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RESEARCH ARTICLE

Interaction effects of eucalyptus biochar and chlorantraniliprole on soil carbon mineralization under different soil moistures

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ABSTRACT

Biochar can provide beneficial organic matter and habitat for microorganisms in soils polluted with insecticides, potentially mitigating some of the adverse effects on microbial activity. This study aimed to assess the effectiveness of eucalyptus feedstock biochar in remediation of a clayey soil polluted with chlorantraniliprole (CAP) under laboratory conditions through the determination of microorganism activity. The CAP as commercial formulation at recommended dose and tenfold dose (C1 and C10), eucalyptus biochar (produced at 550 °C) at 0.5% and 1% (B05 and B1) and their combinations (B05+C1, B05+C10, B1+C1 and B1+C10) were applied on a clayey soil while control treatment has neither biochar nor insecticide application. Soil C mineralization (Cm) was monitored for 46 d at a constant temperature (28 °C) under two soil moisture conditions: 50% (50FC) and 80% (80FC) field capacity. Treatments B05, B1, C1, and C10 decreased Cm by for 3.5%, 5.5%, 8.4%, and 14.1% under 50FC and for 3.2%, 6.0%, 7.9%, and 14.6% under 80FC compared to control without biochar and insecticide ($P < 0.05$). However, co-application of eucalyptus biochar mitigated the negative effects of CAP for more than 4% on Cm. B05+C10 significantly decreased C mineralization compared to B05 and B05+C1 while B1+C10 was significantly lower than B1 but higher than C10 ($P < 0.05$). Treatments CAP and biochar alone and their combinations significantly decreased Cm compared to control in this study but it is suggested that eucalyptus biochar might have mitigated the negative effects of CAP on Cm by forming complexes with labile organic matter.

Key words: Biochar, carbon mineralization, chlorantraniliprole, insecticide, soil microbial activity, soil moisture.

INTRODUCTION

The application of insecticides remains one of the most significant strategies in crop protection, particularly for the management of insect pests. During a single cropping season, insecticides are often applied multiple times to ensure effective control. Many insecticides applied in plant production usually reach the soil through misapplication and/or indirectly by leaching from the green parts of plants. In addition, extended and repeated applications of insecticides may cause their residues or metabolites to accumulate in plant and soil ecosystems (Zhang et al., 2025).

One of the main insecticides belonging to the anthranilic and phthalic diamide group is chlorantraniliprole (CAP). It has the ability to control a broad spectrum of insects and acts as a mobile and persistent molecule in aquatic and soil ecosystems. Repeated applications of CAP may lead to its accumulation in soils and cause harmful effects on non-target soil organisms (Wu et al., 2017; 2018). It has been reported that CAP had no negative effect on soil ecosystem through its effects on soil enzymatic activity, soil respiration and phospholipid fatty acids (PLFA) profile in a paddy soil when it was used at recommended field doses (Wu et al., 2017). In contrast, the application of high field doses of CAP has been reported to have toxicological effects on soil bacterial communities and microbial activities (Wu et al., 2018).

Carbon is one of the fundamental elements of all living beings, and its cycling has vital impacts on soil health and climatic regimes. Soil organic matter (SOM) represents the sequestration of C products through photosynthesis by plants, while about half of these products are released back into the atmosphere as carbon dioxide through the respiration of microorganisms or plants, or through SOM decomposition. Disruptions in SOM turnover may lead to significant effects on soil ecosystems and organic C reserves on Earth. Short term C mineralization in soil can be determined under laboratory conditions constant temperature and soil moisture regimes which has the ability to reflect soil respiration under field conditions. Many studies have been reported that optimum microbial activity such as C mineralization can be measured in soils under Mediterranean climate at 28 °C and monitored for more than 30 d (Sagliker and Cevik, 2016; Koçak and Ortaş, 2021; Koçak, 2022).

Biochar is a form of black C and can be obtained from the pyrolysis of organic matter in high temperatures under limited oxygen conditions. One of the remediation methods for pesticide-polluted soils is the application of biochar, which has attracted much attention for more than 15 yr (Zhang et al., 2020). In many countries, the production of biochar from eucalyptus feedstocks has become easier due to their abundance, and it is strongly suggested that this production can also make use of residues obtained from the forestry products sector (Heidari et al., 2019). When biochar applied to the soil ecosystems, it can significantly influence the degradation, adsorption, leaching and bioavailability of pesticides (Liu et al., 2018a). For instance, Nag et al. (2011) reported that addition of biochar at rates of 0%, 0.5% and 1% to the soils had a positive effect on the survival of ryegrass against two popular herbicides (atrazine and trifluralin).

