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Organic matter mitigates biotic impact of copper in fruit orchard soil☆

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ABSTRACT

Inorganic copper (Cu) fungicides and bactericides are widely used to control disease in fruit and vegetable crops and has led to widespread accumulation of the metal in soil beyond regulatory thresholds. We aimed to elucidate the impacts of Cu on soil health within cherry orchard soils in New Zealand, focusing on three biological indicators: earthworm behaviour, soil respiration, and plant growth. We sampled soils from four blocks of different ages within a single orchard, varying in amounts of accumulated soil Cu (7–263 mg kg^{-1}) but also in Soil Organic Matter (SOM) content (3–10 %). Experimental work was designed to isolate the impacts of both Cu and SOM on three critical biological descriptors: earthworm behaviour, soil respiration and root growth. Soils were amended to standardise both variables in laboratory and glasshouse experiments. The results demonstrated a pronounced inhibition of soil respiration and root development, as well as adverse effects on earthworm behaviour, with increasing Cu concentrations. SOM played a mitigating role, reducing the bioavailability and toxicity of Cu to soil organisms. However, the buffering capacity of SOM is limited and long-term reliance on SOM to mitigate Cu toxicity is not sustainable. Currently Cu continues to accumulate in most orchard soils. This study highlights the importance of assessing Cu bioavailability and soil health in the context of orchard management.

1. Introduction

The horticultural sector in Aoteroa-New Zealand (NZ) is reliant on clean-green credentials and sustainable management practices. Fruit orchards constitute an important component both of production landscapes and the national economy (StatisticsNZ, 2021), and cherries in the Central Otage region of South Island provide one such high value export crop. Similar to most other horticultural crops, cherry production necessitates the control of diseases (principally *Pseudomonas syringae*, Blast Bacterial Spot and *Monilinia fructicola,* Brown Rot fungus in cherries) for which regular and routine crop spraying with inorganic formulations of Cu is used in combination with a range of products with synthetic organic active ingredients (Farmlands, 2024). Copper reaches the ground through leaching by rainfall, falling plant debris and spray drift, and the metal accumulates rather than dissipates in surface soil (Jeon, 2024). Copper is an essential nutrient for plants and animals in trace amounts, but it becomes acutely or chronically toxic in excess (Ameh and Sayes, 2019).

The global use of Cu-based products to control fungal and bacterial disease has led to long-term accumulation and restrictions on use, although Cu is generally still acceptable for use under organic certification (Besnard et al., 2001; Fagnano et al., 2020; Komárek et al., 2010; Mirlean et al., 2009; Morgan and Taylor, 2004; Ruyters et al., 2013; Wightwick et al., 2008; Yang et al., 2005). In NZ, as in many other countries with temperate climates, regulatory limits of Cu applications are 8 kg Cu ha^{-1} yr^{-1} in conventional orchards and 3 kg Cu ha^{-1} yr^{-1} in organic orchards (Zespri Group Limited, 2023; Jeon, 2024). Despite these restrictions, substantially elevated soil Cu from long-term spraying at higher application rates is frequently recorded and continues to increase in a range of fruit crops including cherries, kiwifruit, apples, avocados and grapes. Across a range of these orchard soils we have recorded mean total soil Cu concentrations of 248 mg Cu kg^{-1} at 0–0.15 m depth (Jeon, 2024). Most published threshold limits use concentrations >100 mg Cu kg^{-1} as evidence of contamination and potential environmental risks (Schramel et al., 2000).

Soil organic matter (SOM) is a crucial component of soil health, composed of plant and animal residues at various stages of decomposition and substances synthesized by soil organisms. SOM improves soil structure, water retention and nutrient supply, and supports a diverse microbial community, and can also influence the mobility of

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contaminants such as Cu in soil. More than 90 % of Cu added to a soil is typically bound to SOM (Weng et al., 2002). The interaction between SOM and Cu is complex; SOM can bind Cu, reducing its bioavailability and toxicity to soil organisms (Antoniadis et al., 2017; Kaplan et al., 2016). This binding capacity can be affected by factors such as low soil pH although this is of less significance in most production soils that require less acidic soils.

