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Biochar

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Biochar application enhances ant (*Formica japonica*) ecological functions as indicated by their social behaviors

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Abstract

Soil fauna activity is crucial for soil ecosystem functioning and health, but soil remediation techniques, such as biochar application, are seldom examined for their influence on soil fauna activity. This study systematically evaluated the effects of rice straw biochar application on the social behaviors of ants, *Formica japonica*, and elucidated how these behavioral changes enhance ant ecological functional traits. At moderate concentrations (2.5–5%), biochar significantly enhanced several ant colony functions: nest site selection specificity increased by 73.4%, nest architecture complexity improved by 2.8-fold, foraging efficiency accelerated by 2 fold, and social recognition accuracy enhanced by 3.5-fold. The improved colony performance was manifested through sophisticated behavioral responses, including strengthened territorial defense (3.5-fold increase in aggressive behavior duration) and enhanced intraspecific cooperation (4 fold increase in peaceful touch frequency). These behavioral enhancements coincided with specific soil physicochemical conditions at the application rate of 5% biochar. However, higher biochar doses (10%) reduced colony-level survival to 60 ± 5.44% and diminished functional enhancement of behavioral performance, in part due to the presence of persistent free radicals and high pH. Our results indicate that ant social behavior may be altered during soil remediation, which should be carefully considered prior to engineering practices.

Highlights

- Biochar application altered social behaviors of the ant *Formica japonica*.
- Moderate doses (2.5–5%) enhanced foraging and species recognition of ants.
- High biochar dose (10%) reduced survival and behavioral efficiency.

Keywords Rice straw, Social behavior, Nesting, Foraging, Species recognition, Ecosystem engineering

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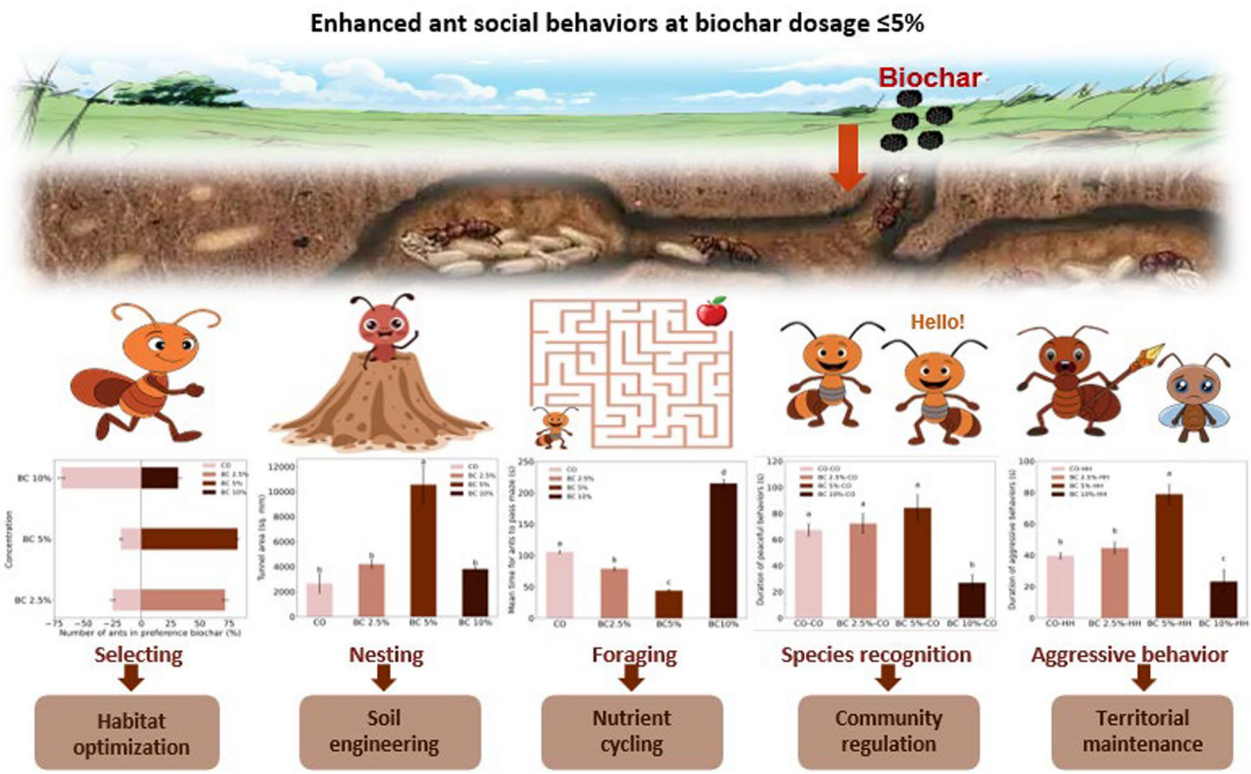
Graphical Abstract



1 Introduction

Soil degradation has become a global ecological crisis, with 33% of Earth's soils experiencing accelerated deterioration of biological functions and a 69% decline in global biodiversity (including soil fauna) since 1970 (Dirzo and Raven 2003; Montanarella et al. 2015; Pennock et al. 2015). This degradation has triggered widespread ecosystem service failures, widely reported as reduced soil fertility, decreased carbon sequestration capacity, and disrupted nutrient cycling (Lal 2015). Particular concern is the reduction in soil ecosystem function, threatening the fundamental stability of terrestrial ecosystems (Lal 2015; Bender et al. 2016). A major cause is considered to be the loss of soil organic matter due to increased erosion and photodegradation (Häring et al. 2013; Hancock et al. 2019; Li et al. 2019). In response, biochar, defined as a carbon-rich and stable product of biomass pyrolysis, has emerged as a promising strategy for soil restoration (Biederman and Harpole 2013). For example, biochar has attracted worldwide attention due to its demonstrated potential to improve soil properties (Laghari et al. 2016) and other ecosystem services (such as increasing plant growth (Hussain et al. 2017) and changing soil microbiological composition and abundance (Lehmann et al. 2011)), as well as sequestering carbon to mitigate climate change (Brassard et al. 2016).

Emerging research suggests that prominent soil fauna could be substantially impacted by biochar (Liu et al. 2020; Ma et al. 2024), driven by changes in soil pH, humidity, and the structure of microbial communities (Noyce et al. 2015; De Tender et al. 2021). Biochar may release toxic substances (such as polycyclic aromatic hydrocarbons or persistent free radicals) and thus inhibit nematode activities, for example (Lieke et al. 2018); however, other studies support that biochar can increase soil organic matter and temperature to enhance ant species abundance and activity (e.g., *Tetramorium caespitum* Linnaeus, 1758) (Castracani et al. 2015). Thus, the biological effect always appears to be a trade-off between adverse and beneficial effects. Therefore, it is necessary to evaluate biochar safety from ecological functions instead of individual toxicity endpoints.