When insecticides reach or are introduced into the soil through repeated applications, even at field rates, they can have positive (stimulatory), negative (inhibitory), or neutral effects on soil microorganisms. For instance, applications of hexachlorocyclohexane, carbofuran, fenvalerate and phorate at field rates enhanced the mineralization and availability of organic C in soil (Wołejko et al., 2020). In contrast, chlorpyrifos and cartap hydrochloride at field rates reduced C mineralization during the first 30 d, but increased it afterward under laboratory conditions (Dhuldhaj et al., 2023). Similarly, the recommended dose of emamectin benzoate, along with its twofold and fourfold doses, inhibited soil C mineralization under different temperatures and soil moisture levels (Cenkseven et al., 2019). Deltamethrin and lambda-cyhalothrin, when applied at field, twofold, and fourfold doses, also inhibited soil C mineralization under laboratory conditions (Sagliker and Cevik, 2016). In another study, ten and hundred folds of deltamethrin applications caused changes in soil microbial parameters in a clay-loam soil under laboratory conditions (Muñoz-Leoz et al., 2013). High field rates of spirotetramat showed low toxic effects on soil C mineralization, but increases in soil moisture counteracted these negative effects (Koçak, 2022). Overall, such insecticide impacts alter soil microbial activity and may disrupt soil C turnover.

The objectives of this study were to evaluate the individual and combined effects of the recommended and tenfold doses of CAP, along with eucalyptus biochar (0.5% and 1%, produced at 550 °C), on C mineralization and its rate in a clayey soil under two moisture levels (50% and 80% field capacity). We hypothesized that high dose of CAP would reduce C mineralization, but that biochar would mitigate these negative effects on soil microbial activity.

MATERIALS AND METHODS

Biochar synthesis

Eucalyptus (*Eucalyptus camaldulensis* Dehn.) feedstocks were collected in October 2021 from the Cukurova University Agricultural Research Farm, located in the southeastern Mediterranean region of Turkey. The air-dried feedstocks were pyrolyzed at 550 °C for 2 h in a sealed container with limited oxygen using a muffle furnace (RD50, Refsan, Istanbul, Türkiye). After pyrolysis, the resulting biochar was ground and passed through a 2 mm sieve prior to organic C and total N analyses. Finally, the prepared biochar was stored in a desiccator under dry conditions to prevent moisture absorption.

Study area, soil sampling and analysis

Soil samples were collected from the Agricultural Research Station of Cukurova University (37°00′54″ N, 35°21′27″ E; 32 m a.s.l.) at a depth of 0-10 cm in October 2021 in Adana, Türkiye. A single surface soil sample was taken from each of the three corners of the study area due to its shape. The site had no previous history of chlorantraniliprole or biochar application.

The soil is classified as Vertisols and belong to the Arık series, which is generally distributed in nearly flat terrains. With an A-C horizon arrangement, the Arık series has a relatively thick A horizon. It differs from other series in the region by its high clay content and shiny shear surfaces (Turgut and Koca, 2019).

The study site is located in a Mediterranean climate zone, with an average annual precipitation of 668.8 mm. July and August are typically the hottest months, with mean daily temperatures of 28.2 and 28.7 °C, respectively (Koç and Topaloğlu-Paksoy, 2025). This climate type is characterized by extremely hot summers and mildly cold winters.

Soil samples were initially air-dried, passed through a 2 mm mesh sieve, and thoroughly mixed prior to analysis. The following soil and biochar properties were determined according to Kacar (2016): Soil texture was measured using the Bouyoucos hydrometer method, and soil and biochar pH were recorded in a 1:2.5 soil-to-distilled water suspension with a pH meter (Orion Star A211; Thermo Fisher Scientific, Waltham, Massachusetts, USA). The electrical conductivity (EC) of biochar was then determined using a portable conductivity meter (Orion Star A222, Thermo Fisher Scientific). Calcium carbonate ($CaCO_3$) content was estimated using a Scheibler calcimeter, while field capacity (FC, %) was determined using a vacuum pump at one-third atmospheric pressure. Organic C (SOC%) and total N (TN%) contents of soils and biochar were determined by the modified Walkley Black method and the Kjeldahl method, respectively.

