhibited lower but more sustained respiration activity, suggesting that biochar contributed to microbial stabilisation rather than an immediate increase in microbial activity. The combined treatment showed the highest microbial respiration (qCO₂) levels, implying that biochar moderated microbial activity while promoting carbon retention.

All amendments significantly increased respiration rates in Entisol, which initially had the lowest microbial activity and organic content. Black cumin residue alone led to the highest initial peak, reflecting its role as a fast-decomposing organic amendment. Biochar treatments resulted in lower respiration rates but prolonged microbial activity, indicating a shift toward more efficient microbial carbon use. The combined treatment resulted in moderate respiration rates with prolonged microbial activity, suggesting an optimal balance between nutrient availability and carbon stabilisation.

In Mollisol, where organic content was already high, respiration rates were initially high in the control group. Amendments led to more moderate increases in microbial respiration, as the existing organic matter content buffered the effects of additional inputs. Biochar alone resulted in the lowest qCO₂ values, supporting the idea that biochar acts more as a stabilising agent than an immediate carbon source. The combination of black cumin residue and biochar led to slightly higher qCO₂ values, but the response was less pronounced than Alfisol and Entisol.

3.6 | Mineralisable Carbon (MEC), Mineralisable Nitrogen (MEN) and MBC/MBN Ratio Response to Soil Amendments

The application of black cumin residue, biochar and their combination significantly influenced mineralisable extractable carbon (MEC) and mineralisable extractable nitrogen (MEN) across different soil types (Alfisol, Entisol and Mollisol) (Figure 5). The control treatments exhibited the lowest MEC and MEN values, whereas organic amendments led to notable changes, with variations depending on the amendment type and soil characteristics.

In Alfisol, MEC levels were initially low in the control group. Black cumin residue application significantly increased MEC, with the highest values observed in the early incubation period. Biochar treatments (BC1 and BC2) resulted in a moderate increase in MEC, with BC2 showing a more sustained release over time. The combined treatment exhibited the highest overall MEC values, suggesting that biochar helped stabilise carbon inputs from black cumin residue, preventing rapid mineralisation losses. Similarly, MEN levels followed the same trend, with the highest microbial nitrogen mineralisation occurring in the combined treatment, indicating enhanced nitrogen cycling.

In Entisol, which initially had low carbon and nitrogen content, MEC and MEN values showed the largest relative increases following amendments. The highest MEC levels were observed in black cumin residue alone, suggesting rapid microbial decomposition of plant residues and release of mineralisable carbon. Biochar treatments exhibited a slower but more sustained increase in MEC, indicating improved carbon stabilisation. MEN levels were highest in the combined treatment, demonstrating that biochar contributed to nitrogen retention, reducing nitrogen losses through leaching.

MEC and MEN values exhibited less pronounced relative changes in Mollisol, where organic matter content was naturally high. Biochar treatments resulted in moderate increases in MEC and MEN, while the combined treatment still exhibited the highest sustained levels, reinforcing biochar's role in stabilising mineralisable carbon and nitrogen.

The microbial biomass carbon to microbial biomass nitrogen (MBC/MBN) ratio varied significantly across different soil types and treatments, reflecting changes in microbial efficiency and nutrient cycling dynamics (Figure 5). The control groups exhibited relatively stable MBC/MBN values. At the same time, amendments led to distinct changes, with differences observed between biochar, black cumin residue and their combination.

In Alfisol, the control MBC/MBN ratio remained at moderate levels. Biochar applications (BC1 and BC2) led to a notable decrease in MBC/MBN values over time, with BC2 exhibiting a stronger reduction. The combined treatment showed the highest stability, indicating that biochar moderated microbial nitrogen uptake while promoting carbon retention.

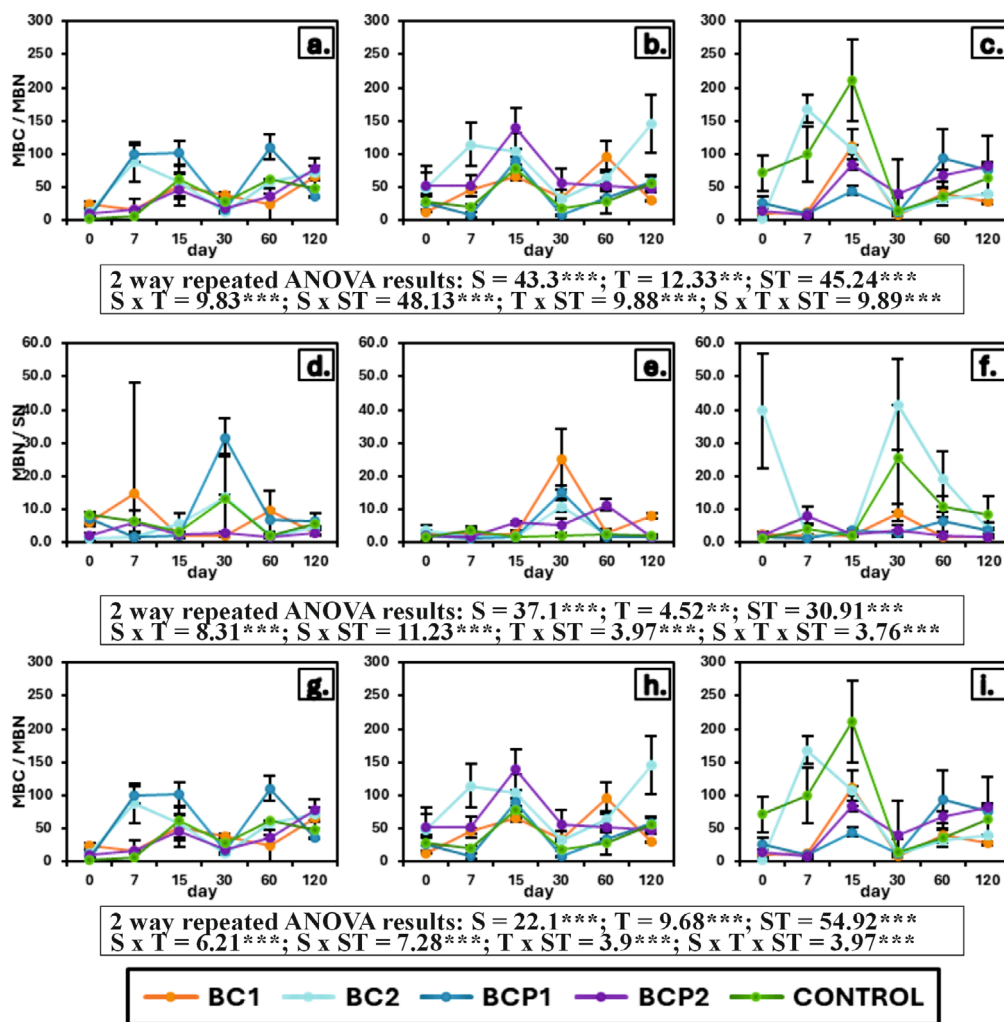


FIGURE 5 | Evolution of mineralisable C ratio (a: Alfisol, b: Entisol and c: Mollisol), mineralisable N ratio (d: Alfisol, e: Entisol, and f: Mollisol), and microbial biomass C to microbial biomass N ratio (g: Alfisol, h: Entisol, and i: Mollisol) incubated for 120 days with black cumin residue, its biochar, and control. Error bars denote standard deviation ($n=3$). F values and significance of the two-way repeated measures ANOVA are shown in each graph. S=soil type, ST=sampling time, and T=treatment. Significant at ** $p < 0.01$ and *** $p < 0.001$. BC1=black cumin biochar dose 1, BC2=black cumin biochar dose 2, BCP1=black cumin residue dose 1, and BCP2=black cumin residue dose 2.

In Entisol, which had the lowest initial organic content, MBC/MBN ratios exhibited the most significant changes following amendments. The application of black cumin residue increased MBC/MBN ratios, suggesting enhanced microbial activity and carbon utilisation efficiency. In contrast, biochar treatments led to lower MBC/MBN values, particularly at higher doses, indicating that biochar limited excessive microbial nitrogen mineralisation and favoured more efficient nitrogen cycling.

MBC/MBN ratios were more stable across treatments in Mollisol, which naturally has high organic matter. Biochar alone did not significantly alter MBC/MBN values. Still, the combination of biochar and black cumin residue led to a slight increase, suggesting improved microbial balance and nitrogen assimilation over time.

3.7 | Total Carbon Sequestration (TCS) and Carbon Use Efficiency (CUE) Response to Soil Amendments

The application of black cumin residue, biochar and their combination significantly influenced total carbon sequestration

(TCS) and carbon use efficiency (CUE) across Alfisol, Entisol and Mollisol (Figure 6). The control groups exhibited the lowest TCS values. At the same time, organic amendments contributed to substantial increases, with variations depending on amendment type and soil characteristics.

In Alfisol, biochar treatments (BC1, BC2) resulted in higher TCS values, with BC1 having the most pronounced effect at early stages. The combined treatment of biochar and black cumin residue exhibited the highest TCS levels, suggesting that biochar facilitated long-term carbon retention while organic residues enhanced microbial activity. CUE values followed a similar trend, with the combined treatment yielding the most efficient microbial carbon use over time.

In Entisol, which initially had low organic matter, the increase in TCS was the most significant relative to the control, particularly under biochar treatments. The combined treatment resulted in the highest TCS values, indicating enhanced carbon stabilisation in a soil system that otherwise has high carbon loss potential. CUE values in Entisol were moderate

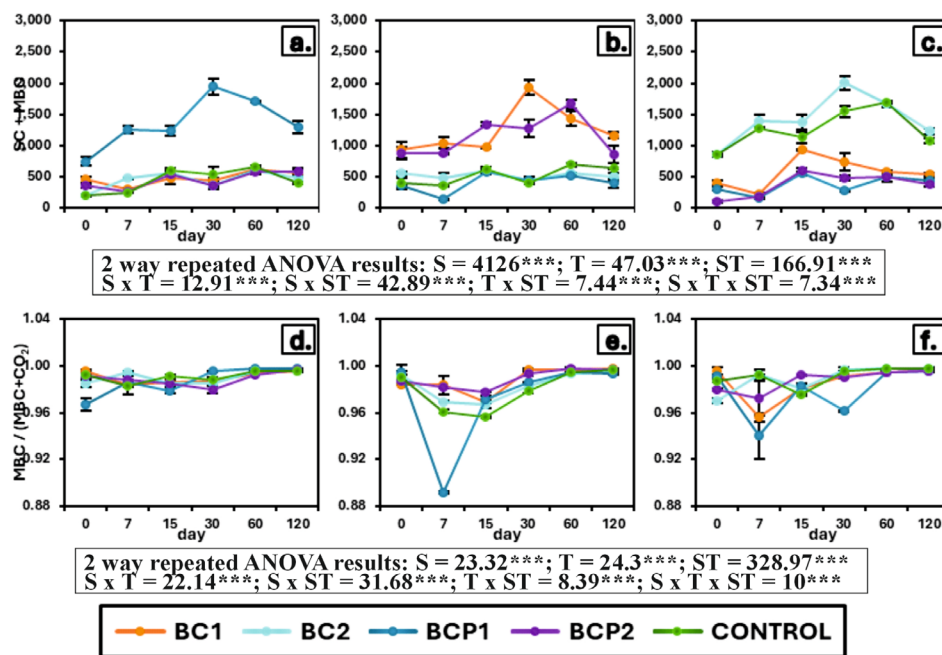


FIGURE 6 | Evolution of total C sequestration (a: Alfisol, b: Entisol and c: Mollisol), and C use efficiency (d: Alfisol, e: Entisol and f: Mollisol) incubated for 120 days with black cumin residue, its biochar and control. Error bars denote standard deviation ($n=3$). F values and significance of the two-way repeated measures ANOVA are shown in each graph. S = soil type, ST = sampling time, and T = treatment. Significant at *** $p < 0.001$. BC1 = black cumin biochar dose 1, BC2 = black cumin biochar dose 2, BCP1 = black cumin residue dose 1, and BCP2 = black cumin residue dose 2.

in biochar-only treatments but increased in the presence of organic residues, reflecting an improvement in microbial carbon retention.