Once soils become contaminated with Cu, dissipation rates are too slow to be significant (Aikpokpodion et al., 2010; Morgan and Johnston, 1991). For example, Dickinson and Lepp (1985) estimated that 80–120 years would be required to achieve a 10 % reduction in Cu concentrations through natural processes. High concentrations of residual Cu in soil adversely affect biological indicators of soil health (Doran and Timothy, 1994), but improved knowledge is required to evaluate and predict critical Cu concentrations and the extent and magnitude of risks to soil organisms and soil functionality (Nunes et al., 2016). Plant growth responses, earthworm activity and soil respiration are particularly useful indicators for assessing environmental impacts (Cruz et al., 2022; Li et al., 2020; Raiesi and Salek-Gilani, 2020; Rich et al., 2015; Zornoza et al., 2015), especially relating to effective management strategies to mitigate potential risks.

The aim of our study was to investigate the interplay between SOM and Cu contamination to understand whether SOM masks the biotic impact of Cu and thus misinforms the risks associated with its accumulation. We investigated the effects of Cu on earthworm behaviour, soil respiration, and plant root growth.

2. Materials and methods

We sampled four soils from different locations of a single soil type within the same cherry orchard in Cromwell, Central Otago. The 34.7 ha orchard was established in 1982 with Cu-based fungicides and bactericides usually applied 2–3 times per year, particularly to manage Blast Bacterial Spot (*Pseudomonas syringae*). Application rates recommended for cherry production are up to 27.3 kg ha $^{-1}$ of copper oxychloride in four applications through the year (Farmlands, 2024). The collected soils (S1-S4) were experimentally amended with additional Cu and organic matter (Fig. 1).

2.1. Soils and ecological determinants

2.1.1. Soils

The four bulk soils were collected in July 2021 from three different-aged blocks (S1-S3) and another unsprayed location (S4 reference) at the periphery of the orchard. S4 is the reference location that had never been planted. Approximately 100 kg of each soil was collected at 0–0.2 m depth and transported to Lincoln University. Each soil was thoroughly mixed using a clean cement mixer, then sieved (<4 mm) to remove small amounts of plant residues and stones. Soils were stored in open steel bins indoors at the university nursery prior to their use for experimental work. For physico-chemical analysis, five replicated sub-samples were air dried at 35–40 °C overnight and crushed to pass through a 2 mm screen, then commercially analysed (Hill Laboratories, Christchurch) for soil pH (potentiometric), Olsen phosphorus (Molybdenum Blue colorimetry), potassium, calcium, magnesium, and sodium (all ICP-OES from 1 M neutral ammonium acetate extracts), and bulk density (Core method). At Lincoln University, total copper (Cu), soil organic matter (SOM), and total nitrogen (N) were quantified by the first author (Jeon, 2024) using atomic absorption spectrophotometry (AAS) following microwave digestion, loss on ignition (LOI), and a CN analyser, respectively (Table 1).

2.1.2. Earthworms

A native anecic species of New Zealand earthworm, *Maoridrilus transalpinus*, was collected from Ahuriri Scenic Reserve on the Christchurch Port Hills following the method described and as identified using DNA barcoding by Kim et al. (2017, 2022) and used for earthworm preference tests. This species was selected for its ease of collection in large enough numbers by digging and the abundance of adults. Before use, adult earthworms were maintained in their original soils for one month in an incubator (15 °C, 30–35 % soil moisture). Adults with an easily observable clitellum were selected, individuals weighing 1–3 g.

2.1.3. Plants

Hop plants (*Humulus lupulus*, cultivar Cascade) were used as an alternative to cherries, since the latter are slow growing and difficult to grow uniformly. The selection of the hop cultivar Cascade was justified by their uniformity in height and widespread popularity, ensuring experimental consistency and relevance to current agricultural