Incubation setup and monitoring

Soil (100 g) was placed in 750 mL incubation vessels. Obtained eucalyptus biochar materials were added to the soils at 0.5% (B05) and 1% (B1). Chlorantraniliprole (CAP; 3-bromo-*N*-[4-chloro-2-methyl-6-(methylcarbamoyl)phenyl]-1-(3-chloro-2-pyridinyl)pyrazole-5-carboxamide) was bought as a commercial formulation (200 g L^{-1} active ingredient) and applied on soils at the recommended dose (C1, 200 mL ha^{-1}) and at ten times higher (C10). The insecticide was applied to the soil assuming a penetration depth of 1 mm and based on the soil bulk density (1.31 g cm^{-3}). A control treatment (A), without insecticide or biochar additions, was included to assess baseline soil microbial activity. Soil, biochar, and insecticide mixtures were adjusted at 50% (50FC) and 80% (80FC) of soil field capacity and incubated at 28 °C for 46 d. There was a total of nine treatments for each field capacity in this experiment: 1) Control (A), 2) biochar at 0.5% (B05), 3) biochar at 1% (B1), 4) CAR at recommended field dose (C1), 5) CAR at tenfold of recommended field dose (C10), 6) B05 + C1, 7) B05 + C10, 8) B1 + C1, 9) B1 + C10. The incubation experiment was conducted under laboratory conditions following a completely randomized design with three replicates and was provided in Figure 1. The CO_2 generated by the activity of soil microorganisms was periodically captured in 0.5 M NaOH solution contained in small tubes positioned above the soil within the incubation vessels. The amount of CO_2 produced by soil microorganisms was measured by titration with hydrochloric acid (Alef, 1995) on the following days of incubation: 1, 4, 8, 12, 17, 24, 32, and 46.

Cumulative C mineralization (Cm, mg C-CO_2 100 g^{-1} air-dried soil) was calculated by summing the CO_2 released on all sampling days throughout the 46 d incubation period. The mineralization rate was then determined by dividing the cumulative C mineralized by the soil organic C content of both control and treated samples (Koçak and Darici, 2016).

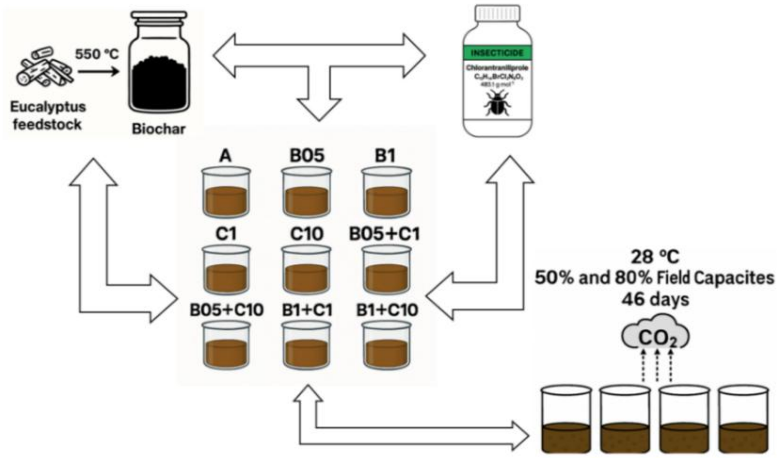

Figure 1. Experimental design. A: Control; B05: biochar at 0.5%; B1: biochar at 1%; C1: chlorantraniliprole at recommended field dose; C10: chlorantraniliprole at tenfold of recommended field capacity. Imagen debe decir capacities en lugar de capacites

Statistical analyses

A three-way ANOVA was performed to test the effects of moisture, biochar dose, insecticide dose, and their interactions on soil C mineralization and its rate. The assumptions of normality and homogeneity of variances were tested using the Shapiro-Wilk and Levene's tests, respectively, prior to the ANOVA test. Because there was found no interaction of all factors (Moisture × Biochar dose × Insecticide dose combined), two-way ANOVA was applied to evaluate the effects of biochar and insecticide doses and their interaction on C mineralization. Least significant differences (LSD) test was used to show differences between the means of biochar doses, insecticide doses and their combined doses in C mineralization, separately. All statistical analyses were carried out using JMP version 9 (JMP Statistical Discovery, Cary, North Carolina, USA). Results are presented in tables and figures as mean values ± standard errors of three replicates, and differences between the data were declared as significant at $P < 0.05$.