In Mollisol, where organic matter content was naturally high, biochar and organic amendments still contributed to further carbon accumulation, but the relative increase was smaller than in the other soils. The highest TCS values were recorded in the biochar-only treatment, suggesting that biochar contributed to long-term carbon sequestration rather than short-term microbial activity. CUE values remained relatively stable, indicating that microbial communities in Mollisols were already efficient in utilising organic carbon, with biochar having a more passive role in microbial carbon turnover.

3.8 | Nitrogen Mineralisation Rate (NMR) and Respiration Ratio Response to Soil Amendments

The nitrogen mineralisation rate (NMR) varied significantly across soil types and treatments, indicating differences in microbial nitrogen cycling dynamics (Figure 7). The control groups exhibited the lowest NMR values, while biochar, black cumin residue and their combination influenced nitrogen mineralisation in distinct ways depending on soil characteristics.

In Alfisol, the control treatment exhibited low nitrogen mineralisation rates, reflecting moderate organic matter decomposition. Applying black cumin residue alone resulted in a rapid increase in NMR, suggesting that organic nitrogen from plant residues was quickly converted into mineral forms. Biochar treatments (BC1 and BC2) moderated nitrogen mineralisation, with BC2 leading to a more gradual and sustained increase. The combined

treatment resulted in the highest NMR values, indicating that biochar played a role in slowing down nitrogen losses while enhancing microbial nitrogen cycling.

In Entisol, which initially had low organic nitrogen content, all amendments significantly increased NMR. The highest increase was observed in black cumin residue treatments, where microbial communities rapidly decomposed plant-derived nitrogen, making it available for plant uptake. Biochar treatments exhibited a slower but more stable increase in NMR, suggesting that biochar adsorbed and gradually released nitrogen, preventing excessive leaching. The combined treatment exhibited moderate nitrogen mineralisation, balancing short-term availability and long-term retention.

The control group already had relatively high NMR values in Mollisol, which naturally has higher nitrogen content. Amendments led to more stable increases in NMR, with biochar alone contributing to a slower release of mineral nitrogen. The combined treatment resulted in slightly increased nitrogen mineralisation, reinforcing biochar's role in nitrogen stabilisation rather than immediate mineralisation enhancement.

The respiration ratio (Resp_R) varied across soil types, indicating differences in microbial activity and carbon mineralisation potential (Figure 7). Respiration ratios ranged from 0.0038 to 0.6346, with a mean of 0.0476 and a median of 0.0146. The standard deviation was 0.1051, suggesting moderate variability in Alfisol. The highest respiration ratios were observed in this soil type, ranging from 0.0056 to 0.9171. The mean was 0.0656, with a median of 0.0171. The high standard deviation (0.1585) indicates substantial variation among treatments in Entisol. Respiration ratios were the lowest among the soil types, ranging from 0.0030 to 0.0349, with a mean of 0.0151 and a median of

0.0143. The low standard deviation (0.0098) suggests more consistent microbial activity in this Mollisol.

3.9 | Correlation Coefficient Analysis of Between Soil Parameters and Alfisol, Entisol, and Mollisol Soils

The correlation matrices illustrate the relationships between key soil biochemical parameters, including soluble carbon (SC),

microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), microbial respiration rate (Resp_R), nitrogen mineralisation rate (NMR), total carbon sequestration (TCS) and microbial efficiency indicators across Alfisol, Entisol and Mollisol (Figure 8). The observed correlations provide insights into how biochar and black cumin residue amendments influence soil carbon and nitrogen cycling and microbial activity.

In Mollisol, where organic matter content is naturally high, SC and MBC showed a strong positive correlation ($r=0.46$),

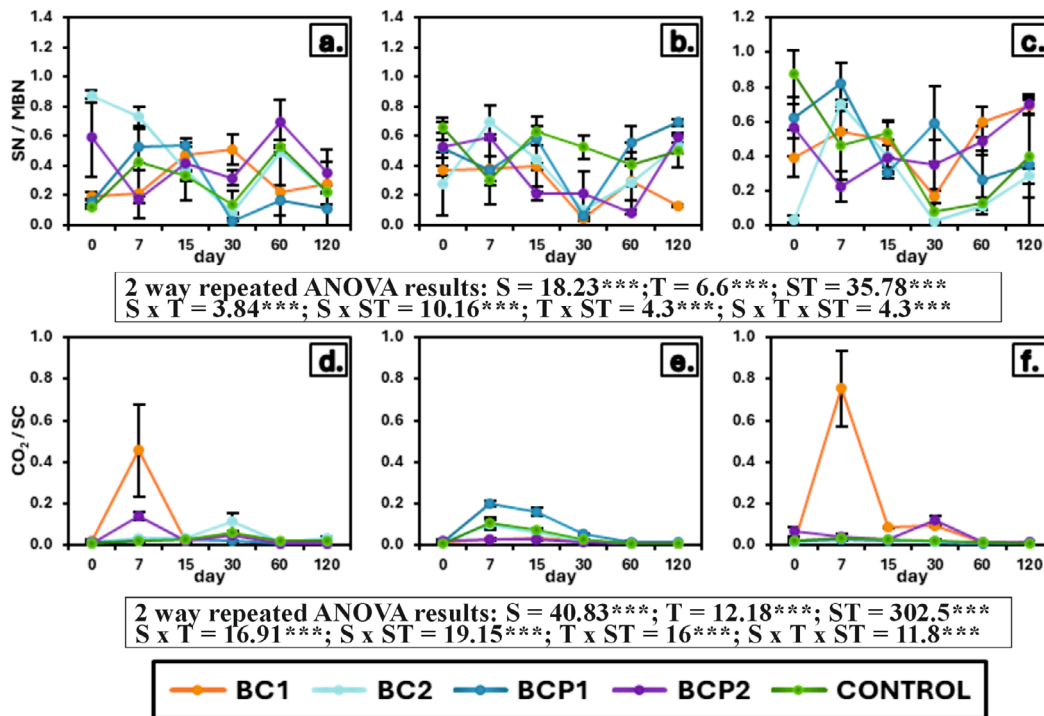


FIGURE 7 | Evolution of nitrogen mineralisation ratio (a: Alfisol, b: Entisol and c: Mollisol), and Evolution of respiration ratio (d: Alfisol, e: Entisol and f: Mollisol) incubated for 120 days with black cumin residue, its biochar, and control. Error bars denote standard deviation ($n=3$). F values and significance of the two-way repeated measures ANOVA are shown in each graph. S = soil type, ST = sampling time, and T = treatment. Significant at *** $p < 0.001$. BC1 = black cumin biochar dose 1, BC2 = black cumin biochar dose 2, BCP1 = black cumin residue dose 1, and BCP2 = black cumin residue dose 2.

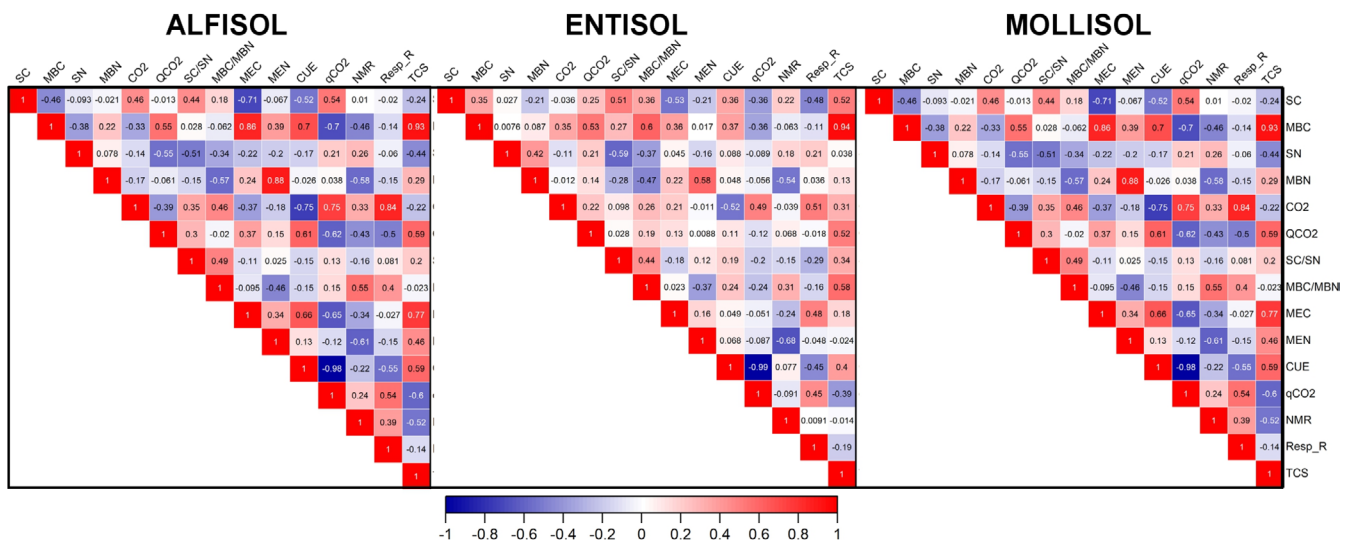


FIGURE 8 | Correlation coefficient analysis of soil parameters in Alfisol, Entisol and Mollisol soils.

indicating that higher soluble carbon availability directly supported microbial biomass growth. Conversely, the relationship was weaker in Alfisol ($r=0.18$) and Entisol ($r=0.35$), suggesting that factors beyond soluble carbon, such as microbial substrate quality and nutrient availability, influenced microbial biomass formation.

Across all soils, MBC/MBN showed a positive correlation with total carbon sequestration (TCS), suggesting that microbial communities contributed to long-term carbon retention. This correlation was the strongest in Entisol ($r=0.58$), followed by Alfisol ($r=0.44$) and the weakest in Mollisol, where pre-existing organic matter may have reduced microbial dependency on external nitrogen sources.

A strong negative correlation between Resp_R and CUE was observed in Mollisol ($r=-0.55$), followed by Alfisol ($r=-0.48$) and Entisol ($r=-0.45$). It indicates that higher microbial respiration rates led to reduced microbial carbon retention, increasing carbon losses via CO₂ emissions.

CO₂ emissions (qCO₂) were positively correlated with nitrogen mineralisation (NMR) across all three soils, with the strongest correlation in Entisol ($r=0.49$), indicating that as microbial activity increased, nitrogen availability also rose. In Alfisol and Mollisol, the correlation was weaker, suggesting that biochar amendments may have moderated nitrogen mineralisation rates by adsorbing and gradually releasing nitrogen.

SC and TCS showed a strong positive correlation ($r=0.69$), highlighting the role of carbon-rich amendments in enhancing long-term carbon storage in Alfisol. The correlation between MBC and TCS was highest ($r=0.94$), suggesting that microbial biomass stability was a key factor in carbon sequestration in Entisol. MBC and CO₂ emissions were positively correlated ($r=0.7$), indicating that high microbial respiration rates contributed to organic matter decomposition in Mollisol.