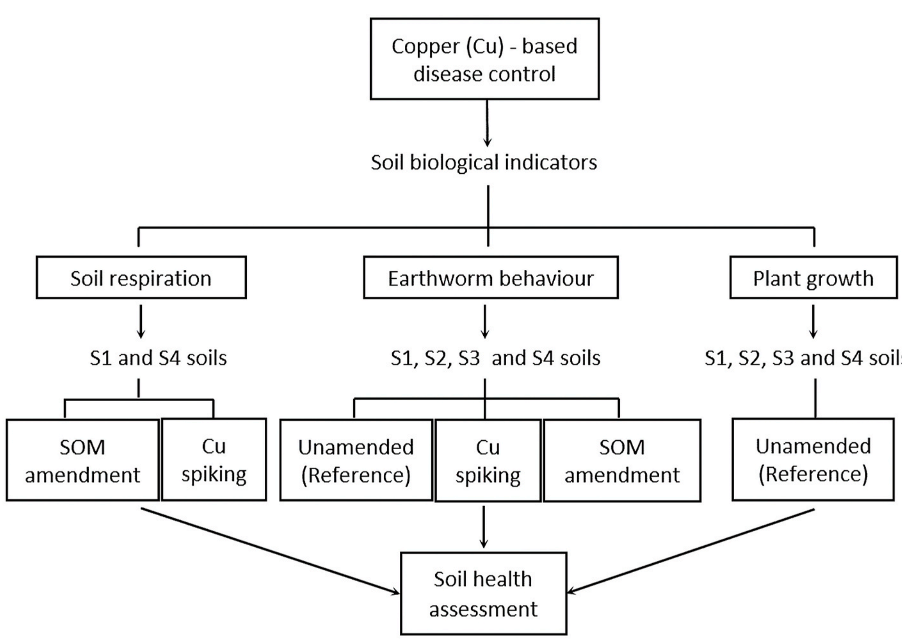

Fig. 1. Methodology outline using four soils (S1-S4) amended using copper (Cu) and organic matter (SOM).

Table 1
Chemical analysis of the four soils collected from four locations in the cherry orchard (means ± s.d.; n = 5).

Determinant	S1	S2	S3	S4	Optimal range[c]
Soil total Cu (mg kg^{-1}) [b]	263 ± 37.5a	160 ± 10.7b	91.0 ± 11.4c	7.70 ± 0.50d	–
$pH_{1:2}$ [b]	6.55 ± 0.06b[d]	7.20 ± 0.15a	6.45 ± 0.05b	7.15 ± 0.16a	5.8–6.5
Soil organic matter (%) [b]	6.10 ± 1.05b	12.0 ± 1.50a	7.30 ± 0.87b	4.00 ± 0.98c	n.a.[e]
Total Nitrogen (g kg^{-1}) [b]	2.30 ± 0.05b	4.30 ± 0.09a	2.00 ± 0.10b	1.8 ± 0.06b	n.a.[e]
CN ratio [b]	16.0 ± 3.05	16.4 ± 2.98	20.0 ± 4.06	13.8 ± 4.21	n.a.[e]
Olsen Phosphorus (mg L^{-1}) [b]	38.0 ± 2.4b	90.0 ± 3.70a	34.5 ± 3.50b	11.0 ± 1.05c	25–50
CEC [b]	13.1 ± 1.72b	23.9 ± 3.05a	12.8 ± 1.05b	9.12 ± 1.26b	12–25
Potassium (me $100g^{-1}$) [b]	0.50 ± 0.05b	1.45 ± 0.12a	0.30 ± 0.02c	0.80 ± 0.05ab	0.50–1.0
Calcium (me $100g^{-1}$) [b]	10.1 ± 1.01b	20.4 ± 1.10a	9.31 ± 0.58b	6.62 ± 0.42c	6.0–12
Magnesium (me $100g^{-1}$) [b]	1.24 ± 0.06a	3.02 ± 0.08b	0.90 ± 0.02a	0.91 ± 0.06a	0.60–1.2
Sodium (me $100g^{-1}$)	<0.05	<0.05	<0.05	<0.05	0.00–0.5
Bulk density (g mL^{-1}) [a]	1.32 ± 0.04a	0.95 ± 0.05b	1.23 ± 0.06a	1.1 ± 0.02ab	0.60–1.0

[a] Significant at the 0.01 probability level.
[b] Significant at the 0.001 probability level.
[c] The optimum range guidelines relate to sampling protocols as per Hill's Laboratory crop guides and are based on reference values where these are published.
[d] Values with the same lower-case letters in a row are not significantly different at $P < 0.05$.
[e] n. a. = not applicable.

practices. Cascade is one of the world's top ten most popular hop cultivars, and it now represents around 10 % of all hops grown in the United States (Healey, 2021). Cascade is a successful and well-established aroma hop (USDA U.S. Department of Agriculture, 2007) that is cultivated in New Zealand. One-year old plants (50–100 mm tall) were transferred from cultivation beds into soils in rhizoboxes (five replicates).

2.2. Experimental design

2.2.1. Earthworm preference test

Preference tests were conducted following Kim et al. (2017, 2022). Simple choice chambers were assembled using five polypropylene containers (450 mL). Holes in the jars allowed free movement of earthworms within each set of containers (Fig. 2). The four outer containers were filled with 100 g of either S1, S2, S3 or S4 soil maintained at 30–35 % moisture. Earthworms were added into each set of choice chambers for behavioural assays. Gauze covering the central container provided ventilation. Soil moisture was replenished every three days, with the amount added assessed by weight of the containers. Five sets of choice chambers were maintained in an incubator at 15 °C for 14 days, in a randomized arrangement.