RESULTS

Some properties of soil and biochar

The physical and chemical characteristics of the soil and the C and N contents of the biochar are summarized in Table 1. The soil was classified as clay with a slightly basic pH. Field capacity and lime content were 26.86% and 29.07%, respectively. Soil organic C (SOC), total N (TN), P (P_2O_5), K (K_2O), and Mg levels were 1.48%, 0.114%, 77.2 kg ha^{-1}, 1153.1 kg ha^{-1}, and 1489.8 kg ha^{-1}, respectively. The biochar contained 72.33% organic C and 0.81% total N while EC and pH of biochar was 1.38 and 9.06 mS cm^{-1}, respectively.

Table 1. Selected physical and chemical properties of the soil and C and N contents of the eucalyptus biochar. Data are expressed as mean ± standard error (n = 3). EC: Electrical conductivity.

	Mean
Clay, %	42.80 ± 0.40
Silt, %	36.20 ± 0.35
Sand, %	21.00 ± 0.17
Texture	Clay
pH (1:2.5)	8.50 ± 0.01
EC, mS cm^{-1}	0.23 ± 0.00
$CaCO_3$, %	29.07 ± 0.06
Field capacity, %	26.86 ± 1.13
C, %	1.48 ± 0.015
N, %	0.114 ± 0.005
P, P_2O_5 kg ha^{-1}	77.2 ± 0.60
K, K_2O kg ha^{-1}	1153.1 ± 18.7
Mg, kg ha^{-1}	1489.8 ± 8.8
Biochar pH	9.06 ± 0.03
Biochar EC, mS cm^{-1}	1.38 ± 0.04
Biochar C, %	72.33 ± 0.21
Biochar N, %	0.81 ± 0.007

Responses of C mineralization to treatment combinations

The incubation experiment procedure selected for this study provided insights about the effects of high and recommended field rates of CAP, eucalyptus biochar and soil moistures on SOC turnover under laboratory conditions in short term. Therefore, 46 d of soil + insecticide + biochar additions incubations under constant temperature (28 °C) and moistures (50% and 80% of field capacity) were long enough to observe the responses of insecticide contamination SOC mineralization in this study (Figures 2 and 3).

According to the ANOVA results (Table 2), cumulative C mineralization at 46 d incubation was significantly affected by moisture (F = 2310.74, P < 0.001, η^2 = 0.984) and insecticide dose (F = 92.42, P < 0.001, η^2 = 0.837). In contrast, biochar alone, as well as the interactions of Moisture × Biochar, and Moisture × Insecticide, did not show significant effects on cumulative C mineralization. However, the interaction between Biochar × Insecticide was significant. In contrast, biochar alone (F = 1.19, P = 0.315, η^2 = 0.062), as well as the interactions of Moisture × Biochar (F = 0.018, P = 0.982, η^2 = 0.001) and Moisture × Insecticide (F = 2.88, P = 0.069, η^2 = 0.138), did not show significant effects on cumulative C mineralization. However, the interaction between Biochar × Insecticide was significant (F = 11.17, P < 0.001, η^2 = 0.554). The three-way interaction (Moisture × Biochar × Insecticide) was nonsignificant for cumulative mineralization (F = 0.53, P = 0.714, η^2 = 0.056). For the rate of C mineralization, all main effects including moisture (F = 2408.99, P < 0.001, η^2 = 0.985), biochar (F = 1643.31, p < 0.001, η^2 = 0.989), and insecticide dose (F = 111.56, P < 0.001, η^2 = 0.861) were significant. Moreover, the interactions of Moisture × Biochar (F = 31.84, P < 0.001, η^2 = 0.639), Moisture × Insecticide (F = 3.45, P = 0.043, η^2 = 0.161), and Biochar × Insecticide (F = 21.17, P < 0.001, η^2 = 0.702) also had significant effects. In contrast, the three-way interaction of Moisture × Biochar × Insecticide was nonsignificant (F = 0.73, P = 0.579, η^2 = 0.075) for mineralization rate.