3.10 | Hierarchical Clustering Heatmap Between Treatments and Soil Properties

The heatmap clustering results for Alfisol, Entisol and Mollisol provide insights into the relationships between microbial activity, carbon and nitrogen cycling, and soil amendment effects (Figure 9). Each soil type exhibited distinct clustering patterns, revealing how different treatments (control, biochar and black cumin residue) influenced soil biochemical parameters.

The clustering analysis revealed a strong association between microbial biomass nitrogen (MBN) and mineralisable extractable carbon (MEC) in BCP1 treatments, suggesting that black cumin residue enhanced nitrogen cycling and microbial activity in Alfisol. Treatments containing biochar (BC1 and BC2) were grouped separately, indicating a stabilising effect on soil properties rather than immediate microbial stimulation.

Respiration rate (Resp_R) and CO₂ emissions (qCO₂) clustered closely in the control and high-dose biochar treatments (BC2),

suggesting that in the absence of plant residues, biochar did not significantly enhance microbial respiration. Soluble carbon (SC) and microbial biomass carbon (MBC) clustered closely in the BCP1 treatment, reinforcing that plant-derived organic matter contributed to short-term microbial activity.

In Entisol, the heatmap revealed a strong correlation between total carbon sequestration (TCS) and microbial biomass carbon (MBC), particularly in the BCP2 treatment. It suggests that the combined application of biochar and black cumin residue effectively enhanced microbial carbon retention in this low-fertility soil.

The clustering of SC, MBN and nitrogen mineralisation rate (NMR) in biochar-treated soils (BC1, BC2) suggests that biochar played a role in moderating nitrogen mineralisation and reducing nitrogen losses. The control treatment exhibited weak microbial activity, as indicated by the lower correlation between respiration rate and microbial biomass parameters.

The Mollisol heatmap displayed a strong relationship between microbial efficiency (CUE) and nitrogen availability in biochar-treated soils (BC1 and BC2). It indicates that biochar enhances microbial nitrogen efficiency in high-organic-matter soils without triggering excessive respiration-driven carbon losses.

The clustering of CO₂ emissions (qCO₂) with microbial respiration rate (Resp_R) in the control and BCP1 treatments suggests that plant residues in the absence of biochar contributed to increased microbial decomposition, leading to higher CO₂ emissions. The BCP2 treatment exhibited the most balanced clustering, with microbial biomass carbon (MBC), nitrogen mineralisation (NMR) and TCS forming a stable network, indicating that the combined amendments improved long-term soil stability.

3.11 | The Principal Component Analysis (PCA)

The principal component analysis (PCA) biplots revealed distinct clustering of treatments across all three soil types (Alfisol, Entisol and Mollisol), indicating strong treatment effects on microbial and biochemical parameters (Figure 10). The first two principal components (PC1 and PC2) explained a substantial proportion of the variance: Alfisol: PC1 (32.7%), PC2 (19.7%); Entisol: PC1 (31.1%), PC2 (17.7%); and Mollisol: PC1 (38.1%), PC2 (20.6%). These variations highlight the influence of soil type on microbial community responses to different treatments.

Soil carbon pools, represented by soil carbon (SC) and microbial biomass carbon (MBC), showed contrasting responses among soil types. In Alfisol and Entisol, MBC had a stronger association with microbial efficiency (MEC) and microbial biomass nitrogen (MBN), suggesting that microbial growth was closely tied to nitrogen availability. However, in Mollisol, the weaker correlation between MBC and respiration parameters (CO₂, qCO₂) indicated greater carbon retention and a more stable microbial biomass pool.

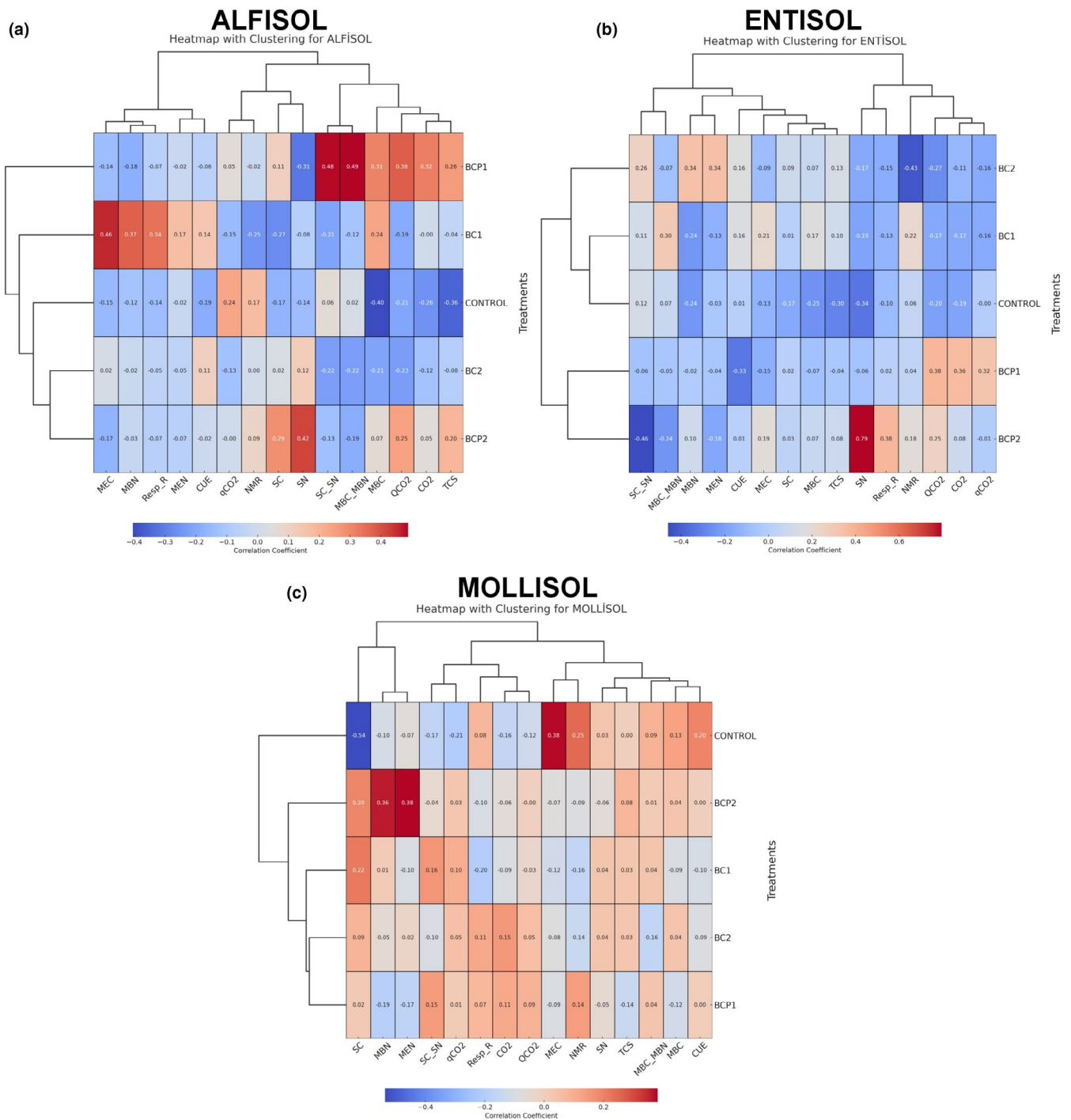


FIGURE 9 | Hierarchical clustering heatmap between treatments and soil properties in (a) Alfisol, (b) Entisol and (c) Mollisol soils.

The respiration ratio (Resp_R) varied significantly between soil types. Entisol exhibited the highest Resp_R values, indicating higher microbial turnover and potential carbon loss through mineralisation. In Alfisol, Resp_R was closely linked with MBN, suggesting nitrogen-dependent microbial activity. Mollisol had the lowest Resp_R values, indicating more efficient microbial carbon use and greater organic matter stability.

The metabolic quotient (qCO₂), an indicator of microbial stress and efficiency, showed higher values in Entisol and Alfisol,

indicating more active but potentially less efficient microbial metabolism. In Mollisol, qCO₂ was lower, suggesting a more stable microbial community with better carbon use efficiency.

Nitrogen-related parameters, including soil nitrogen (SN), microbial biomass nitrogen (MBN) and microbial nitrogen efficiency (MEN), had a strong positive correlation with respiration in Alfisol and Entisol. It suggests that microbial growth was nitrogen-limited in these soils. In contrast, Mollisol had a weaker correlation between nitrogen parameters and respiration,

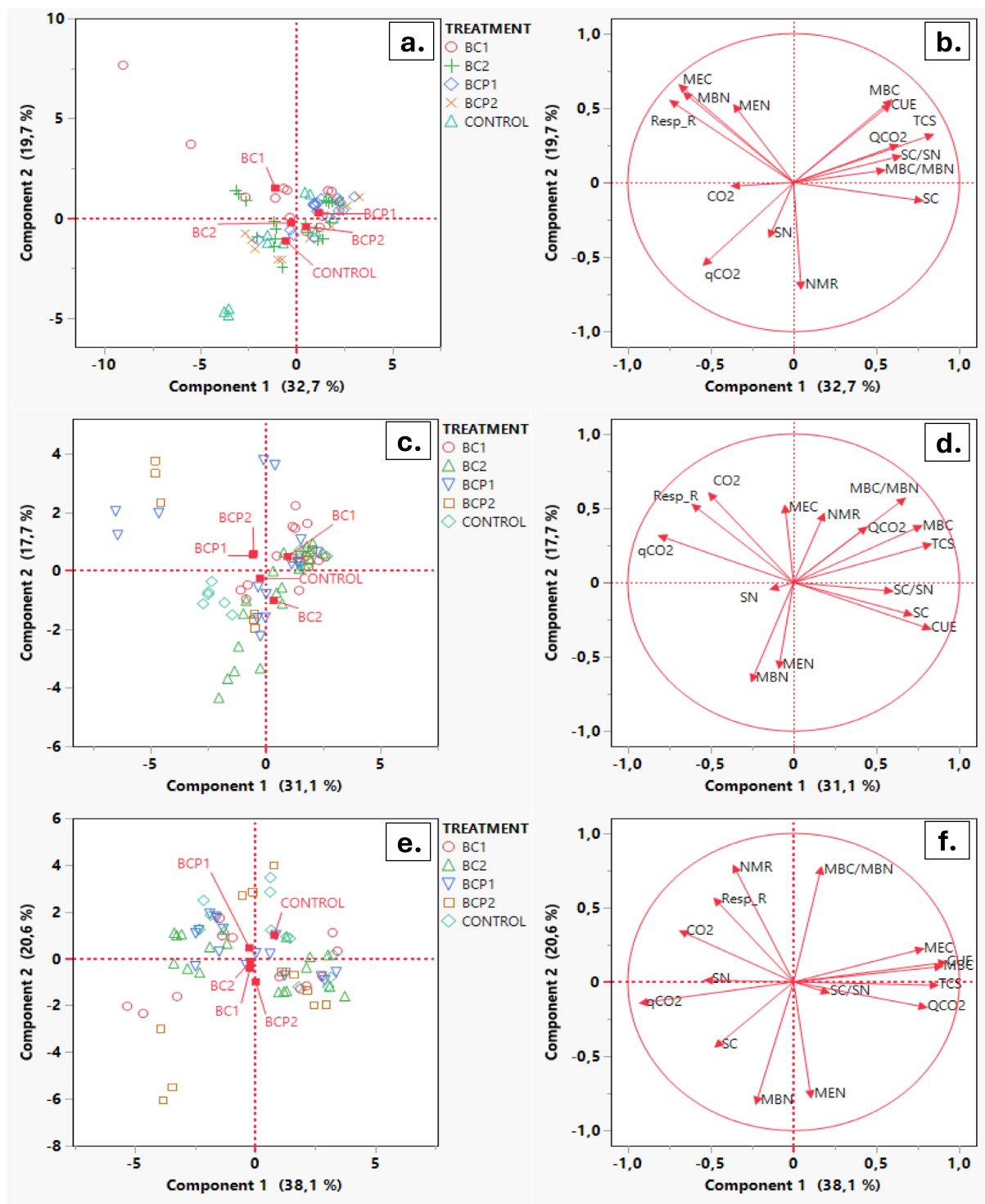


FIGURE 10 | Principal component analysis (PCA) between treatments in (a) Alfisol, (c) Entisol and (e) Mollisol soils; and soil properties in (b) Alfisol, (d) Entisol and (f) Mollisol soils.

implying greater nitrogen availability and more stable microbial activity.