Ants (Hymenoptera, Formicidae) are specialized soil social insects with more than 12,500 species (Bolton 1995). They live in colonies organized according to complex age- and caste-specific relationships. Ants

execute complex social behaviors, with colony-level activities creating cascading impacts on ecosystem processes. These social behaviors are manifested through sophisticated behavioral networks. For example, their foraging patterns regulate nutrient redistribution and seed dispersal, significantly influencing soil nutrient heterogeneity. Their defensive behaviors influence community structure through trophic regulation, playing a key role in mediating local invertebrate dynamics. Furthermore, collective nest construction modifies soil physical properties, substantially enhancing soil porosity and aeration in various ecosystems. Their predation and competition control the pest and the structuring of invertebrate communities (Carvalho Campos 2012; Perfecto and Philpott 2023). The highly complex social nature of ant colonies results in social behavior that is critical to colony health and survival (Bernadou et al. 2015). Crucially, these social interactions rely on precise chemical communication, particularly cuticular hydrocarbons (CHCs), which serve as recognition cues. Since CHC profiles are modulated by environmental factors such as nest soil properties and the microbiome (Bos et al. 2011), biochar-induced changes in the soil physicochemical matrix could disrupt these vital signaling pathways. However, little attention has been paid to the effect of biochar application on the social behavior of ants.

We hypothesize that the effects of biochar application on the ecological functions of ant colonies are biochar-dose-dependent. Excessive doses of biochar have negative effects, while low-dose application increases social activities and subsequently increases overall soil functions. Therefore, a regulatory strategy is needed and essential for massive biochar applications. We designed a comprehensive experimental framework using *Formica japonica* Motschoulsky, 1866 across four biochar concentration gradients (0%, 2.5%, 5%, 10%), which were selected to represent typical agricultural application rates. The applied biochar was thoroughly mixed with native soil to create specific exposure environments. We compared behavioral responses of ants in these treatments, integrating multiple behavioral dimensions: habitat selection preferences, nest architecture efficiency, foraging performance, and social recognition. Additionally, physicochemical properties of the treated soils were analyzed to elucidate the underlying environmental drivers. This multifaceted approach systematically describes ant behavioral plasticity and establishes links between soil amendments and ant ecological functions. The results of this work will provide critical insights for the application of biochar in soil restoration while maintaining soil fauna function.

2 Materials and methods

2.1 Biochar and soil preparation

Rice straw is frequently recommended for biochar production due to its ubiquity and abundance. In our experiment, biochar was produced from rice straw obtained from Wujiaying County, Kunming, China (24.8°N; 102.8°E), as presented in our previous study (Liu et al. 2024). Briefly, the collected rice straw was washed, dried, chopped, milled, and then passed through a 100-mesh sieve. This processed material was then placed into a muffle furnace (SX-4-10, Beijing Ever Bright Medical Treatment Instrument, China) for pyrolysis. The furnace was purged with N_2 at 1.5 L min^{-1} and 99.99% purity for 30 min. The temperature was then increased to 500 °C at a rate of 15 °C min g^{-1} and held for 2 h. Biochar characterization revealed a pH of 9.1 ± 0.01 (mean ± SD, n = 6) and an ash content of 38.7 ± 0.42%. Elemental analysis revealed contents of 50.7 ± 0.57% C, 2.79 ± 0.04% H, 1.16 ± 0.07% N, and 16.9 ± 0.17% O. The specific surface area of the biochar was 38.3 ± 0.66 m^2 g^{-1}, with a total pore volume of 0.079 ± 0.01 m^3 g^{-1}.

The soil was collected from Wujiaying County, Kunming, which is classified as plateau red soil (typically acidic and barren) with a pH of 5.7 ± 0.76 and a TOC content of 0.11 ± 0.01 mg g^{-1} (dry weight basis, mean ± SD, n = 6). The soil was dried and passed through a 100-mesh sieve. It was then mixed with 100-mesh quartz sand at a ratio of 1:2. Biochar was incorporated into the experimental boxes at 0% (control, CO), 2.5%, 5%, and 10%, which was a critical component of the ant nest environment. To maintain consistent nest volumes (approximately 170 cm^3) across all treatments, we adjusted the amount of the base materials (biochar and soil) accordingly (Sappington 2018). Each treatment was repeated three times in a randomized block design, and environmental conditions (temperature and humidity) were maintained consistently across all experimental units (Figure S1).

2.2 Physicochemical analysis of biochar-amended soils

To characterize the chemical environment exposed to ants, soil samples were collected from each treatment group (CO, BC 2.5%, BC 5%, and BC 10%) at the beginning of the experiment. All measurements were performed in triplicate (n = 3) for each treatment. EPFRs were detected using an electron paramagnetic resonance (EPR) spectrometer (A300-6-1, Bruker, USA) at room temperature. Samples (0.2 mg) were loaded into quartz capillaries (I.D. 1 mm, O.D. 1.5 mm, length 125 mm; 707-SQ-250M, Wilmad, USA), sealed with vacuum grease, and placed in the resonance cavity. The measurement parameters were: microwave frequency 9.86 GHz,

microwave power 10 mW, center field 3518 G, sweep width 100 G, modulation frequency 100 kHz, modulation amplitude 2.00 G, resolution 1024 points, time constant 40.96 ms, and sweep time 83.97 ms. The signal intensity was normalized by sample mass (mg) and converted to spins g^{-1} using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a standard. Data were processed using Bruker WinEPR Acquisition software. Total carbon (TC) and total nitrogen (TN) contents were determined using an elemental analyzer (Vario Micro Cube, Elementar, Germany). Samples (2 ± 0.2 mg) were wrapped in tin boats, compacted to remove air, and analyzed via high-temperature combustion (combustion furnace at 1150 °C, and reduction furnace at 850 °C). Soil pH was measured in a 1:10 (w v^{-1}) soil-water suspension using a pH meter (Leici Instruments, Shanghai, China). Samples were mixed with ultrapure water, shaken at room temperature for 30 min, and measured after calibration with standard buffer solutions (pH 4, 7, and 10).

2.3 Ant collection and rearing

Formica japonica, a common ant species widely distributed across Asia, was used for all behavioral tests. Adult worker ants (5–7 mm) originating from a single large population stock were purchased from Spark Pet Ant Inc. (Shanxi, China) to minimize genetic variation. All workers were tested regardless of their specific task specialization (e.g., nurses or foragers), and individuals were randomly allocated to treatment groups. Ants were acclimated for 7 d in polyethylene containers (20 × 30 × 12 cm) lined with 2 cm of clean sand. Apple chunks and water-soaked cotton balls served as food and water sources, respectively. Temperature was maintained at 20 ± 1 ℃ and humidity at 60–70%. After acclimation, ants were randomly transferred to new experimental containers separated into the four distinct soil treatments (0%, 2.5%, 5%, and 10% biochar). A total of 120 workers were used (30 per treatment). To ensure statistical independence, each treatment consisted of three replicate containers (10 ants per container). The ants were housed in clean plastic containers filled with the prepared biochar-soil substrate mixtures and fed with 10% honey water. Survival was monitored daily for 10 d in an incubator maintained at 20 °C and 65% humidity. This duration was selected to assess acute to subacute toxicity following our preliminary experiments and the established protocols for soil invertebrates (Hassan et al. 2022; Fu et al. 2024).