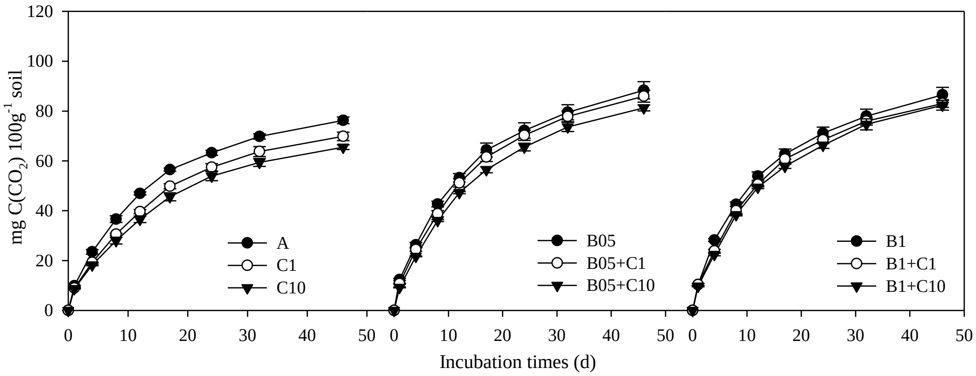

Figure 2. Cumulative C mineralization. A: Control; B05: biochar at 0.5%; B1: biochar at 1%; C1: chlorantraniliprole at recommended field dose; C10: chlorantraniliprole at tenfold of recommended field dose and their combinations (B05+C1; B05+C10; B1+C1; B1+C10) under 50% field capacity (*n* = 3).

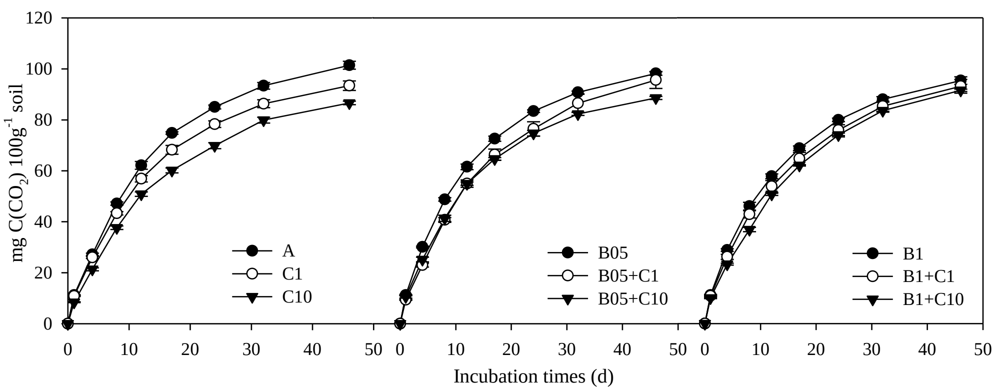

Figure 3. Cumulative C mineralization. A: Control; B05: biochar at 0.5%; B1: biochar at 1%; C1: chlorantraniliprole at recommended field dose; C10: chlorantraniliprole at tenfold of recommended field dose and their combinations (B05+C1; B05+C10; B1+C1; B1+C10) under 80% of field capacity (*n* = 3).

Table 2. Effects of moisture, biochar doses, insecticide doses and their interactions on soil C mineralization (Cm) and rate.

		df	Mean square	*F*	*P*	Partial eta squared (η^2)
Moisture	C_m	1	7306.13	2310.83	< 0.001	0.984
	Rate of C_m	1	22.71	2408.99	< 0.001	0.985
Biochar	C_m	2	3.767	1.191	0.315	0.062
	Rate of C_m	2	15.49	1643.31	< 0.001	0.989
Insecticide	C_m	2	292.27	92.44	< 0.001	0.837
	Rate of C_m	2	1.052	111.56	< 0.001	0.861
Moisture × Biochar	C_m	2	0.056	0.020	0.982	0.001
	Rate of C_m	2	0.300	31.84	< 0.001	0.639
Moisture × Insecticide	C_m	2	9.080	2.87	0.069	0.138
	Rate of C_m	2	0.033	3.45	0.043	0.161
Biochar × Insecticide	C_m	4	35.31	11.17	< 0.001	0.554
	Rate of C_m	4	0.199	21.17	< 0.001	0.702
Moisture × Biochar × Insecticide	C_m	4	1.681	0.53	0.714	0.056
	Rate of C_m	4	0.007	0.73	0.579	0.075

Soil C mineralization under 50% and 80% of field capacity

At the end of the incubation period, cumulative C mineralization was influenced by both soil moisture levels (50% and 80% field capacity; FC), as well as biochar (B05 and B1), insecticide (C1 and C10), and their combinations (Figures 2 and 3). All treatments resulted in lower cumulative C mineralization compared to the control (A) under both moisture regimes.