Microbial efficiency coefficients (MEC and MEN) varied by soil type. Alfisol and Entisol showed a positive association between MEC, MBN and microbial biomass efficiency, indicating that microbial communities in these soils benefit from organic inputs. MEC and MEN remained stable across treatments in Mollisol, suggesting a more resilient microbial ecosystem.

4 | Discussion

4.1 | SOM

The observed increases in SOM across all soil types following the application of black cumin residue, biochar and their combination align with existing literature on organic amendments enhancing soil health (Pandian et al. 2024; Pamuru 2024). Biochar, produced through the pyrolysis of organic materials,

is known for its high carbon content and stability, contributing to long-term carbon sequestration in soils. Its porous structure improves soil aeration and water retention and provides habitats for beneficial microorganisms. Studies have shown that biochar application can enhance soil organic carbon and total nitrogen, thereby improving soil fertility (Tian et al. 2021; Lorenz and Lal 2014).

Applying plant residues, such as black cumin residue, supplies readily decomposable organic matter, which serves as a substrate for soil microbes, thereby enhancing microbial activity and nutrient cycling (Thakur et al. 2024). This rapid decomposition leads to a quicker, albeit shorter-term, increase in SOM compared to biochar. Research indicates that black cumin residues can significantly reduce soil pathogens and improve plant growth parameters, suggesting their role in enhancing soil health (Yang et al. 2018).

Combining biochar with plant residues appears to synergise, leading to the highest SOM increase observed in this study (Fischer and Glaser 2012). The stable carbon framework of biochar, coupled with the nutrient-rich profile of black cumin residue, creates an environment conducive to sustained microbial activity and organic matter accumulation. This combination provides immediate nutrient availability from the black cumin residue and ensures long-term carbon retention through biochar application. Such integrated approaches have been recommended to improve soil structure, fertility, and productivity (Shah and Wu 2019).

The differential responses among the soil types can be attributed to their inherent properties. With their already high organic matter content, Mollisols showed a relatively smaller percentage increase compared to Entisols, which had the lowest initial SOM. It suggests soils with lower baseline organic matter may benefit more substantially from such amendments. Tailoring amendment strategies to specific soil characteristics is crucial for optimising soil health and fertility outcomes (Gram et al. 2020).

The differential responses of Alfisol, Entisol and Mollisol soils to biochar application can be partly attributed to their inherent physicochemical properties. For instance, Alfisols with higher clay content (42.4%) and near-neutral to alkaline pH (7.76) likely enhanced biochar–soil interactions by increasing the cation exchange capacity (CEC) and supporting microbial colonisation within biochar pores. In contrast, Mollisols, although rich in organic matter (10.18%), displayed less dramatic shifts, possibly due to organic matter saturation buffering the microbial response to external inputs. The relatively acidic pH (7.15) may have also moderated biochar reactivity in these soils. Entisols, with low organic matter and clay, showed the most responsive behaviour to both carbon and nitrogen inputs, aligning with prior findings that nutrient-poor soils exhibit stronger responses to biochar amendments (Lehmann et al. 2011; Glaser et al. 2002). These observations underscore the importance of considering soil-specific context when applying biochar-based strategies.

In conclusion, applying black cumin residue, biochar and their combination positively influences SOM content across different soil types, with combined treatments offering the

most significant benefits. These findings support the integration of organic amendments into soil management practices to enhance soil health and agricultural productivity (Das et al. 2015).

4.2 | Functional Group Changes and Implications

The FTIR results confirm that biochar and plant residues influence soil organic matter composition differently (Hagemann et al. 2018). The black cumin residue treatments promoted more labile organic compounds, enhancing microbial activity and increasing potential carbon losses through decomposition (Pan and Huang 2024). In contrast, biochar contributed to more stabilised carbon structures, reducing rapid mineralisation and enhancing long-term carbon sequestration potential (Li et al. 2020).

The combined application of biochar and black cumin residue resulted in the most balanced spectral response, with moderate peak intensities across all functional groups (Xue et al. 2016). It suggests that biochar adsorbs and stabilises fresh organic inputs, slowing down their decomposition while maintaining nutrient availability for microbial communities (Chen et al. 2024).

The FTIR spectral analysis reveals that organic amendments significantly modify soil organic matter composition, with biochar promoting stabilisation and plant residues enhancing microbial activity (Zhang et al. 2017). The observed changes vary by soil type, with Entisol showing the most pronounced effects due to its low initial organic matter content (Grossman 1983). The findings support the use of biochar-organic matter co-application to improve soil carbon retention while maintaining microbial activity, particularly in degraded or nutrient-poor soils (Fatima et al. 2021).

4.3 | Soluble Carbon (SC) and Microbial Biomass Carbon (MBC)

The observed SC trends highlight the differential impact of biochar and plant-derived organic residues on soil carbon availability. The application of black cumin residue provided readily decomposable organic matter. It led to an initial surge in SC levels and a gradual decline due to microbial respiration and organic matter mineralisation. This pattern aligns with findings from previous studies, where labile organic inputs promote microbial activity, enhancing short-term carbon availability but leading to rapid depletion (Lehmann and Joseph 2015).

In contrast, biochar treatments resulted in more stable SC increases, particularly at higher doses. Biochar's porous structure and high surface area contribute to carbon adsorption and reduced microbial degradation, leading to longer retention of soluble organic carbon (Sun et al. 2021; Büyükkılıç Yanardağ et al. 2020). The ability of biochar to stabilise dissolved organic matter may also explain why SC levels remained elevated for longer periods in biochar-treated soils, particularly in Mollisols.

Soil type played a crucial role in the response of SC levels to amendments. In Entisol, which has low initial organic matter,

the increase in SC was the most pronounced, indicating that carbon-deficient soils benefit the most from organic amendments (Bekchanova et al. 2024). Conversely, additional amendments had a smaller relative impact on Mollisol, which naturally contains high organic matter. It supports the hypothesis that soils with high carbon saturation have a limited capacity to retain additional soluble carbon (Paustian et al. 1997). The moderate response in Alfisol suggests that soil texture and mineralogy influence organic carbon dynamics, as clay-rich soils can protect organic carbon from rapid microbial decomposition (Six et al. 2002).

The highest SC levels were observed in the combined biochar and black cumin residue treatment, indicating a synergistic effect where biochar stabilised the labile carbon derived from plant residues, preventing its rapid mineralisation. This finding is consistent with reports that combining biochar with fresh organic inputs enhances both short-term microbial activity and long-term carbon sequestration (Jeffery et al. 2011). These results emphasise that biochar should not be viewed solely as a carbon source but as a tool for enhancing carbon retention and stabilisation in agricultural soils.

The significant increase in MBC following the application of black cumin residue is consistent with previous findings that plant residues provide easily degradable organic matter, boosting microbial activity and biomass production (Kögel-Knabner 2002). The rapid decomposition of plant-based amendments contributes to short-term microbial stimulation, which explains why black cumin residue treatments exhibited early stage MBC peaks followed by declines over time.

In contrast, biochar application alone led to more gradual and sustained microbial biomass increases, particularly in Alfisols and Mollisols (Xu et al. 2020; Tamilselvi et al. 2015). Biochar's high porosity and adsorption capacity create favourable microhabitats for microbial colonisation and influence nutrient retention (Lehmann and Rondon 2006). However, because biochar is a more recalcitrant carbon source, its impact on MBC was less immediate compared to black cumin residue alone.

The combined application of biochar and black cumin residue resulted in the highest MBC values across all soil types, suggesting a synergistic effect where biochar stabilised labile organic inputs, preventing rapid mineralisation and enabling sustained microbial proliferation (Molina-Herrera and Romanya 2015). It supports the concept that co-application of fresh organic matter with biochar enhances microbial growth by providing both energy sources and stable habitats (Wang et al. 2024).

Soil type played a crucial role in determining MBC response to amendments. The low initial organic content in Entisol meant that microbial biomass responded more dramatically to external carbon inputs, particularly from labile organic amendments (Ma et al. 2021). In Alfisol, which has moderate SOM levels, MBC increases were more balanced, reflecting a combination of microbial stimulation and organic carbon stabilisation (Bajgai et al. 2015). In Mollisol, where organic matter content was already high, biochar's impact on MBC was more associated with microbial habitat formation rather than nutrient supplementation, reinforcing previous findings that biochar benefits

microbial activity indirectly by influencing soil structure and moisture retention rather than acting as a direct energy source (Yanardağ et al. 2017).

4.4 | Soluble Nitrogen (SN) and Microbial Biomass Nitrogen (MBN)

The increase in SN following black cumin residue application aligns with findings that fresh organic residues release nitrogen rapidly through microbial decomposition (Cheng et al. 2012). The initial spike in SN followed by a decline in black cumin residue treatments indicates that microbial uptake and leaching processes are at play, a pattern commonly observed in plant-based organic amendments (Zhou et al. 2017).

In contrast, biochar treatments exhibited a more stable SN increase, suggesting that biochar adsorbs ammonium and nitrate, reducing nitrogen leaching while making it available for microbial uptake over time (Major et al. 2012). It is particularly evident in the combined biochar and black cumin residue treatment, which showed the highest and most sustained SN and MBN levels across all soils. These findings align with reports that biochar improves nitrogen retention and facilitates microbial nitrogen cycling (Liu et al. 2018).

Soil type played a significant role in determining SN and MBN responses to amendments. In Entisol, the large relative increase in SN and MBN indicates that low-nitrogen soils benefit most from organic amendments (Bekchanova et al. 2024). In contrast, in Mollisol, where nitrogen levels were already high, biochar focused more on stabilising nitrogen rather than increasing its availability, reducing microbial nitrogen losses, and enhancing long-term nitrogen use efficiency (Paustian et al. 1997). The response in Alfisol suggests that clay-rich soils can help retain biochar-adsorbed nitrogen, reducing leaching losses and making nitrogen available for microbial and plant uptake (Lehmann et al. 2011).

4.5 | CO₂ and Cumulative CO₂ Emissions (qCO₂)

Soil CO₂ emissions are a key indicator of microbial respiration and soil organic matter turnover. Higher CO₂ emissions indicate active microbial decomposition, whereas lower emissions may suggest either reduced microbial activity or enhanced carbon stabilisation (Liu et al. 2018).