2.4 Preference experiments

A preference device was constructed using two pots (6 × 6 × 7 cm) connected by a rod (10 cm in length and 1.5 cm in diameter) 1 cm from the bottom (Figure S2). One pot contained 10 g of biochar-amended soil, while the other contained 10 g of control soil. Apple pieces were provided in both pots. Three biochar concentration gradients (2.5%, 5%, 10%) were tested in triplicate. Ten *F. japonica* workers were initially placed in the biochar-amended pot. The pots were kept in the dark at 20 ± 1 ℃ and 60–70% relative humidity. Ants were counted daily in each pot for 10 d (Chen et al. 2022).

2.5 Social behavior experiments

2.5.1 Nesting experiment

After acclimation, 30 ants per treatment were transferred to formicaria (20 × 1.5 × 15 cm) with three replicates per treatment (BC 2.5%, BC 5%, BC 10%, and CO). The formicaria contained holes covered with screen mesh to allow for airflow. The mixture was initially sprayed with 20 mL of water, which was found to be necessary to initiate nest building. In addition, the formicaria contained two attached test tube chambers, one with a cotton ball saturated with approximately 5 mL of spiked honey solution and the other with spiked water (Figure S1) (Sappington 2018). Food and water were replenished three times a week with fresh stock solutions prepared weekly and stored in the dark.

Ants were observed for 7 d during nest construction. Temperature (19–22° C) and ant behavior were recorded daily under a 14L:10D light cycle (7 d in formicaria + 7 d pre-exposure in containers). On day 14, digital images were taken of both sides of the formicaria to assess the amount of tunneled area (Figure S1). Images were calibrated to a known scale and analyzed using the ImageJ™ software. Subsequently, a 2 d starvation period was initiated, during which ants had access only to treated water, in order to promote ant foraging in subsequent trials.

2.5.2 Foraging experiment

On day 16, artificial mazes were used to assess ant foraging success. The T-shaped mazes were constructed using clear, plastic tubes with an inner diameter of 13 mm and T-connectors with a total length of 20 cm (Figure S3A) (Poissonnier et al. 2023). Food was placed at the inner end of the maze (left side), which required the ants to navigate between 2 paths (passing the maze without eating/eating food and staying in the maze). Prior to the foraging trial, ants were individually placed in 1 mL EP tubes connected to the maze. During the trial, foraging success was described by the latency to reach the food source and number of ants feeding at the food station in the maze within 10 min (a total of 120 ants were tested). The number of ants that never left the maze was recorded.

We also assessed the minimum time for ants to discover food (Figure S3B) (Glaser and Grüter 2023). Following the same pretreatment and starvation protocols, five worker ants of similar size from each experimental

group (60 ants in total) were carefully transferred to a clean, smooth Petri dish (10 cm in diameter). Food was placed at the maximum linear distance from the ants. The time of first food detection and whether the ants consumed the food within the allotted time (180 s) were recorded (Figure S3C).

2.5.3 Worker encounter and aggression

Workers of the invasive red fire ant (*Solenopsis invicta* Buren, 1972) were chosen as the interspecific contact/attack object. They were acclimated under standardized conditions for one week to ensure experimental consistency. To assess the effect of biochar application on social behavior, one-on-one worker–ant encounters were used for both intraspecific (within *F. japonica* treatment groups) and interspecific (*F. japonica* vs. *S. invicta*) interactions (peaceful: Figure S3D; aggressive: Figure S3E) (Krapf et al. 2023). Social behavior experiments were conducted between *F. japonica* in different treatments and between *F. japonica* and *S. invicta*. Each encounter combination had 3 replicates.

One-on-one worker encounters were performed in vials (3 cm inner diameter) coated with ant escape liquid (70% ethanol and 10,000 mesh talcum powder). Each glass vial was used once after washing to avoid interference from pheromone residues that could alter worker behavior. All encounters were filmed for 180 s at 4K resolution (3840 × 2160 pixels) using a HUAWEI Honor smartphone (HUAWEI, Beijing, China). For each encounter, the analysis began when the second worker was introduced to the glass vial.

To exclude observer bias, encounters were analyzed by a blinded observer. Behaviors were scored on a scale of −4 to 5, with −1 to −4 considered peaceful, 0 neutral, and 1–5 aggressive. This scoring system, detailed in Table S1, allowed for the calculation of a Mean Behavior Index (MBI) for aggressive (MBI_{agg}) and non-aggressive (MBI_{pcf}) interactions, providing a quantitative summary of the predominant behaviors during an encounter (Table S1) (Krapf et al. 2019). Non-aggressive interactions included behaviors such as antennation and allogrooming, while aggressive behaviors ranged from mandibular threats to biting and gaster flexion. All behaviors without a nearby counterpart were scored as "ignoring". When multiple behaviors occurred simultaneously, the highest score (i.e., the more aggressive grade) was recorded. The first 10 s of each encounter were discarded to eliminate handling-induced aggression (Krapf et al. 2019, 2023).

Following Krapf et al. (2019), the MBI was calculated based on a specific time threshold (99 s) to determine the predominant behavior. Specifically, the MBI was assigned the observed maximum score when the total duration of aggressive behaviors exceeded the threshold while peaceful behaviors did not; conversely, the minimum score was assigned when only the peaceful duration exceeded the threshold. In cases where both or neither duration met the threshold, the MBI was calculated as the average of the maximum and minimum scores or set to zero, respectively. The subscripts "agg" and "pcf" denote the specific indices derived for aggressive interactions (towards *S. invicta*) and peaceful interactions (towards conspecifics).

2.6 Statistical analysis

Data normality and homogeneity of variance were assessed using the Shapiro–Wilk and Levene's tests, respectively. The impacts of soil physicochemical properties (including pH, C, N, and EPFRs) were analyzed using one-way Analysis of Variance (ANOVA). Survival analysis was performed using the Kaplan–Meier estimator, and differences between survival curves were determined using the Log-rank (Mantel-Cox) test. For nest building, foraging, and social behavior metrics, data were analyzed using two-way ANOVA with biochar dose and experimental block as factors. Tukey's HSD post-hoc test was used for multiple comparisons when significant effects were detected. Detailed statistical results, including the analysis of block effects, are provided in Table S2. All statistical analyses were performed using Python (packages lifelines and statsmodels), and figures were generated using Python (matplotlib). Statistical significance was set at $p < 0.05$. All data analyses were performed with R 4.0.2 statistical software (Team 2010), and figures were generated with the R package ggplot2 (Wickham and Wickham 2016).