Microbial respiration followed similar trends when biochar and CAP were considered singly and co-applied under both moisture regimes (Figures 2 and 3). Soil respiration increased from 1 to 8 d after incubation at all biochar, insecticide and their combined treatments. In general, the peak of soil microbial respiration was observed at 9th day of incubation period and thereafter there was a decrease and data of soil microbial respiration obtained follow a consistent pathway. Both eucalyptus biochar and CAP has influenced microbial respiration all the days from the beginning of the incubation to the ending of the experiment. However, results indicated that eucalyptus biochar, CAP doses and their combined treatments generally inhibited soil microbial respiration from the 9th day of incubation to the end of incubation under both moisture regimes (Figures 2 and 3).

Under 50FC, cumulative C mineralization ranged from 65.52 mg C-CO_2 (C10) to 76.31 mg C-CO_2 (A), while under 80FC, it ranged from 86.66 mg C-CO_2 (C10) to 101.45 mg C-CO_2 (A) (Table 3). Compared to the control, decreases were 3.5%, 5.5%, 8.4%, and 14.1% for B05, B1, C1, and C10 under 50FC, and 3.2%, 6.0%, 7.9%, and 14.6% for B05, B1, C1, and C10 under 80FC, respectively. Under both soil moisture levels, B05+C10 significantly reduced C mineralization compared to B05 and B05+C1 ($P < 0.05$). Similarly, B1+C10 was significantly lower than B1 alone but remained higher than C10 ($P < 0.05$).

The higher C10 dose of insecticide had the strongest suppressive effect on C mineralization, although co-application with biochar partially mitigated this impact. Increasing soil moisture from 50FC to 80FC generally enhanced cumulative C mineralization across all treatments. Nevertheless, soils amended with biochar and insecticide consistently showed lower mineralization values compared to the control.

Table 3. Cumulative C mineralization of Biochar × Insecticide treatment combinations under two soil moisture levels (50% FC and 80% FC) ($n = 3$). FC: Field capacity. Under 50FC, Biochar: ns, Insecticide: $P < 0.001$, Biochar × Insecticide: $P < 0.05$. Under 80FC, Biochar: ns, Insecticide: $P < 0.001$, Biochar × Insecticide: $P < 0.001$. ns: Nonsignificant.

	Soil moisture	
Treatmens	50FC	80FC
Control (A)	76.31 ± 0.79^{a}	101.45 ± 0.9^{a}
C1	69.88 ± 0.98^{ce}	93.44 ± 1.07^{cd}
C10	65.52 ± 0.55^{f}	86.66 ± 0.39^{f}
B0.5	73.64 ± 1.66^{ab}	98.24 ± 0.42^{b}
B1	72.12 ± 1.43^{bc}	95.36 ± 0.85^{bc}
B0.5+C1	71.66 ± 1.14^{bd}	95.62 ± 1.90^{bc}
B0.5+C10	67.74 ± 0.58^{ef}	88.62 ± 0.35^{ef}
B1+C1	69.21 ± 0.75^{ce}	93.13 ± 1.56^{cd}
B1+C10	68.64 ± 0.94^{de}	91.58 ± 0.27^{de}

Rates of soil C mineralization

Soils treated with biochar, insecticide, or their combination exhibited lower C mineralization rates than the control (A) under both 50% FC and 80% FC at the end of incubation (Figure 4). Under 50% FC, rates ranged from 3.12% in B1+C10 to 5.16% in A, while under 80% FC, rates ranged from 4.16% in B1+C10 to 6.85% in A. Significant differences between all treatments showed similar trends under both field capacities: B05, B1, C1 and C10 were significantly lower than A ($P < 0.05$). Treatment B05+C10 was significantly lower than B05 ($P <$

0.05). There were found nonsignificant differences between B1, B1+C1 and B1+C10 under FC50, whereas B1+C10 was significantly lower than B1 under 80FC ($P < 0.05$).