The application of black cumin residue led to the highest CO₂ emissions, particularly in Entisol and Alfisol, due to its high degradability and rapid microbial metabolism. It aligns with previous research showing that easily decomposable organic amendments stimulate microbial activity and increase soil respiration rates (Luan et al. 2020).

Biochar treatments resulted in lower CO₂ emissions, suggesting a more efficient microbial system with enhanced carbon retention. Biochar is known to improve microbial habitat conditions while reducing the loss of organic carbon via respiration, leading to more stable soil carbon pools (Lorenz and Lal 2014).

The combined application of biochar and black cumin residue produced the lowest qCO_2 values, indicating greater microbial carbon use efficiency. Lower qCO_2 values suggest that microbial communities utilised carbon more effectively for biomass production rather than respiration, reducing carbon losses (Karimzadeh et al. 2025).

Soil type influenced CO_2 dynamics, with Entisol showing the largest relative increase in respiration, highlighting the importance of organic inputs in carbon-poor soils (Lal 2004). In Mollisol, where SOM levels were already high, biochar's impact focused more on carbon stabilisation than respiration enhancement, preventing excessive carbon loss.

4.6 | Respiration Rate and Microbial Respiration (qCO_2)

The observed differences in respiration rates and microbial respiration (qCO_2) values across soil types and treatments provide insight into microbial carbon use efficiency and the stability of organic inputs (Yu et al. 2025).

The sharp initial increase in respiration rate following black cumin residue application aligns with previous findings that fresh organic residues serve as a readily available carbon source, stimulating microbial metabolism and organic matter decomposition (Wang et al. 2015). The rapid decline in respiration over time suggests that microbial communities quickly utilise available labile carbon, leading to a temporary increase in respiration but not long-term carbon sequestration (Cleveland et al. 2007).

Biochar treatments led to lower but more sustained respiration rates, indicating greater microbial efficiency in utilising carbon and nitrogen resources. Biochar is known for its high stability and surface area, which enhances microbial habitat conditions and nutrient retention, leading to longer-term improvements in microbial activity rather than immediate respiration bursts (Joseph et al. 2021).

Combining biochar and black cumin residue resulted in the most balanced respiration and qCO_2 values, suggesting synergistic effects between labile and stable carbon pools. Studies have shown that co-application of biochar and plant residues can enhance microbial metabolism by providing both energy sources and a stable environment for microbial growth (Zhang, Qu, et al. 2022).

Soil type had a strong influence on respiration patterns. In Entisol, which initially had low microbial biomass and nutrient content, respiration rates increased significantly following amendments, demonstrating that microbial communities in nutrient-poor soils respond strongly to organic inputs (Lal 2004). In Alfisol, respiration responses were moderate, suggesting that clay content played a role in organic matter retention and microbial efficiency (Weil and Magdoff 2004). In Mollisol, where organic matter content was naturally high, biochar helped moderate microbial respiration, preventing excessive carbon losses (Yan et al. 2022).

4.7 | Mineralisable Carbon (MEC), Mineralisable Nitrogen (MEN) and MBC/MBN Ratio

The observed trends in MEC and MEN highlight the different roles of biochar and plant residues in soil carbon and nitrogen cycling. Rapid carbon and nitrogen release from black cumin residue aligns with findings that fresh organic matter decomposes quickly, leading to short-term increases in microbial activity and nutrient availability (Song et al. 2017). The decline in MEC and MEN over time suggests that microbial communities rapidly consumed available organic substrates, resulting in a short-lived burst of mineralisation (Zhou et al. 2017).

Biochar treatments led to lower but more sustained MEC and MEN values, indicating greater carbon stabilisation and nitrogen retention. Biochar is known for its ability to adsorb labile carbon and nitrogen compounds, reducing their rapid loss and promoting long-term soil fertility improvement (Lehmann and Rondon 2006). This effect was particularly evident in Entisol, where biochar prevented excessive nitrogen losses while maintaining microbial carbon use efficiency.

The highest MEC and MEN levels in the combined treatment confirm a synergistic effect between biochar and black cumin residue. Biochar acted as a stabilising agent, preventing rapid carbon loss, while black cumin residue provided readily available nutrients, promoting both short-term microbial stimulation and long-term carbon sequestration (Steiner 2008).

Soil type strongly influenced mineralisation patterns. The highest relative increase in MEC and MEN was observed in Entisol, suggesting that degraded soils benefit most from organic amendments (Lal 2004). In Alfisol, mineralisation responses were more balanced, while in Mollisol, whose carbon and nitrogen content were already high, biochar's role was more focused on nutrient stabilisation rather than mineralisation enhancement (Paustian et al. 1997).

4.8 | MBC/MBN Ratio

The MBC/MBN ratio is a key indicator of microbial metabolic efficiency and nitrogen use. Higher ratios typically suggest greater microbial carbon utilisation efficiency, while lower ratios indicate enhanced nitrogen turnover and mineralisation (Fang et al. 2018).

Biochar treatments generally led to a decrease in MBC/MBN ratios, particularly in Alfisol and Entisol. It suggests that biochar promotes microbial nitrogen conservation while slowing down carbon decomposition, a known effect of biochar's high stability and carbon sequestration properties (Chen et al. 2024).

Black cumin residue application increased MBC/MBN ratios in Entisol, likely due to the rapid availability of labile organic matter, which encouraged microbial growth and increased microbial biomass carbon (Rousk et al. 2016).

The combined treatment resulted in the most balanced MBC/MBN values across all soils, indicating that biochar effectively

moderated nitrogen cycling while supporting microbial biomass stability (Yu et al. 2018).

4.9 | Total Carbon Sequestration (TCS) and Carbon Use Efficiency (CUE)

The differences in TCS and CUE trends across soil types and treatments highlight the contrasting roles of biochar and organic residues in soil carbon dynamics. Higher TCS values in biochar treatments confirm biochar's role in carbon stabilisation (Fang et al. 2014). Biochar is known for its high resistance to microbial degradation, resulting in greater long-term carbon sequestration compared to plant residues (Yin et al. 2022). The strong response in Entisol suggests that biochar amendments are most beneficial in soils with low organic matter, as they reduce carbon loss through respiration while providing a stable carbon pool (Joseph et al. 2021). CUE values were highest in combined treatments, indicating a more efficient balance between microbial carbon assimilation and respiration losses (Liu et al. 2020). While biochar alone improved TCS, it had a lower impact on microbial efficiency unless combined with labile organic matter. Adding black cumin residue provided microbial communities with an immediate carbon source, promoting higher CUE and greater microbial biomass retention.

Soil type influences the extent of the effects of biochar and organic residue. In Alfisol and Entisol, biochar improved TCS, but its greatest impact was observed when paired with plant residues (Hurtado et al. 2017). In Mollisol, biochar was more passive, contributing to carbon stability rather than direct microbial activity enhancement (Jiang et al. 2024).

4.10 | Nitrogen Mineralisation Rate (NMR) and Respiration Ratio Response to Soil Amendments

Both organic nitrogen inputs and the stability of soil amendments influence the nitrogen mineralisation rate. Plant residues such as black cumin residue provided readily decomposable nitrogen, leading to higher short-term mineralisation rates. The sharp NMR increases in plant residue treatments suggest rapid microbial activity and nitrogen turnover, which is beneficial for quickly improving soil fertility (Zhao et al. 2018). However, this also increases the risk of nitrogen leaching if not managed properly (Gómez-Garrido et al. 2018).

Biochar treatments exhibited lower but more sustained nitrogen mineralisation rates, indicating that biochar plays a role in nitrogen retention. Biochar's ability to adsorb ammonium and nitrate ions helps moderate nitrogen mineralisation, reducing excessive losses while ensuring nitrogen remains available for microbial and plant uptake over time (Khan et al. 2023; Gómez-Garrido et al. 2018).

The combined application of biochar and plant residues resulted in the most balanced NMR trends, demonstrating that biochar helps stabilise organic nitrogen inputs, preventing rapid depletion while promoting microbial nitrogen cycling (Clough and Condon 2010).

Soil type strongly influenced nitrogen mineralisation patterns. In Entisol, nitrogen availability was initially low, making amendments highly effective in improving NMR (Lal 2004). In Alfisol, NMR responses were more balanced, reflecting its moderate fertility and ability to retain nitrogen (Six et al. 2002). In Mollisol, where nitrogen content was naturally high, biochar helped regulate nitrogen mineralisation, preventing excessive nitrogen losses (Ding and Yu 2024).

The respiration ratio is a key indicator of microbial activity and carbon utilisation efficiency. The observed differences across soil types highlight the influence of soil properties on microbial metabolism. Entisol exhibited the highest respiration ratios, possibly attributed to its lower organic matter stability and microbial community structure, leading to greater CO₂ emissions. In contrast, Mollisol, known for its high organic matter content and stable microbial biomass, had the lowest respiration ratios, suggesting efficient microbial carbon use and reduced carbon loss (Liu et al. 2018; Sinsabaugh et al. 2013). Alfisol, with intermediate respiration values, likely reflects a balance between microbial carbon use efficiency and substrate availability (Wardle and Ghani 1995).

The high variability in respiration ratios within Entisol and Alfisol suggests that different soil amendments significantly impact microbial metabolism. Organic amendments such as biochar or compost have been shown to either stimulate microbial respiration through increased substrate availability or suppress it by modifying soil physical and chemical properties (Lehmann et al. 2011; Wang et al. 2021). Mollisol's more stable respiration ratios indicate that microbial communities in these soils might be less affected by short-term amendments.

Optimising soil management practices to maintain balanced microbial respiration is essential for carbon sequestration and soil fertility. Excessive microbial respiration can accelerate organic matter depletion, while overly suppressed respiration may indicate microbial dormancy and reduced nutrient cycling (Blagodatskaya et al. 2007). Long-term studies integrating microbial respiration with soil organic matter turnover are necessary to develop sustainable soil fertility strategies.

4.11 | Correlation Coefficient Analysis of Between Soil Parameters and Alfisol, Entisol and Mollisol Soils

The observed correlations suggest that biochar and plant residue amendments influenced microbial dynamics and soil carbon stability differently across soil types. In low-carbon soils such as Entisols, biochar had the most significant role in carbon stabilisation, reducing carbon losses through respiration and enhancing microbial biomass retention (Chen et al. 2018). The strong correlation between MBC and TCS in Entisol ($r=0.94$) suggests that biochar amendments increased microbial carbon efficiency, improving carbon sequestration (Liu et al. 2020). Additionally, the positive relationship between qCO₂ and NMR ($r=0.49$) indicates that organic amendments accelerated microbial decomposition, increasing nitrogen mineralisation rates (Marzi et al. 2020).

In Alfisol, microbial efficiency was crucial in carbon and nitrogen cycling. The positive correlation between SC and TCS ($r=0.69$) suggests that carbon sequestration was largely driven by organic matter availability. However, the negative correlation between Resp_R and CUE (-0.48) indicates that higher respiration rates reduced microbial carbon efficiency, reinforcing the role of biochar in stabilising organic matter (Zhang, He, et al. 2022).

In Mollisol, the primary influence of amendments was on microbial respiration rather than carbon sequestration. The strong correlation between MBC and CO₂ emissions ($r=0.7$) suggests that microbial activity is more closely tied to carbon mineralisation than stabilisation in high organic matter soils. It aligns with findings by Brookes et al. (2017), who reported that in carbon-rich soils, microbial activity is driven by organic matter decomposition rather than new carbon inputs.