3 Result

3.1 Physicochemical property change after biochar application

The addition of biochar to the soil resulted in significant changes in key soil properties. Soil pH, N content, C content, and EPR signals altered significantly with biochar application (Fig. 1A). A positive correlation between biochar concentration and soil pH was observed. The CO showed pH around 6.5, while the pH values increased to 7.5, 8.2, and 9.3 for BC 2.5%, BC 5%, and BC 10% systems, respectively (Fig. 1B). Physiologically, the mild alkalinization (pH 7.5–8.2) observed at intermediate doses may improve water balance and chemosensory reliability at the cuticular interface, whereas the very high pH (9.3) at 10% biochar poses a risk of disrupting ion homeostasis and neuromuscular performance. This indicates that biochar application significantly alkalized the soil. Both soil N and C contents increased with biochar concentrations (Fig. 1C and D). This enrichment in organic matter

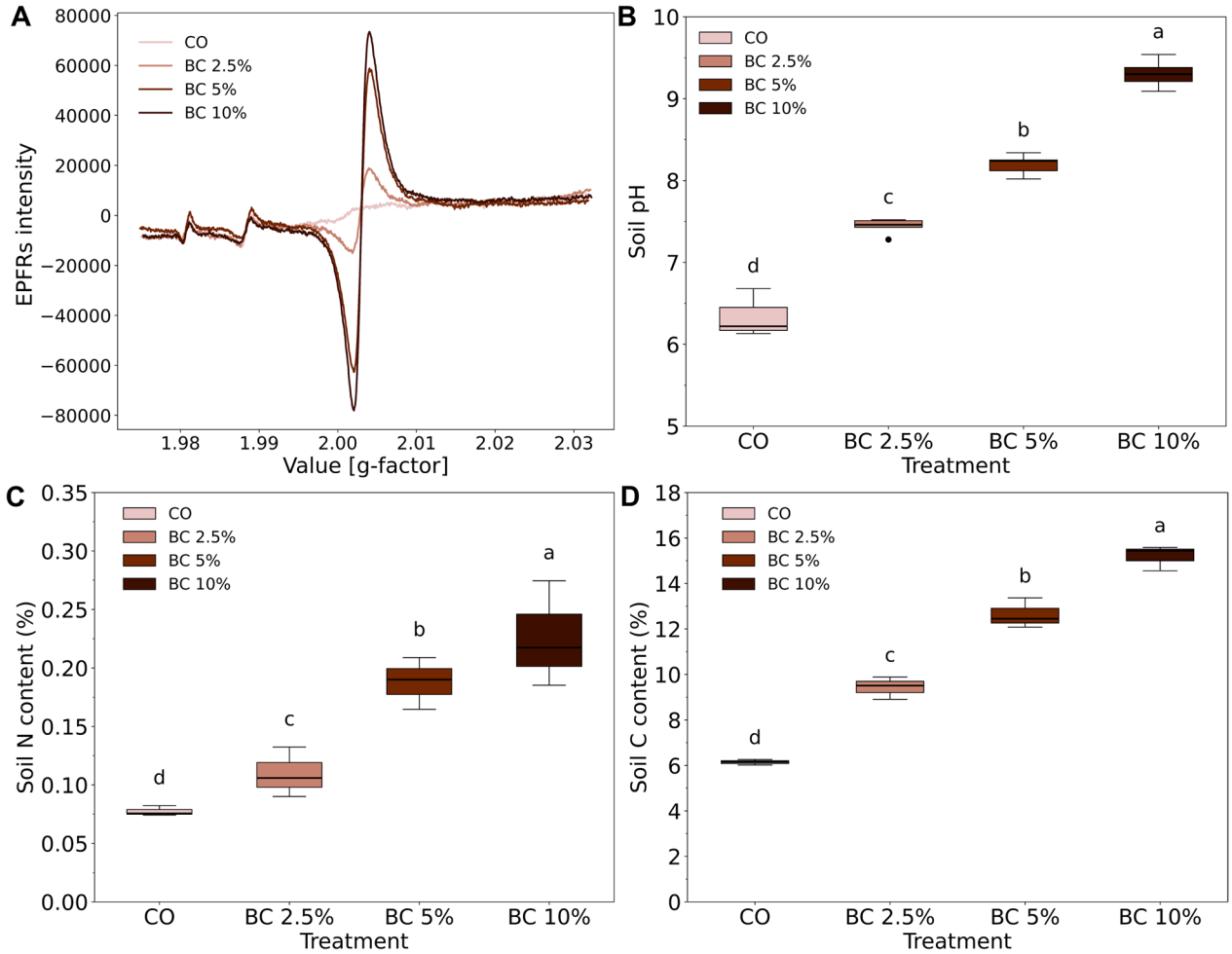


Fig. 1 EPR signals (**A**), pH values (**B**), C (**C**) and N (**D**) contents of soils amended with biochar. CO: control group (0% biochar); BC 2.5%, BC 5%, and BC 10%: soil amended with 2.5%, 5%, and 10% biochar, respectively; EPFRs, environmentally persistent free radicals. Data are presented as mean ± SD (n = 9). Different letters indicate significant differences based on one-way ANOVA ($p < 0.05$)

likely increases water-holding capacity and substrate friability, thereby reducing the mechanical cost of digging, which helps explain the optimized nest-building behavior observed at intermediate doses.

It has been reported that biochar contains significant EPR signals, which is associated with the environmentally persistent free radicals (EPFRs) generated during biochar production (Ruan et al. 2019). Similar EPR signals were observed in the investigated biochar in this study, and the application of this biochar in soil resulted in the significant increase of soil EPR signals. The gradually increasing sharp and symmetrical peaks around g-factor 2.00–2.01—most likely a mixture of carbon- and oxygen-centered radicals—with biochar application were similar to the signal observed in biochar (Fig. 1A). Previous studies have reported that EPFRs in biochar are reactive and may pose adverse effects on soil organisms. Specifically, the dose-dependent increase in EPFRs is consistent with induced oxidative stress and neurotoxicity (Lieke et al. 2018). Therefore, the elevated pH and high EPFR concentrations, particularly in the 10% treatment, represent significant changes in the chemical environment of the ants and are plausible contributing factors to the negative biological responses observed in this study. This observation aligns with our findings that the optimal habitat for the selected ant species requires a pH of 6.5–8.0, along with humid conditions (Liu et al. 2024).

3.2 Habitat selection and survival

Results showed that biochar application significantly altered *F. japonica* habitat selection patterns. Figure S4 shows the dose-dependent effect of biochar on ant survival. Figure 2A shows the survival dynamics of *F. japonica* over the 10-d exposure period. Kaplan–Meier survival analysis revealed significant differences among treatments (Log-rank test, $p < 0.001$). Ants in the control