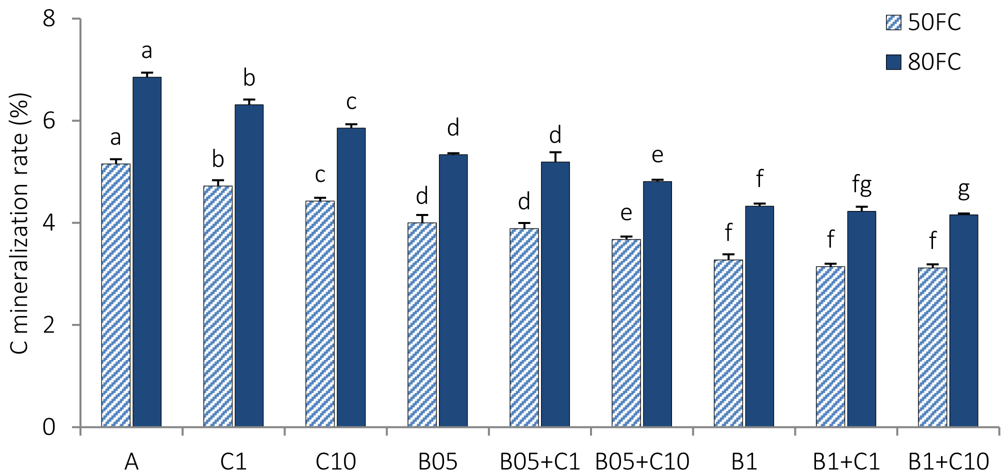

Figure 4. Rate of C mineralization. A: Control; B05: biochar at 0.5%; B1: biochar at 1%; C1: chlorantraniliprole at recommended field dose; C10: chlorantraniliprole at tenfold of recommended field dose and their combinations (B05+C1; B05+C10; B1+C1; B1+C10) under 50% and 80% of field capacity. Data are expressed as mean ± standard error. Different letters denote significant differences among control. biochar and insecticide doses according to LSD analysis for each individual field capacity ($P < 0.05$) ($n = 3$).

DISCUSSION

Effects of chlorantraniliprole

In consistency with our hypothesis, in general, the applications of recommended and tenfold doses of chlorantraniliprole (CAP), combined with and without biochar, significantly decreased soil C mineralization (Cm) and its rate under both field capacities. These findings are similar to Zhang et al. (2025), who suggested that repeated application of CAP suppressed the accumulation of soil N and organic matter and decreased some soil enzymatic activities in soil ecosystems. Furthermore, the same authors reported that CAP inhibited metabolic pathways of C-N and altered soil microbial communities (Zhang et al., 2025). In contrast, it was suggested that different concentrations of CAP applications did not change soil fungal and bacterial diversities, but there were interactive effects between time and CAP applications that significantly affected the diversity of soil microorganisms (Tang et al., 2023). Additionally, Sahu et al. (2019) suggested that the field rate dose and high dose of CAP had no inhibitory effect on fungi, microorganisms in N fixation and phosphate-solubilizing bacteria, although heterotrophic bacteria populations significantly varied between different doses of this insecticide. They also reported that clay content is a major factor in the dissipation of the insecticide in soil and claimed that the field rate dose had no toxic effects on soil microbes under controlled environments (Sahu et al., 2019). However, the recommended dose (C1) significantly reduced soil microbial activity by more than 8% under both 50% (50FC) and 80% soil moisture (80FC) in this study. Wu et al. (2017) reported that CAP decreased microbial soil respiration and metabolic quotient (qCO_2) in paddy soils during the first 14 d incubation, but after the 14th day, soils treated with CAP were significantly equivalent to the control. These authors claimed that their results indicated CAP ultimately had no harmful effects on activities of soil microorganisms and microbial compositions in paddy soils (Wu et al., 2018). In contrast, in this study, there were generally nonsignificant differences between all doses of insecticide and control with and without biochar addition until the 8th day of incubation. This finding suggests that CAP might have had no harmful or toxic effects on soil microbial activity during the first week of incubation. Povedano et al. (2025) suggested that CAP significantly decreased soil microbial respiration in the long-term incubation experiment under laboratory conditions in clay soil by 56% and in sandy clay loam soil by 88%, compared to blank soil. Based on this latter study, residues or metabolites of CAP may possibly threaten soil Cm after the 8th day by inhibiting the activity of soil microorganisms in this study.