These findings highlight the importance of tailoring biochar and organic amendments based on soil type. Biochar significantly improves microbial carbon retention and reduces respiration-driven carbon losses in low-carbon soils like Entisols. A balance of biochar and plant residues is required to optimise microbial efficiency and nutrient cycling in moderate-carbon soils like Alfisols. In high-carbon soils like Mollisols, amendments enhance microbial respiration but do not significantly alter carbon sequestration patterns.

4.12 | Hierarchical Clustering Heatmap Between Treatments and Soil Properties

The heatmap clustering analysis confirms that biochar and black cumin residue amendments affect microbial activity and soil biochemical processes differently depending on soil type. In Alfisol, the strong clustering of MBN, MEC and Resp_R in BCP1 treatments suggests that plant residues had a significant short-term impact on microbial nitrogen cycling (Mary et al. 1996). However, biochar treatments (BC1 and BC2) clustered separately, indicating their role in long-term carbon stabilisation rather than immediate microbial activation. It supports the findings by Zhang, Qu, et al. (2022), which show that biochar enhances soil carbon retention but does not always promote immediate microbial respiration.

In Entisol, the clustering of TCS and MBC in BCP2 treatments suggests that combining biochar with plant residues significantly improved microbial carbon retention. It aligns with previous studies demonstrating that biochar is most effective in low-carbon soils, enhancing microbial efficiency and preventing carbon losses through respiration (Steiner 2008). The strong association between SC, MBN and NMR in biochar-treated soils (BC1, BC2) indicates that biochar moderated nitrogen cycling, reducing rapid nitrogen mineralisation and leaching (Li et al. 2020).

In Mollisol, the clustering of CUE and nitrogen availability in biochar treatments suggests that biochar improved microbial efficiency by stabilising organic matter, leading to reduced carbon losses through respiration. The close association between CO₂ emissions (qCO₂) and Resp_R in plant residue treatments confirms that microbial decomposition rates were

higher without biochar, increasing CO₂ emissions. This finding aligns with research showing that plant residues enhance microbial respiration, whereas biochar moderates organic matter decomposition, leading to more stable carbon retention (Cheng et al. 2017).

These results show that biochar is most effective in stabilising soil organic matter and enhancing microbial efficiency in low-carbon soils (Entisols). In contrast, plant residues have a stronger immediate effect in moderate-carbon soils (Alfisols). In high-carbon soils (Mollisols), biochar mainly regulates microbial activity rather than significantly altering carbon sequestration. Future studies should focus on long-term field applications to assess biochar's persistence and its role in mitigating CO₂ emissions under different soil conditions.

4.13 | The Principal Component Analysis (PCA)

The PCA results emphasise that microbial respiration and efficiency depend heavily on soil type and treatment. Entisol exhibited the highest respiration ratios, indicating higher microbial carbon turnover and potential carbon loss (Blagodatskaya and Kuzyakov 2008). In contrast, Mollisol showed lower respiration ratios and more stable microbial efficiency, suggesting better carbon retention and more sustainable microbial activity (Cotrufo et al. 2013).

The higher qCO₂ values in Entisol and Alfisol suggest that microbial communities in these soils are under greater metabolic stress. It aligns with previous findings indicating that low organic matter stability leads to inefficient microbial metabolism and increased CO₂ emissions (Biswas and Kole 2017). The lower qCO₂ values in Mollisol suggest that microbial communities in this soil are more adapted to organic matter decomposition and have a higher carbon use efficiency.

The biochar treatments (BC1, BC2, BCP1 BCP2) significantly impacted microbial biomass and respiration. In Alfisol, BC1 and BC2 increased microbial biomass carbon (MBC), suggesting improved microbial growth conditions. It is consistent with previous studies indicating that biochar can enhance microbial habitats by increasing nutrient retention and water-holding capacity (Atkinson 2018).

In Entisol, biochar treatments had mixed effects, with BCP2 leading to higher respiration and carbon loss. It suggests that biochar-induced priming effects may have stimulated microbial respiration and accelerated organic matter decomposition (Lu et al. 2014). In Mollisol, microbial biomass and respiration remained relatively stable across treatments, indicating greater resilience to soil amendments.

The strong relationship between microbial nitrogen parameters (MBN, MEN) and Alfisol and Entisol's respiration suggests that these soils' microbial communities are nitrogen-limited. It supports the idea that organic amendments, such as biochar, improve microbial nitrogen use efficiency and support microbial biomass growth (Zhang et al. 2020). In Mollisol, nitrogen availability was more stable, suggesting that microbial activity in this soil is less dependent on nitrogen inputs.

The findings suggest that Entisol requires management strategies to reduce carbon losses and improve microbial efficiency, while Alfisol benefits from nitrogen-enhancing treatments. Biochar treatments (particularly BC1 and BCP2) could improve microbial biomass efficiency but may lead to higher respiration and carbon turnover in certain soils (Pei et al. 2021). Mollisol appears to be the most stable soil type, with microbial communities exhibiting greater disturbance resilience.

The PCA analysis confirmed that soil type and treatment strongly influence soil microbial respiration, nitrogen availability, and carbon use efficiency (Qiao et al. 2019). Future research should focus on the long-term effects of biochar and organic amendments on soil microbial stability and carbon sequestration, especially in high-respiration soils such as Entisol.

5 | Conclusion

This study demonstrated that black cumin residue and biochar amendments significantly influenced soil microbial activity, carbon sequestration and nitrogen cycling across different soil types. The effects varied depending on soil properties, with Entisol exhibiting the highest microbial respiration and nitrogen mineralisation, indicating a need for organic amendments to improve fertility. In Alfisol, biochar contributed to long-term carbon retention, while black cumin residue accelerated nitrogen availability. Mollisol showed minimal changes, suggesting an inherently stable microbial community with efficient carbon use.

Biochar effectively enhanced microbial biomass and reduced CO₂ emissions, making it a promising tool for long-term carbon sequestration. In contrast, black cumin residue provided a short-term microbial energy source, increasing respiration. Combining biochar and residue balanced microbial activity and carbon stability, optimising nutrient availability and organic matter retention.

The microbial efficiency indicators showed that biochar increased carbon use efficiency (CUE) and microbial biomass carbon (MBC), reducing unnecessary carbon losses. The metabolic quotient (qCO₂) confirmed that Entisol and Alfisol soils experienced greater microbial stress, while Mollisol exhibited more stable microbial efficiency.

These findings suggest that soil-specific management strategies are necessary to maximise amendment benefits. Higher biochar doses are recommended for degraded Entisols, while a balanced biochar and organic residue approach suits Alfisols. As naturally rich in organic matter, Mollisols benefit primarily from biochar's stabilising effect. Future research should explore long-term microbial responses and biochar interactions with other organic amendments to develop more effective carbon sequestration and soil fertility strategies.

Ethics Statement

This study did not involve human participants, animals or sensitive environmental research requiring ethical approval.

Consent

This study did not involve human participants requiring informed consent.

Conflicts of Interest

The author declares no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

- Ahmad, R., N. Ahmad, F. Aljhis, et al. 2020. "Variation in Black Cumin Quality and Its Standardization via Instrumental Analysis: A Study Based on Geographical Origin." *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 48, no. 3: 1141–1154.
- Anderson, T. H., and K. H. Domsch. 1990. "Application of Eco-Physiological Quotients (qCO₂ and qD) on Microbial Biomasses From Soils of Different Cropping Histories." *Soil Biology and Biochemistry* 22, no. 2: 251–255.
- Anderson, T. H., and K. H. Domsch. 1995. "Soil Structure—Its Development and Function." In *Effects of Soil Structure on Microbial Biomass*, 325–345. Lewis Publishers.
- Atkinson, C. J. 2018. "How Good Is the Evidence That Soil-Applied Biochar Improves Water-Holding Capacity?" *Soil Use and Management* 34, no. 2: 177–186.
- Bajgai, Y., P. Kristiansen, N. Hulugalle, M. McHenry, and B. McCorkell. 2015. "Soil Organic Carbon and Microbial Biomass Carbon Under Organic and Conventional Vegetable Cropping Systems in an Alfisol and a Vertisol." *Nutrient Cycling in Agroecosystems* 101: 1–15.
- Bekchanova, M., T. Kuppens, A. Cuypers, M. Jozefczak, and R. Malina. 2024. "Biochar's Effect on the Soil Carbon Cycle: A Rapid Review and Meta-Analysis." *Biochar* 6, no. 1: 1–19.
- Biswas, T., and S. C. Kole. 2017. "Soil Organic Matter and Microbial Role in Plant Productivity and Soil Fertility." In *Advances in Soil Microbiology: Recent Trends and Future Prospects: Volume 2: Soil-Microbe-Plant Interaction*, 219–238. Springer.
- Blagodatskaya, E., and Y. Kuzyakov. 2013, April. "Active Microorganisms as Drivers of Dynamic Processes in Soil: Integration of Basic Teaching Into Research." In *EGU General Assembly Conference Abstracts* (EGU2013-3495).
- Blagodatskaya, E. V., S. A. Blagodatsky, T. H. Anderson, and Y. Kuzyakov. 2007. "Priming Effects in Chernozem Induced by Glucose and N in Relation to Microbial Growth Strategies." *Applied Soil Ecology* 37, no. 1–2: 95–105.
- Blagodatskaya, E., and Y. Kuzyakov. 2008. "Mechanisms of Real and Apparent Priming Effects and Their Dependence on Soil Microbial Biomass and Community Structure: Critical Review." *Biology and Fertility of Soils* 45: 115–131.
- Bolan, N., S. A. Hoang, J. Beiyuan, et al. 2022. "Multifunctional Applications of Biochar Beyond Carbon Storage." *International Materials Reviews* 67, no. 2: 150–200.
- Bouyoucos, G. J. 1962. "Hydrometer Method Improved for Making Particle Size Analyses of Soils 1." *Agronomy Journal* 54, no. 5: 464–465.
- Brookes, P. C., Y. Chen, L. Chen, G. Qiu, Y. Luo, and J. Xu. 2017. "Is the Rate of Mineralization of Soil Organic Carbon Under Microbiological Control?" *Soil Biology and Biochemistry* 112: 127–139.