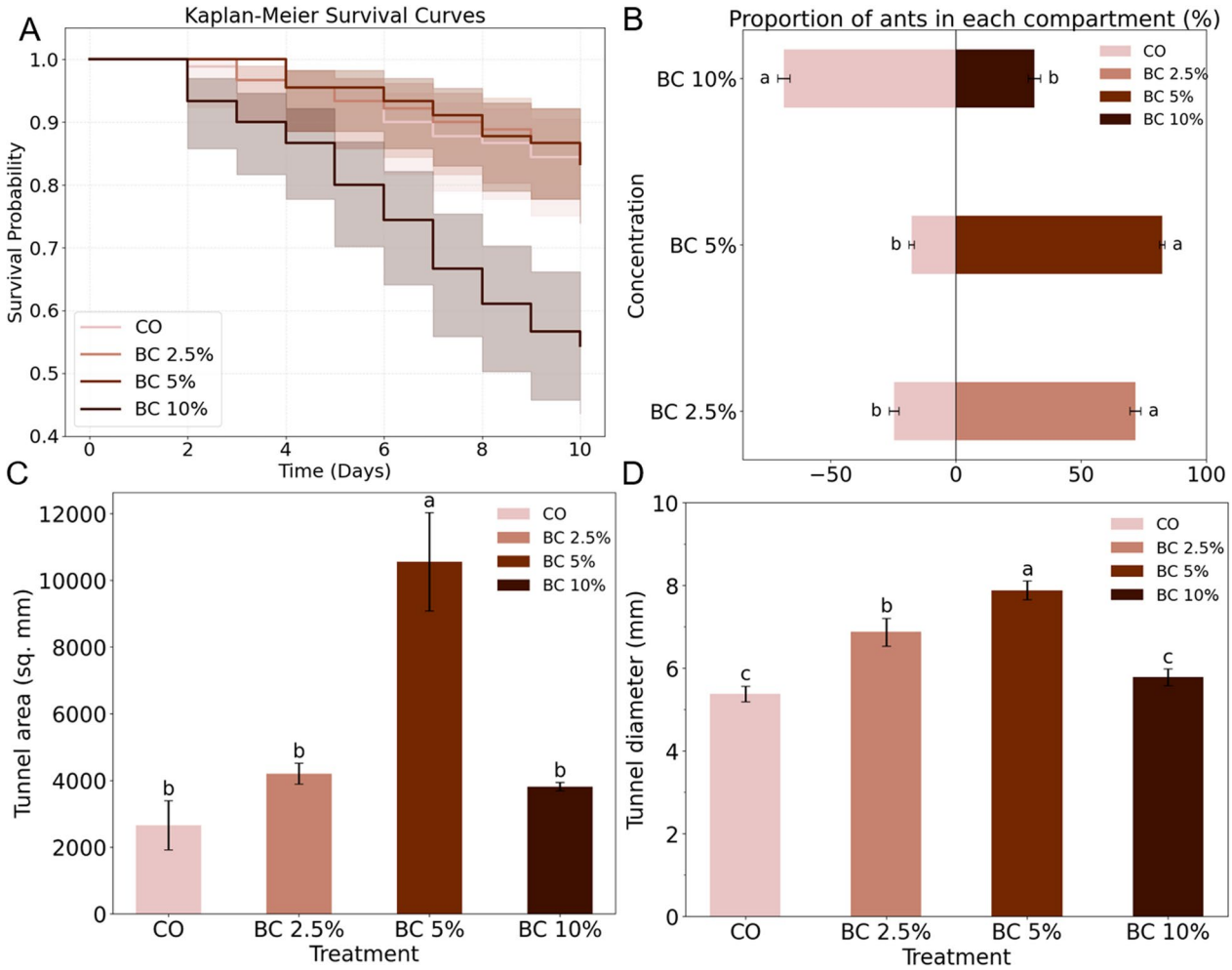


Fig. 2 Ant (*F. japonica*) survival (**A**) and preference analysis (**B**) in biochar-amended habitats, and the impact of biochar concentrations on tunnel area (**C**) and diameter (**D**) of ant nests. Kaplan–Meier survival curves were plotted in Panel **A**, and the shaded areas represent 95% confidence intervals. Significance was determined using the Log-rank (Mantel-Cox) test ($p < 0.001$). CO: control group (0% biochar); BC 2.5%, BC 5%, and BC 10%: soil amended with 2.5%, 5%, and 10% biochar, respectively. Different letters indicate significant differences based on Two-way ANOVA followed by Tukey's HSD test ($p < 0.05$). Error bars represent standard deviation

(CO), BC 2.5%, and BC 5% groups exhibited high survival rates (>83%) with no statistically significant differences among them ($p > 0.05$). In contrast, the BC 10% treatment caused a sharp decline in survival, dropping to approximately 55% by day 10, which was significantly lower than the control group ($p < 0.001$, Log-rank test). Treatment with BC ≤ 5% revealed over 80% survival, while BC 10% resulted in a decrease in survival to 55% in 10 d. The sharp decline in survival at the 10% concentration corresponds to the highest EPFR intensity (Fig. 1A) and extreme alkalinity (pH 9.3, Fig. 1B). Mechanistically, the dose-dependent increase in EPFRs is consistent with induced oxidative stress and neurotoxicity (Lieke et al. 2018), while very high pH levels can disrupt ion homeostasis. These combined physiological stressors likely overwhelmed the ants' tolerance, leading to the observed mortality.

Regarding habitat selection, ants exhibited a statistically significant preference for biochar at dosages ≤ 5% (Fig. 2B), with preference rates of 72.0 ± 11.5% (BC 2.5%) and 82.3 ± 6.40% (BC 5%) over the CO ($p < 0.01$). This attraction to low-dose biochar soils may be driven by mild alkalinization (pH 7.5–8.2, Fig. 1B), which can optimize the microhabitat's moisture retention and potentially improve chemosensory reliability at the cuticular and sensillar interfaces. However, this preference disappeared at the 10% concentration, where ants were distributed almost equally, consistent with the observed toxicity.

The observed ant habitat preference for optimal biochar concentrations may enhance ant-mediated soil processes, including improved soil aggregation, organic matter decomposition, and nutrient distribution—potentially key processes for accelerating ecosystem restoration (Auclerc et al. 2022). Notably, the decreased ant survival in BC 10% system suggests a potential disruption of natural soil processes. Consistent with previous findings, soil faunal responses to soil amendments are concentration-dependent, with 2–7% (often below 5%) biochar being the most beneficial for selected soil invertebrates (da Costa et al. 2024; Ma et al. 2024). Overall, soil faunal responses to biochar vary by animal type, with herbivores and macrofauna being more sensitive (Ma et al. 2024).

3.3 Nesting behavior

At biochar dosages ≤ 5%, both tunnel area and diameter increased compared to the CO group, providing strong evidence that biochar enhanced ant nesting efficiency. For example, the tunnel area was approximately 10,500 mm^2 at BC 5%, which is more than twice the area observed under other conditions (Fig. 2C). This substantial increase in tunnel area ($p < 0.05$) supports the hypothesis that ants can construct more extensive and complex nests under these conditions. The enhanced nesting behavior of the ants may be due in part to the improved soil structure or easier excavation (e. g., tunnel area and diameter, and the soil area of ant nest ground, Figure S1) (Leite et al. 2018). The decreased tunnel area for BC 10% was consistent with the decreased survival (Fig. 2), likely reflecting the physiological stress and neurotoxicity induced by high EPFR concentrations (see Sect. 3.2).

The largest tunnel diameter [mean tunnel diameter: 7.95 ± 0.6 mm (n = 3; $p < 0.05$)] was also observed for BC at 5% (Fig. 2D). This increase in tunnel diameter may indicate that ants are able to construct more robust and potentially more functional tunnels. One potential explanation for this observation is that biochar altered the physical properties of the substrate, such as friability or particle aggregation, which may have facilitated excavation (Lee et al. 2022). This hypothesis is supported by the significant increase in soil C and N concents observed in the biochar-amended soils (Fig. 1C and D). Higher organic matter content is typically associated with reduced bulk density and improved soil structure, which likely lowered the mechanical resistance for digging (Rasa et al. 2024; Zhao et al. 2025). Larger tunnels can facilitate ant movement, resource transport, and nest ventilation of the nest (Tschinkel 2021). Larger tunnel diameters and networks likely increase soil macroporosity and connectivity, improving water infiltration and gas exchange, and enhancing ant-mediated ecosystem services such as organic matter incorporation, nutrient cycling, and microbial microhabitat creation (Lu et al. 2019; Zhang et al. 2024).