Effects of biochar and CAP combinations

There is no general consensus on the true effects of biochar on soil Cm in the literature. Biochar addition has shown beneficial (Zhang et al., 2022) and harmful (Liu et al., 2018b) effects on soil Cm (Chen et al., 2024). In general, the 1% biochar dose (B1) significantly reduced cumulative Cm under 50FC and 80FC after the 46th day. Suppression of eucalyptus biochar on soil microbial activity can be caused by: 1) The formation of complexes through the combination of labile organic matter and biochar components, thus inhibiting mineralization, 2) inhibition of microbial activity through organic components of biochar, and/or 3) suppression of microbially available labile substrates in biochar (Zhang et al., 2022). Dempster et al. (2012) suggested that eucalyptus biochar amendment decreased CO_2 evolution at a 5 t ha^{-1} dose but not at 25 t ha^{-1} dose in a soil with 95% sand. On the other hand, Singh and Cowie (2014) suggested that biochar obtained from eucalyptus wood and leaves (pyrolyzed at 550 °C) increased organic Cm in a low-C clayey soil, although this impact decreased over time. Koçak and Ortaş (2021) claimed that eucalyptus biochar significantly increased Cm in a clayey soil by more than 20%, but decreased it in a silty loam soil.

Nonsignificant differences were found between combinations of the recommended dose of insecticide and biochar (B05+C1 and B1+C1) and the counterpart controls with only biochar (B05 and B1) under 50FC and 80FC in cumulative Cm. Treatment B1+C10 showed significantly higher mineralization compared to tenfold CAP dose (C10) under 80FC. These results indicate that eucalyptus biochar doses may have decreased the negative effects of the recommended dose of this insecticide compared to the C1 treatment. Wang et al. (2012) suggested that additions of eucalyptus biochar (produced at 450 and 850 °C) caused a strong reduction in the biological availability of CAP in soil ecosystem. In addition, Wang et al. (2015) suggested that sorption of CAP was stimulated by eucalyptus biochar (produced at 850 °C) in soils. These latter studies are consistent with the results of this study, suggesting that eucalyptus biochar had a beneficial effect on soil microorganisms decomposing the recommended dose of CAP. In contrast, Sun et al. (2021) claimed that walnut shell biochar (produced at 550 °C) did not alter the degradation rate of residue or half-life of CAP in soil under field conditions.

In this study, eucalyptus biochar may have reduced the negative effects of the recommended dose of CAP on soil microbes by binding with the CAP. There are reports that biochar produced from Eucalyptus species has promising effects for the removal of insecticides. Srikhaow et al. (2022) showed that eucalyptus wood chip biochar has high adsorption rates for the insecticides imidacloprid, acetamiprid, and methomyl at commercial grade. In the latter study, they explained that chemical bonds between biochar and the insecticides, as well as between their aromatic C, may have caused the high adsorption of the insecticides. Fernandes et al. (2022) suggested that the amendment of eucalyptus biochar did not change hexazinone (herbicide) mineralization, but biochar produced at 850 and 950 °C effectively restrained the leaching of this herbicide into the soil.

Effects of soil moisture

Two of the main factors that regulate soil biological activities are water content and water uptake by soil microorganisms, it is closely associated with energy obtained from organic matter through mineralization (Cenkseven et al., 2017). In this study, 50FC significantly reduced cumulative Cm by about 20% more than 80FC in all treatments. Cenkseven et al. (2019) reported that recommended field dose, twofold, and fourfold doses of emamectin benzoate insecticide were significantly higher at 80% of field capacity than at 60% and 40%. Wirsching et al. (2023) claimed that limiting environmental conditions such as decreasing soil moisture can reduce the mineralization of pesticides. Overall, soil moisture is a more important factor than biochar and CAP in soil organic matter mineralization in this study.

CONCLUSIONS

This study examined the influence of the recommended field dose and high dose of the insecticide chlorantraniliprole (CAP) on soil C mineralization (Cm), with and without the addition of eucalyptus biochar under two soil moisture conditions: 50% and 80% of field capacity. Increasing soil moisture generally enhanced cumulative Cm for all treatments. It found that the recommended dose and tenfold dose of CAP applications significantly suppressed soil Cm, whereas biochar partially alleviated these inhibitory effects. In general, all biochar and CAP treatments significantly decreased the rate of Cm in the studied soil. These results suggest that eucalyptus biochar may help mitigate the negative effects of CAP on soil microbial activity, potentially by binding the insecticide in the soil. However, further research is needed to fully understand the interactions among biochar, soil microorganisms, and CAP, particularly with respect to the biodegradation of CAP and its metabolites.

Author contribution

The author confirms sole responsibility for the following: Study conception and design, data collection, analysis and interpretation of results, and manuscript preparation.

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