- Büyükkılıç Yanardağ, A., A. Faz Cano, A. Mermut, İ. H. Yanardağ, and M. Gomez Garrido. 2020. "Organic Carbon Fluxes Using Column Leaching Experiments in Soil Treated With Pig Slurry in SE Spain." *Arid Land Research and Management* 34, no. 2: 136–151.
- Chen, J., X. Sun, J. Zheng, et al. 2018. "Biochar Amendment Changes Temperature Sensitivity of Soil Respiration and Composition of Microbial Communities 3 Years After Incorporation in an Organic Carbon-Poor Dry Cropland Soil." *Biology and Fertility of Soils* 54: 175–188.
- Chen, Y., K. Sun, Y. Yang, B. Gao, and H. Zheng. 2024. "Effects of Biochar on the Accumulation of Necromass-Derived Carbon, the Physical Protection and Microbial Mineralization of Soil Organic Carbon." *Critical Reviews in Environmental Science and Technology* 54, no. 1: 39–67.
- Cheng, H., P. W. Hill, M. S. Bastami, and D. L. Jones. 2017. "Biochar Stimulates the Decomposition of Simple Organic Matter and Suppresses the Decomposition of Complex Organic Matter in a Sandy Loam Soil." *GCB Bioenergy* 9, no. 6: 1110–1121.
- Cheng, L., F. L. Booker, C. Tu, et al. 2012. "Arbuscular Mycorrhizal Fungi Increase Organic Carbon Decomposition Under Elevated CO₂." *Science* 337, no. 6098: 1084–1087.
- Cleveland, C. C., D. R. Nemergut, S. K. Schmidt, and A. R. Townsend. 2007. "Increases in Soil Respiration Following Labile Carbon Additions Linked to Rapid Shifts in Soil Microbial Community Composition." *Biogeochemistry* 82: 229–240.
- Clough, T. J., and L. M. Condron. 2010. "Biochar and the Nitrogen Cycle: Introduction." *Journal of Environmental Quality* 39, no. 4: 1218–1223.
- Cotrufo, M. F., M. D. Wallenstein, C. M. Boot, K. Denef, and E. Paul. 2013. "The Microbial Efficiency-Matrix Stabilization (MEMS) Framework Integrates Plant Litter Decomposition With Soil Organic Matter Stabilization: Do Labile Plant Inputs Form Stable Soil Organic Matter?" *Global Change Biology* 19, no. 4: 988–995.
- Das, D., B. S. Dwivedi, M. C. Meena, V. K. Singh, and K. N. Tiwari. 2015. "Integrated Nutrient Management for Improving Soil Health and Crop Productivity." *Indian Journal of Fertilisers* 11, no. 4: 64–83.
- Deng, Z., J. Wang, Y. He, et al. 2023. "Biochar-Based *Bacillus Subtilis* Inoculant for Enhancing Plants Disease Prevention: Microbiota Response and Root Exudates Regulation." *Biochar* 5, no. 1: 81.
- Deng, Z., J. Wang, Y. Yan, J. Wang, W. Shao, and Z. Wu. 2025. "Biochar-Based *Bacillus subtilis* Inoculants Promote Plant Growth: Regulating Microbial Community to Improve Soil Properties." *Journal of Environmental Management* 373: 123534.
- Ding, J., and S. Yu. 2024. "Impact of Biochar on Nitrogen-Cycling Functional Genes: A Comparative Study in Mollisol and Alkaline Soils." *Life* 14, no. 12: 1631.
- Ding, Y., Y. Liu, S. Liu, et al. 2016. "Biochar to Improve Soil Fertility. A Review." *Agronomy for Sustainable Development* 36: 1–18.
- Fang, Y., B. Singh, B. P. Singh, and E. Krull. 2014. "Biochar Carbon Stability in Four Contrasting Soils." *European Journal of Soil Science* 65, no. 1: 60–71.
- Fang, Y., B. P. Singh, D. Collins, B. Li, J. Zhu, and E. Tavakkoli. 2018. "Nutrient Supply Enhanced Wheat Residue-Carbon Mineralization, Microbial Growth, and Microbial Carbon-Use Efficiency When Residues Were Supplied at High Rate in Contrasting Soils." *Soil Biology and Biochemistry* 126: 168–178.
- Fatima, S., M. Riaz, M. I. Al-Wabel, et al. 2021. "Higher Biochar Rate Strongly Reduced Decomposition of Soil Organic Matter to Enhance C and N Sequestration in Nutrient-Poor Alkaline Calcareous Soil." *Journal of Soils and Sediments* 21: 148–162.
- Fischer, D., and B. Glaser. 2012. *Synergisms Between Compost and Biochar for Sustainable Soil Amelioration*, 167–198. INTECH Open Access Publisher.
- Geyer, K. M., E. Kyker-Snowman, A. S. Grandy, and S. D. Frey. 2016. "Microbial Carbon Use Efficiency: Accounting for Population, Community, and Ecosystem-Scale Controls Over the Fate of Metabolized Organic Matter." *Biogeochemistry* 127: 173–188.
- Gholamnezhad, Z., R. Keyhanmanesh, and M. H. Boskabady. 2015. "Anti-Inflammatory, Antioxidant, and Immunomodulatory Aspects of Black Cumin for Its Preventive and Bronchodilatory Effects on Obstructive Respiratory Diseases: A Review of Basic and Clinical Evidence." *Journal of Functional Foods* 17: 910–927.
- Giagnoni, L., and G. Renella. 2022. "Effects of Biochar on the C Use Efficiency of Soil Microbial Communities: Components and Mechanisms." *Environments* 9, no. 11: 138.
- Glaser, B., J. Lehmann, and W. Zech. 2002. "Ameliorating Physical and Chemical Properties of Highly Weathered Soils in the Tropics With Charcoal—a Review." *Biology and Fertility of Soils* 35: 219–230.
- Gómez-Garrido, M., Á. Faz-Cano, S. Martínez-Martínez, D. M. Carmona-Garcés, and A. Büyükkılıç-Yanardag. 2018. "Nitrogen Dynamic and Leaching in Calcareous Soils Amended With Pig Slurry." In *Soil Management and Climate Change*, 171–187. Academic Press.
- Gram, G., D. Roobroeck, P. Pypers, J. Six, R. Merckx, and B. Vanlauwe. 2020. "Combining Organic and Mineral Fertilizers as a Climate-Smart Integrated Soil Fertility Management Practice in Sub-Saharan Africa: A Meta-Analysis." *PLoS One* 15, no. 9: e0239552.
- Grossman, R. B. 1983. "Entisols." In *Developments in Soil Science*, vol. 11, 55–90. Elsevier.
- Hagemann, N., K. Spokas, H. P. Schmidt, R. Kägi, M. A. Böhler, and T. D. Bucheli. 2018. "Activated Carbon, Biochar and Charcoal: Linkages and Synergies Across Pyrogenic Carbon's ABCs." *Water* 10, no. 2: 182.
- Hurtado, C., N. Cañameras, C. Domínguez, G. W. Price, J. Comas, and J. M. Bayona. 2017. "Effect of Soil Biochar Concentration on the Mitigation of Emerging Organic Contaminant Uptake in Lettuce." *Journal of Hazardous Materials* 323: 386–393.
- Jeffery, S., F. G. Verheijen, M. van der Velde, and A. C. Bastos. 2011. "A Quantitative Review of the Effects of Biochar Application to Soils on Crop Productivity Using Meta-Analysis." *Agriculture, Ecosystems & Environment* 144, no. 1: 175–187.
- Jiang, B. N., Y. Y. Zhang, Y. Wang, et al. 2024. "Sponge Iron-Coupled Biochar Solution Can Achieve the Synergistic Augmentation of Carbon Sequestration, Carbon Sink Capacity, and Denitrification in Ecological Ditches." *Chemical Engineering Journal* 493: 152496.
- Joergensen, R. G., and P. C. Brookes. 1990. "Ninhydrin-Reactive Nitrogen Measurements of Microbial Biomass in 0.5 M K₂SO₄ Soil Extracts." *Soil Biology and Biochemistry* 22, no. 8: 1023–1027.
- Joseph, S., A. L. Cowie, L. Van Zwieten, et al. 2021. "How Biochar Works, and When It Doesn't: A Review of Mechanisms Controlling Soil and Plant Responses to Biochar." *GCB Bioenergy* 13, no. 11: 1731–1764.
- Karimzadeh, Z., A. A. Soltani Toularoud, H. S. Arkhazloo, and T. Rouhi-Kelarlou. 2025. "Analyzing the Impact of Variations in Land Use and Elevation on Selected Soil Microbial Indices and Spatial Distribution." *Environmental Monitoring and Assessment* 197, no. 1: 1–16.
- Khan, Z., X. J. Yang, Y. Fu, et al. 2023. "Engineered Biochar Improves Nitrogen Use Efficiency via Stabilizing Soil Water-Stable Macroaggregates and Enhancing Nitrogen Transformation." *Biochar* 5, no. 1: 52.
- Kögel-Knabner, I. 2002. "The Macromolecular Organic Composition of Plant and Microbial Residues as Inputs to Soil Organic Matter." *Soil Biology and Biochemistry* 34, no. 2: 139–162.
- Lal, R. 2004. "Soil Carbon Sequestration Impacts on Global Climate Change and Food Security." *Science* 304, no. 5677: 1623–1627.