3.4 Foraging behavior

Our data indicated that biochar application was associated with significant changes in the foraging performance of *F. japonica*. In T-maze navigation (Fig. 3A and B), ant from the BC 5% treatment had the highest success rate and the shortest mean transit time, with 87.5% of ants successfully traversing the maze compared to 57.5% in the CO group ($p < 0.001$). Mean transit time was also significantly shorter for BC 5% (47.50 ± 4.93 s vs. 107.50 ± 4.51 s for CO; $p < 0.001$). Similarly, this group showed the highest success rate and lowest latency in food-finding trials (Fig. 3C and D). BC 5% demonstrated superior performance, with 72.5% of ants reaching food (vs. 33.3% in control; $p < 0.001$) and a shorter mean foraging time (45 ± 4.43 s vs. 105 ± 4.36 s for CO; $p < 0.001$), while BC 2.5% showed modest improvements over the CO (e.g., 67.50% maze success; $p < 0.05$). The improved foraging efficiency at moderate doses (2.5–5%) may be attributed to hormetic activation, where mild environmental stress enhances physiological alertness and locomotor activity. However, BC 10% significantly impaired the performance across all metrics (e.g., 40% maze success; $p < 0.01$ vs. CO). Notably, BC 10% increased mean transit and foraging times to 215.0 ± 9.51 s and 217.5 ± 10.2 s, respectively (both $p < 0.001$ vs. all treatments). These findings indicate that moderate biochar applications (up to 5%) enhance ant navigation and foraging efficiency, whereas BC 10% significantly impede these behaviors.

We also recorded the time it took the fastest ant to find the food after passing through the maze (Figure S4). Ants in the BC 5% group found food the fastest (7.62 ± 2.78 s, $p < 0.01$), demonstrating the most efficient foraging. The BC 2.5% group (36.4 ± 11.9 s) also showed improved efficiency compared to the CO (62.5 ± 24.4 s). Our previous observations indicated that 10% BC treatment decreased ant survival, and thus altered the community-based parameters. For this parameter, shown in Fig. 4, the longest food-finding time (153.5 ± 35.45 s) suggested that the surviving ants at high biochar concentrations (10%) may have suffered from EPFRs-induced nerve injury or behavioral damage with subsequent death. This decline aligns with the neurotoxic potential of high EPFR concentrations (Fig. 1A). Oxidative stress from EPFRs can impact key neuromodulators like octopamine and serotonin, which regulate foraging and locomotion (Barbero et al. 2023), leading to the observed sluggishness and inefficiency. Indeed, this treatment had the lowest ant survival

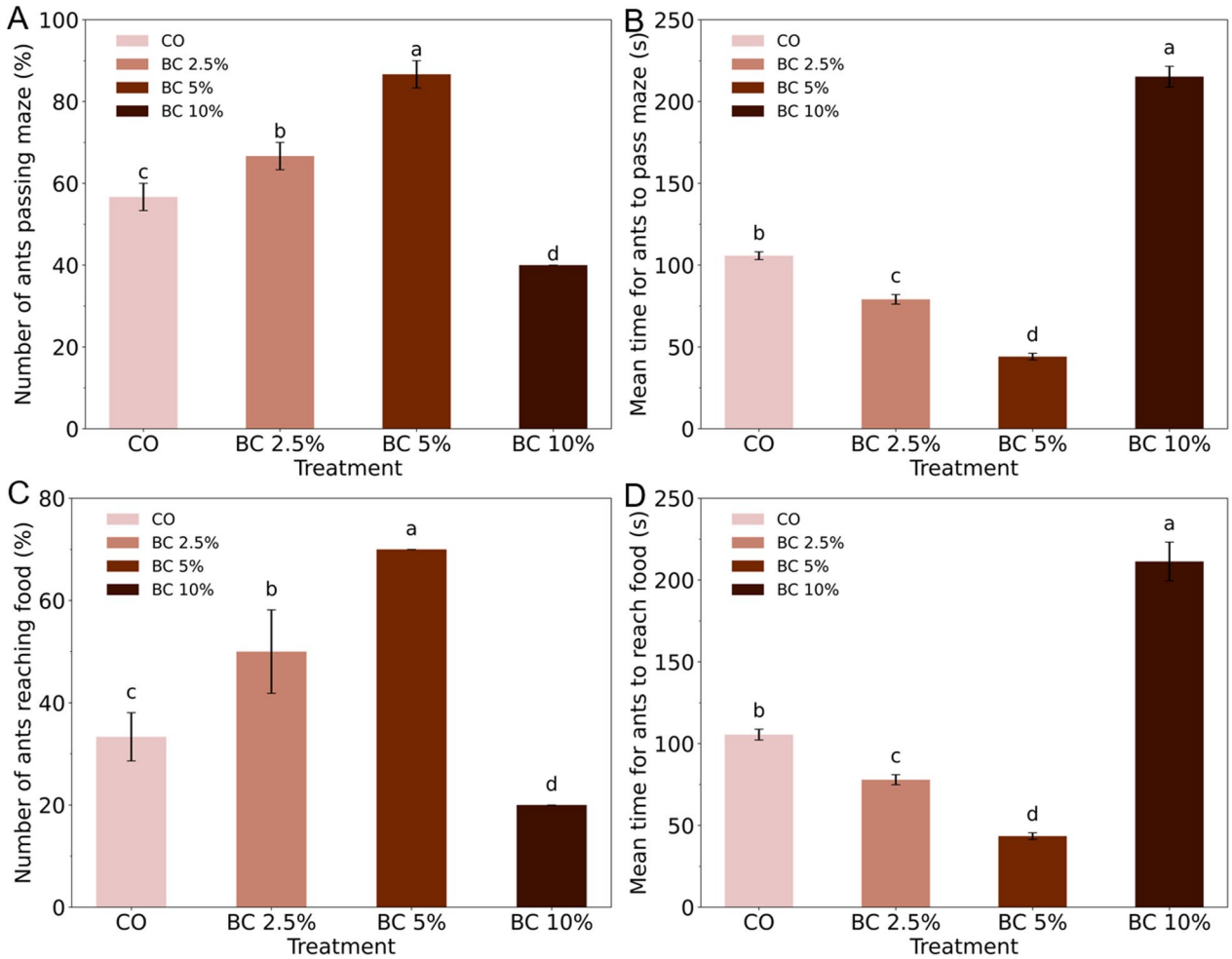


Fig. 3 Ant foraging efficiency in response to various biochar concentrations. Percentage of ants successfully navigating T-maze (**A**), mean navigation time (**B**), percentage of ants reaching food (**C**), and mean time to reach food of the foragers (**D**) were recorded in every 10 min. CO: control (0% biochar); BC: biochar treatments at indicated concentrations (2.5%, 5%, and 10%). Data are presented as mean ± SD. Different letters indicate significant differences based on two-way ANOVA followed by Tukey's HSD test ($p < 0.05$)

(See figure on next page.)

Fig. 4 Duration (**A**) and frequency (**B**) of aggressive behaviors towards an invasive ant species *S. invicta* (labeled as HH), and the duration (**C**) and frequency (**D**) of peaceful behavior to conspecifics of ants exposed in biochar-amended soil (*F. japonica*, labeled as CO). Panels E and F display the mean behavior index calculated for aggression (E, MBI_{agg}) and peaceful interactions (F, MBI_{pcf}). The Y-axis labels in E and F indicate "Treatment-Replicate-Opponent" (e.g., BC 5%-1-HH). CO (treatment): control group (0% biochar); BC 2.5%, BC 5%, and BC 10%: soil amended with 2.5%, 5%, and 10% biochar, respectively; HH refers to the invasive opponent (*S. invicta*), and CO (as suffix) refers to the conspecific opponent (*F. japonica*). Different letters indicate significant differences based on two-way ANOVA followed by Tukey's HSD test ($p < 0.05$)

(Fig. 2). Thus, it should also alter community-based parameters (Li et al. 2024).

3.5 Social behaviors of ants

Both aggressive behavior towards invasive species and peaceful behavior towards conspecifics were examined for the ants exposed to biochar-amended soil for 14 d. In general, biochar treatment increased both aggression duration and frequency of the investigated ants studied. For example, BC 5% treatment showed the highest duration (73.0 ± 12.6 s) and frequency (13.3 ± 4.0 times) of aggressive behaviors among all the treatments (Fig. 4A and B).

The duration and frequency of peaceful behaviors of the biochar-exposed ants compared to unexposed

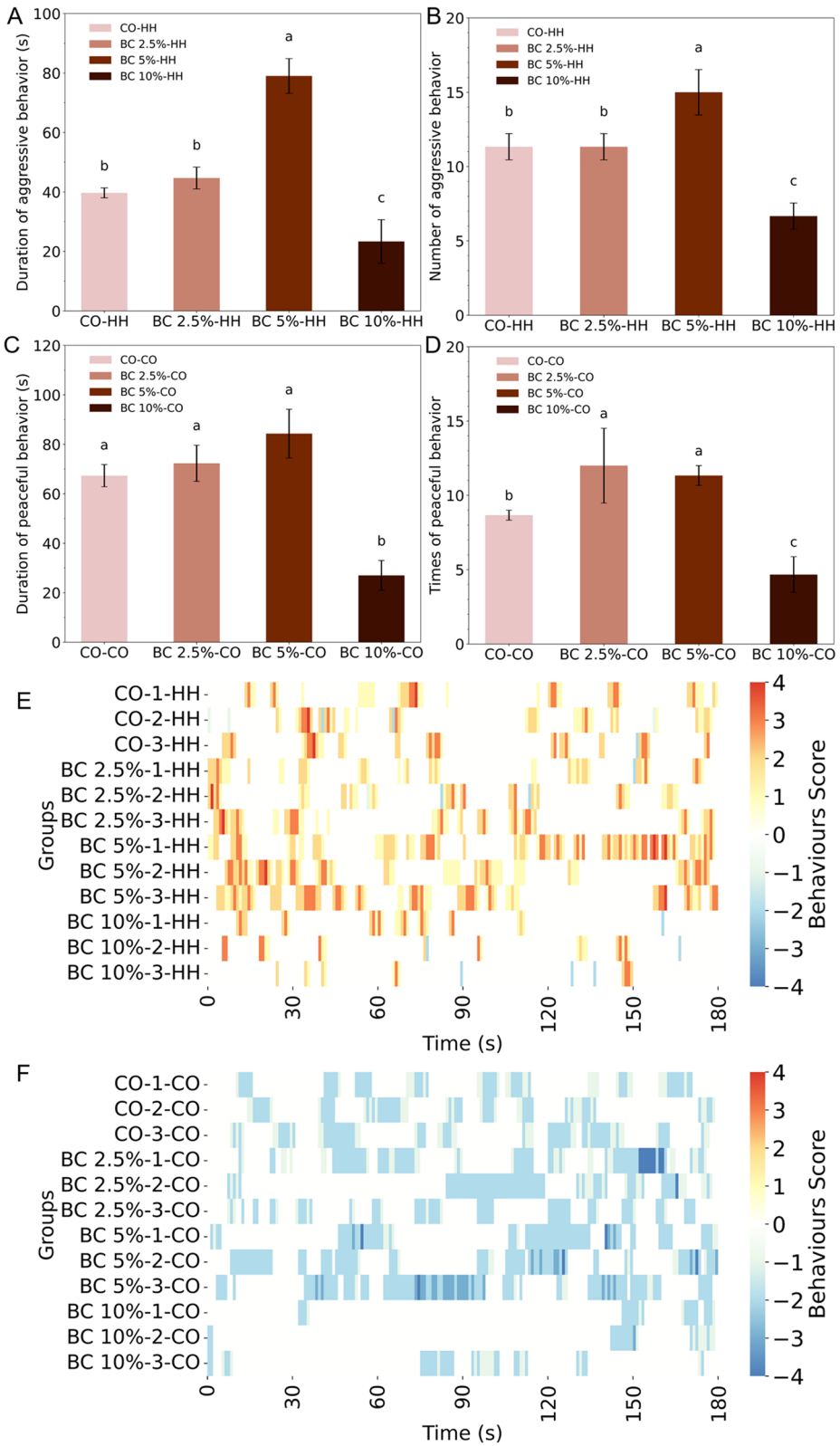


Fig. 4 (See legend on previous page.)

ants (CO group) followed a similar pattern. For example, BC 5% treatment exhibited the longest duration of peaceful behaviors (83.3 ± 13.9 s), followed by BC 2.5% (72.3 ± 10.3 s) (Fig. 4C). The frequency of these peaceful behaviors in the BC 5% (11.3 ± 0.9) and BC 2.5% (12.0 ± 3.60) groups was significantly higher than that in the BC 10% group ($p < 0.05$) (Fig. 4C).

Based on the scoring system of aggression grades, a mean behavior index (MBI) was calculated and compared. The studied ants generally showed aggressive behaviors towards the invasive species after exposure to biochar systems. The MBI_{agg} values were generally 4 (Fig. 4E), suggesting a common aggressive behavior of biting with gaster flexion (Table S2). The MBI_{agg} values for BC 10% decreased to 3, corresponding to biting without gaster flexion, which was also consistent with the decreased activity at high biochar application dosage. This reduction in aggressive intensity at the highest dose is consistent with the physiological stress and decreased locomotor activity caused by neurotoxicity (see Sect. 3.4).

Peaceful behaviors were well maintained in ants exposed in biochar systems. The observed MBI_{pcf} values were in the range of −4 to −2 (Fig. 4F), characterized as peaceful behaviors of trophallaxis (transfer of food/fluids among members of a community), allogrooming (social grooming), and antennation (ants drum each other's antennae in an apparently complex manner).

These alterations in social interactions suggest that biochar modifies the chemical communication landscape. The soil substrate acts as a reservoir and transfer medium for recognition cues (Bos et al. 2011). Recent studies indicate that soil characteristics (e.g., pH and microbial activity) can modulate the profile of ant cuticular hydrocarbons (CHCs), which are essential for distinguishing nestmates from non-nestmates (Hais et al. 2024). The significant shifts in soil pH and chemical composition observed in our study (Fig. 1) likely altered the adsorption of CHCs or shifted the soil microbiome, thereby modulating the chemical signals required for stable social interactions.