- Lehmann, J., and S. Joseph. 2015. "Biochar for Environmental Management: An Introduction." In *Biochar for Environmental Management*, 1–13. Routledge.
- Lehmann, J., M. C. Rillig, J. Thies, C. A. Masiello, W. C. Hockaday, and D. Crowley. 2011. "Biochar Effects on Soil Biota—A Review." *Soil Biology and Biochemistry* 43, no. 9: 1812–1836.
- Lehmann, J., and M. Rondon. 2006. "Bio-Char Soil Management on Highly Weathered Soils in the Humid Tropics." *Biological Approaches to Sustainable Soil Systems* 113, no. 517: e530.
- Li, S., S. Wang, M. Fan, Y. Wu, and Z. Shangguan. 2020. "Interactions Between Biochar and Nitrogen Impact Soil Carbon Mineralization and the Microbial Community." *Soil and Tillage Research* 196: 104437.
- Li, Y., S. Hu, J. Chen, et al. 2018. "Effects of Biochar Application in Forest Ecosystems on Soil Properties and Greenhouse Gas Emissions: A Review." *Journal of Soils and Sediments* 18: 546–563.
- Liang, J., Y. Li, B. Si, et al. 2021. "Optimizing Biochar Application to Improve Soil Physical and Hydraulic Properties in Saline-Alkali Soils." *Science of the Total Environment* 771: 144802.
- Liu, W., C. Qiao, S. Yang, W. Bai, and L. Liu. 2018. "Microbial Carbon Use Efficiency and Priming Effect Regulate Soil Carbon Storage Under Nitrogen Deposition by Slowing Soil Organic Matter Decomposition." *Geoderma* 332: 37–44.
- Liu, Z., X. Wu, W. Liu, et al. 2020. "Greater Microbial Carbon Use Efficiency and Carbon Sequestration in Soils: Amendment of Biochar Versus Crop Straws." *GCB Bioenergy* 12, no. 12: 1092–1103.
- Lorenz, K., and R. Lal. 2014. "Biochar Application to Soil for Climate Change Mitigation by Soil Organic Carbon Sequestration." *Journal of Plant Nutrition and Soil Science* 177, no. 5: 651–670.
- Lu, W., W. Ding, J. Zhang, et al. 2014. "Biochar Suppressed the Decomposition of Organic Carbon in a Cultivated Sandy Loam Soil: A Negative Priming Effect." *Soil Biology and Biochemistry* 76: 12–21.
- Luan, H., W. Gao, S. Huang, et al. 2020. "Organic Amendment Increases Soil Respiration in a Greenhouse Vegetable Production System Through Decreasing Soil Organic Carbon Recalcitrance and Increasing Carbon-Degrading Microbial Activity." *Journal of Soils and Sediments* 20: 2877–2892.
- Ma, Q., T. Watanabe, J. Zheng, and S. Funakawa. 2021. "Interactive Effects of Crop Residue Quality and Nitrogen Fertilization on Soil Organic Carbon Priming in Agricultural Soils." *Journal of Soils and Sediments* 21: 83–95.
- Major, J., C. Steiner, A. Downie, and J. Lehmann. 2012. "Biochar Effects on Nutrient Leaching." In *Biochar for Environmental Management*, edited by J. Lehmann, 303–320. Routledge.
- Manzoni, S., P. Taylor, A. Richter, A. Porporato, and G. I. Ågren. 2012. "Environmental and Stoichiometric Controls on Microbial Carbon-Use Efficiency in Soils." *New Phytologist* 196, no. 1: 79–91.
- Mary, B., S. Recous, D. Darwis, and D. Robin. 1996. "Interactions Between Decomposition of Plant Residues and Nitrogen Cycling in Soil." *Plant and Soil* 181: 71–82.
- Marzi, M., K. Shahbazi, N. Kharazi, and M. Rezaei. 2020. "The Influence of Organic Amendment Source on Carbon and Nitrogen Mineralization in Different Soils." *Journal of Soil Science and Plant Nutrition* 20: 177–191.
- Molina-Herrera, S., and J. Romanya. 2015. "Synergistic and Antagonistic Interactions Among Organic Amendments of Contrasted Stability, Nutrient Availability and Soil Organic Matter in the Regulation of C Mineralisation." *European Journal of Soil Biology* 70: 118–125.
- Pamuru, S. T. 2024. "Assessing the Impacts of Organic Amendments on Disturbed Soil Properties, Water Quality and Vegetation Growth" (Doctoral Dissertation, University of Maryland, College Park).
- Pan, C. C., and C. H. Huang. 2024. "Cow Dung Compost and Vermicompost Amendments Promote Soil Carbon Stock by Enhancing Labile Organic Carbon and Residual Oxidizable Carbon Fractions in Maize Field Soil." *Soil Use and Management* 40, no. 4: e13122.
- Pandian, K., S. Vijayakumar, M. R. A. F. Mustaffa, P. Subramanian, and S. Chitraputhirapillai. 2024. "Biochar—A Sustainable Soil Conditioner for Improving Soil Health, Crop Production and Environment Under Changing Climate: A Review." *Frontiers in Soil Science* 4: 1376159.
- Paustian, K. A. O. J. H., O. Andren, H. H. Janzen, et al. 1997. "Agricultural Soils as a Sink to Mitigate CO₂ Emissions." *Soil Use and Management* 13: 230–244.
- Pei, J., J. Li, S. Mia, B. Singh, J. Wu, and F. A. Dijkstra. 2021. "Biochar Aging Increased Microbial Carbon Use Efficiency but Decreased Biomass Turnover Time." *Geoderma* 382: 114710.
- Qiao, Y., J. Wang, G. Liang, et al. 2019. "Global Variation of Soil Microbial Carbon-Use Efficiency in Relation to Growth Temperature and Substrate Supply." *Scientific Reports* 9, no. 1: 5621.
- Rousk, K., A. Michelsen, and J. Rousk. 2016. "Microbial Control of Soil Organic Matter Mineralization Responses to Labile Carbon in Subarctic Climate Change Treatments." *Global Change Biology* 22, no. 12: 4150–4161.
- Salehi, A., S. Fallah, and A. A. Sourki. 2017. "Organic and Inorganic Fertilizer Effect on Soil CO₂ Flux, Microbial Biomass, and Growth of Black Cumin L." *International Agrophysics* 31, no. 1: 103–116.
- Shah, F., and W. Wu. 2019. "Soil and Crop Management Strategies to Ensure Higher Crop Productivity Within Sustainable Environments." *Sustainability* 11, no. 5: 1485.
- Sinsabaugh, R. L., S. Manzoni, D. L. Moorhead, and A. Richter. 2013. "Carbon Use Efficiency of Microbial Communities: Stoichiometry, Methodology and Modelling." *Ecology Letters* 16, no. 7: 930–939.
- Six, J., P. Callewaert, S. Lenders, et al. 2002. "Measuring and Understanding Carbon Storage in Afforested Soils by Physical Fractionation." *Soil Science Society of America Journal* 66, no. 6: 1981–1987.
- Song, X., J. Zhu, N. He, et al. 2017. "Asynchronous Pulse Responses of Soil Carbon and Nitrogen Mineralization to Rewetting Events at a Short-Term: Regulation by Microbes." *Scientific Reports* 7, no. 1: 7492.
- Steiner, C. 2008. "Soil Charcoal Amendments Maintain Soil Fertility and Establish a Carbon Sink—Research and Prospects." In *Soil Ecology Research Developments*, 1–6. Nova Publishers.
- Sun, Y., X. Xiong, M. He, et al. 2021. "Roles of Biochar-Derived Dissolved Organic Matter in Soil Amendment and Environmental Remediation: A Critical Review." *Chemical Engineering Journal* 424: 130387.
- Tamilselvi, S. M., C. Chinnadurai, K. Ilamurugu, K. Arulmozhiselvan, and D. Balachandar. 2015. "Effect of Long-Term Nutrient Managements on Biological and Biochemical Properties of Semi-Arid Tropical Alfisol During Maize Crop Development Stages." *Ecological Indicators* 48: 76–87.
- Thakur, A., A. Kumar, and E. Berdimurodov. 2024. *Biomass Wastes*, 1. A Waste-to-Wealth Approach.
- Tian, X., Z. Li, Y. Wang, B. Li, and L. Wang. 2021. "Evaluation on Soil Fertility Quality Under Biochar Combined With Nitrogen Reduction." *Scientific Reports* 11, no. 1: 13792.
- Topcagic, A., S. C. Zeljkovic, E. Karalija, S. Galijasevic, and E. Sofic. 2017. "Evaluation of Phenolic Profile, Enzyme Inhibitory and Antimicrobial Activities of Black Cumin L. Seed Extracts." *Bosnian Journal of Basic Medical Sciences* 17, no. 4: 286.
- Vance, E. D., P. C. Brookes, and D. S. Jenkinson. 1987. "An Extraction Method for Measuring Soil Microbial Biomass C." *Soil Biology and Biochemistry* 19, no. 6: 703–707.

- Wang, C., L. Qu, L. Yang, et al. 2021. "Large-Scale Importance of Microbial Carbon Use Efficiency and Necromass to Soil Organic Carbon." *Global Change Biology* 27, no. 10: 2039–2048.
- Wang, H., T. W. Boutton, W. Xu, G. Hu, P. Jiang, and E. Bai. 2015. "Quality of Fresh Organic Matter Affects Priming of Soil Organic Matter and Substrate Utilization Patterns of Microbes." *Scientific Reports* 5, no. 1: 10102.
- Wang, J., Y. Yang, J. Wu, K. Zhao, and X. Zhang. 2024. "The Interaction Between Biochar and Earthworms: Revealing the Potential Ecological Risks of Biochar Application and the Feasibility of Their Co-Application." *Science of the Total Environment* 950: 175240.
- Wardle, D. A. 1992. "A Comparative Assessment of Factors Which Influence Microbial Biomass Carbon and Nitrogen Levels in Soil." *Biological Reviews* 67, no. 3: 321–358.
- Wardle, D. A., and A. Ghani. 1995. "A Critique of the Microbial Metabolic Quotient (qCO_2) as a Bioindicator of Disturbance and Ecosystem Development." *Soil Biology and Biochemistry* 27, no. 12: 1601–1610.
- Weil, R. R., and F. Magdoff. 2004. "Significance of Soil Organic Matter to Soil Quality and Health." In *Soil Organic Matter in Sustainable Agriculture*, 1–43. CRC Press.
- Xu, X., J. Pei, Y. Xu, and J. Wang. 2020. "Soil Organic Carbon Depletion in Global Mollisols Regions and Restoration by Management Practices: A Review." *Journal of Soils and Sediments* 20: 1173–1181.
- Xue, L., P. Zhang, H. Shu, R. Wang, and S. Zhang. 2016. "Agricultural Waste." *Water Environment Research* 88, no. 10: 1334–1369.
- Yan, S., S. Zhang, P. Yan, and M. Aurangzeib. 2022. "Effect of Biochar Application Method and Amount on the Soil Quality and Maize Yield in Mollisols of Northeast China." *Biochar* 4, no. 1: 56.
- Yanardağ, I. H., R. Zornoza, F. Bastida, et al. 2017. "Native Soil Organic Matter Conditions the Response of Microbial Communities to Organic Inputs With Different Stability." *Geoderma* 295: 1–9.
- Yanardağ, İ. H., R. A. U. L. Zornoza, A. F. Cano, A. B. Yanardağ, and A. R. Mermut. 2015. "Evaluation of Carbon and Nitrogen Dynamics in Different Soil Types Amended With Pig Slurry, Pig Manure and Its Biochar by Chemical and Thermogravimetric Analysis." *Biology and Fertility of Soils* 51: 183–196.
- Yang, X., C. Xue, L. Su, et al. 2018. "Exploring Patterns of Camellia Black Cumin Residue Application in Relation to Plant Growth, Soil Nematodes and Microbial Biomass." *Soil Science and Plant Nutrition* 64, no. 2: 253–264.
- Yin, J., L. Zhao, X. Xu, D. Li, H. Qiu, and X. Cao. 2022. "Evaluation of Long-Term Carbon Sequestration of Biochar in Soil With Biogeochemical Field Model." *Science of the Total Environment* 822: 153576.
- Yu, J., J. Yang, L. Qu, et al. 2025. "Soil Microbial Carbon Use Efficiency Differs Between Mycorrhizal Trees: Insights From Substrate Stoichiometry and Microbial Networks." *ISME Communications* 5, no. 1: ycae173.
- Yu, L., M. Yu, X. Lu, et al. 2018. "Combined Application of Biochar and Nitrogen Fertilizer Benefits Nitrogen Retention in the Rhizosphere of Soybean by Increasing Microbial Biomass but Not Altering Microbial Community Structure." *Science of the Total Environment* 640: 1221–1230.
- Zhang, L., Y. He, D. Lin, Y. Yao, N. Song, and F. Wang. 2022. "Co-Application of Biochar and Nitrogen Fertilizer Promotes Rice Performance, Decreases Cadmium Availability, and Shapes Rhizosphere Bacterial Community in Paddy Soil." *Environmental Pollution* 308: 119624.
- Zhang, M., G. Cheng, H. Feng, et al. 2017. "Effects of Straw and Biochar Amendments on Aggregate Stability, Soil Organic Carbon, and Enzyme Activities in the Loess Plateau, China." *Environmental Science and Pollution Research* 24: 10108–10120.
- Zhang, Q., Y. Song, Z. Wu, et al. 2020. "Effects of Six-Year Biochar Amendment on Soil Aggregation, Crop Growth, and Nitrogen and Phosphorus Use Efficiencies in a Rice–Wheat Rotation." *Journal of Cleaner Production* 242: 118435.
- Zhang, R., Z. Qu, L. Liu, et al. 2022. "Soil Respiration and Organic Carbon Response to Biochar and Their Influencing Factors." *Atmosphere* 13, no. 12: 2038.
- Zhao, Y., J. Zhang, C. Müller, and Z. Cai. 2018. "Temporal Variations of Crop Residue Effects on Soil N Transformation Depend on Soil Properties as Well as Residue Qualities." *Biology and Fertility of Soils* 54: 659–669.
- Zhou, X., X. Wang, H. Zhang, and H. Wu. 2017. "Enhanced Nitrogen Removal of Low C/N Domestic Wastewater Using a Biochar-Amended Aerated Vertical Flow Constructed Wetland." *Bioresource Technology* 241: 269–275.
- Zribi, I., N. Ghezal, H. Sbair, G. Richard, M. L. Fauconnier, and R. Haouala. 2019. "Biochemical Composition of Tunisian Black Cumin L. at Different Growth Stages and Assessment of the Phytotoxic Potential of Its Organic Fractions." *Plant Biosystems-An International Journal Dealing With All Aspects of Plant Biology* 153, no. 2: 205–212.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.