Despite these physicochemical shifts, the maintenance of peaceful behaviors suggests a resilience in intraspecific recognition. These behaviors are essential for colony stability (e.g., *via* pathogen removal through allogrooming) and community function (e.g., resource distribution via trophallaxis) (Casillas-Pérez et al. 2023; Meurville et al. 2025). The maintenance of these social bonds, despite the significant physicochemical shifts (e.g., elevated pH and EPFRs, see Sect. 3.4) in biochar-amended soils, suggests a resilience in intraspecific recognition that is vital for sustaining ant-mediated ecosystem services. However, the suppression of these behaviors at 10% biochar highlights the potential ecological risks of excessive soil amendments.

4 Discussion

Our introductory hypothesis that high doses of biochar have negative effects, while low doses increase social activities was supported. The ants' response to increasing doses of biochar in the soil is not linear; rather, it is a hormetic response, as Calabrese and Baldwin (2003a, b) reintroduced more than two decades ago. Hormesis, a dose–response phenomenon characterized by low-dose stimulation and high-dose inhibition, has been frequently observed in properly designed studies and is broadly generalizable because it is independent of the chemical/physical agent, the biological model, and the endpoint measured.

Persistent free radicals in biochar have been shown to develop harmful activity: They can cause reduced germination in corn, wheat, and rice seedlings (Liao et al. 2014), reduced alpha diversity in the soil microbiota (Yang et al. 2023), or lead to neurotoxic symptoms in the nematode *Caenorhabditis elegans* (Lieke et al. 2018). In particular, *C. elegans* showed a hormetic response to exposure to biochar in terms of body bends, relative movement length and, what was only hinted at, defecation. In addition, as described above, the soil invertebrates we studied and their behavior are no exception to the hormesis rule: All behavioral parameters tested followed hormetic curves (Figs. 1, 2, 3, 4). In general, hormesis refers to adaptive responses of biological systems to moderate environmental or self-imposed challenges through which the system improves its functionality and/or tolerance to more severe challenges (Calabrese and Mattson 2017). This implies that the hormetic and toxic exposure range lead to contrasting responses, as shown at different "omics" levels: transcriptomics in larvae of the cotton bollworm (*Helicoverpa armigera*) exposed to the plant secondary metabolite gossypol (Celorio-Mancera et al. 2011), in *C. elegans* exposed to humic substances (Steinberg et al. 2013), in cotton leafworm (*Spodoptera litura*) larvae exposed to a natural insecticide (Yi et al. 2018), zebrafish (*Danio rerio*) exposed the natural polyphenol quercetin (Zhang et al. 2020), or metabolomics in ryegrass (*Lolium perenne*) exposed to sewage sludge biochars (Zhang et al. 2025).

The significant behavioral inhibition observed at the 10% biochar concentration prompts an inquiry into potential underlying toxicological mechanisms. It is well-established that the behavior of social insects (such as ants, bees) is highly sensitive to neurotoxicants. Sublethal exposure to various pesticides, for example, is

known to disrupt neurotransmitter systems in ants, leading to impaired foraging, navigation, and social coordination (Skaldina and Sorvari 2017; Xiao et al. 2024). In this context, the high concentration of persistent free radicals (PFRs) in our 10% treatment represents a potential, yet uninvestigated, source of neurotoxicity. Indeed, studies on other soil invertebrates, such as the nematode *C. elegans*, have demonstrated that PFRs from biochar can induce neurotoxic effects (Lieke et al. 2018). We hypothesize that the behavioral deficits observed in *F. japonica* at high biochar doses are attributable to a dual physiological stressor: (i) the dose-dependent increase in EPFRs likely induces oxidative stress and neurotoxicity, affecting key neuromodulators; and (ii) the extreme alkalinity (pH 9.3) may further disrupt ion homeostasis and neuromuscular performance. These combined factors provide a plausible physiological route explaining the lower survival and slower foraging observed at the highest dose.

While our study demonstrates a clear dose-dependent behavioral response in *F. japonica*, the precise underlying mechanisms and the broader applicability of these findings require further investigation. It should be noted that the test population consisted of a mixed composition of workers (nurses and foragers). While randomization was used to minimize bias, this mixed composition may have introduced additional variance to the behavioral results compared to testing a specific task sub-group. The observed toxicity at high doses, coinciding with elevated pH and PFRs, points towards physiological stress. Future research should explore specific molecular pathways in ants that might be affected, moving beyond the nematode model. For instance, investigating how oxidative stress from PFRs impacts key neuromodulators like octopamine and serotonin, which regulate aggression and foraging, could directly link soil chemistry to behavioral changes (Barbero et al. 2023). Furthermore, it is crucial to recognize that these responses are likely species-specific. The trade-off between physical habitat improvement and chemical stress we observed in a soil-dwelling generalist like *F. japonica* may differ dramatically in other ants. Specialist species, such as seed-harvesters, might be more vulnerable to subtle changes in soil chemistry affecting their food source (Uhey and Hofstetter 2022), while arboreal ants would experience different exposure routes altogether, such as through the trophic transfer of contaminants via their prey (Pohl et al. 2024). Therefore, future work combining mechanistic physiological studies with comparative ecology across various ant functional groups is essential to build a truly predictive understanding of biochar's complex ecological role.

The complex interactions among biochar, ant behavior, and ecosystem functions highlight the potential for targeted biochar application in ecological restoration strategies. Biochar can alter the soil microenvironment, which indirectly affects the physiological states and cognitive abilities of ants. Specifically, appropriate biochar concentrations enhance multiple aspects of ant behavior, including nest building, spatial cognition, and social interactions, thereby enhancing a range of ant-mediated ecosystem services such as nutrient cycling, seed dispersal, and pest control. The enhanced complexity of ant social behavior leads to optimized defensive and cooperative interactions, which are crucial for ecosystem regulation and biodiversity conservation (Suarez and Goodisman 2021).

5 Conclusions

To our best knowledge, this study is the first to quantify the effects of biochar on various social behaviors of large soil fauna. The results underscore the importance of considering ant social behavior in soil restoration efforts and provide valuable insights for developing more effective, biologically driven approaches to ecosystem management. As global efforts to combat land degradation intensify, our findings could provide a promising solution for enhancing the resilience and functionality of degraded ecosystems through the strategic use of biochar and the leveraging of ant-mediated ecological processes.

Supplementary Information

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Supplementary material 1.

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Author contributions
All authors contributed to the study conception and design. Methodology, data collection, software, writing and revising were performed by Sha Liu; Methodology and data curation were performed by Danling Xiong, Liang Zeng, Wei Du and Yang Liu; Methodology, writing and revising were performed by Christian E.W. Steinberg; Supervision, review and editing were performed by Shu Tao; Methodology and formal analysis were performed by Bo Pan and Baoshan Xing; Conceptualization, funding acquisition, writing-review & editing were performed by Bo Pan. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability
The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics statement

This study strictly followed the guidelines for ethical review of experimental animal welfare (GB/T 35892-2018). All ants used in the experiment were carefully reared before and after the experiment, and were provided with adequate food, water, and other necessities. During the experiment, all procedures were performed with care to minimize any potential suffering. After the experiment, the ants were maintained until they died naturally. No violations of animal welfare ethical guidelines occurred.

Competing interests

Baoshan Xing and Bo Pan serve as Editor and EBM of the journal Biochar, respectively, and they were not involved in the peer-review or handling of the manuscript. The authors declare no competing interests.

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