2.2.1.1. Unamended soil. Assays were carried out using the unamended bulk soils with three active and individually weighed earthworms introduced into each set of choice chambers, with five replicate sets of chambers. The four outer containers of each set of choice chambers were filled with 100 g of either S1, S2, S3 and S4 soil. After 14 days incubation, earthworms were extracted, counted and weighed, recording where they were found within each apparatus.

2.2.1.2. SOM amended soil and Cu spiked soil. Soils were also amended to standardise either SOM or soil total Cu concentrations, adjusting each soil to match that with the highest recorded concentrations of each. Thus, SOM was adjusted to 12 % to match S2 using peat moss (Kiwipeat NZ sphagnum peat moss, TNZ Growing Products). Sphagnum moss peat was used as the primary source of soil organic matter (SOM) in this study. According to the manufacturer's specifications (TNZ Growing Products Ltd,), the peat was sterilized, significantly eliminating any native microbial communities present. This sterilization minimized the influence of a non-copper-adjusted microbiome, ensuring that any observed effects on root systems were attributed primarily to copper treatments. $CuSO_4$ stock solution was added to adjust soil Cu to match S1. SOM was adjusted to 12.0 % in three of the soils to match S2. Copper was adjusted to 263 mg kg^{-1} in soils S2-S4 to match S1. Copper stock solution was prepared by dissolving 3.93 g $CuSO_4 \cdot 5H_2O$ in 1000 mL of deionised water, with pH adjusted using sufficient 0.5 M NaOH to avoid precipitation.

2.2.2. Soil respiration

2.2.2.1. SOM and Cu amendments. Different proportions of SOM were added to the soil with highest Cu (S1). Peat moss was used to adjust SOM to 6 % (no addition, reference), 9 %, 12 %, 15 % and 18 %, with amended soils then maintained in the shade for two weeks. Five replicate plastic plant pots (diam. 120 mm, ht. 200 mm) were each filled with 1.5 kg of each prepared soil. A PVC respirometer collar (diameter 100 mm) was inserted to 10 depths, protruding 20 mm above the surface, and soil moisture content was adjusted to 30–40 % with tap water and maintained for three days prior to CO_2 flux measurements.

Additionally, the S4 soil (total Cu: 7.7 mg kg^{-1}; SOM: 4.0 %) was spiked with Cu as above (#2.2.1.2) to concentrations of 7.0 mg kg^{-1} (no addition, reference), 30 mg kg^{-1}, 100 mg kg^{-1}, 200 mg kg^{-1} and 300 mg kg^{-1}, again with five replicates of each, and stabilised in the shade for two weeks. Amended soils were then added to pots, maintained and prepared in the same way.

2.2.2.2. Measurement of CO_2 flux. CO_2 flux was measured using a LICOR-8100 A Automated Soil CO_2 Infrared Gas Analyser (Fig. 3 a), with monitoring of soil temperature and water content (100 mm depth). The IRGA was connected to a Respirometry Chamber on the PVC collars in the plant pots. Prior to CO_2 flux measurement, soils were stabilised for 14 days. Measurement duration of each pot was 120 s, with three replicates per sample, with five replicates per treatment (n = 15), following the instrument manual protocol. Each soil was measured at two time points: after soils were stabilised (t_0) and after 10 days of additional $CuSO_4$ application (t_{10}) (Fig. 3 b). Soil moisture and temperature probes were inserted adjacent to the PVC collar and recorded data automatically during CO_2 flux measurement. Soil CO_2 flux was calculated based on a linear increase in chamber CO_2 concentrations over time (μmol CO_2 C m^{-2} s^{-1}) using standard protocol. Corrections were applied for soil temperature and Water-Filled Pore Space (WFPS), with values standardised to 25 °C and 60 % WFPS (Parkin et al., 1996). Soil bulk density was measured by using soil sample rings (0.0906 L) at the end of the experiment. Soil moisture content (%) was used to calculate volumetric water content (Jeon, 2024).

2.2.2.3. Additional Cu spiking. The impact of repeated doses of the equivalent of 3 Cu kg ha^{-1} yr^{-1} was investigated using ten further applications (daily) of a stock solution for ten days (Fig. 3 b). The experimental set up was maintained in a shade house with a randomised arrangement of the containers which were irrigated every two days. Soils were sampled before and after the experiment for chemical analysis.

Fig. 2. Choice chambers used for earthworm preference tests (n = 5). Each connected polypropylene container (70 mm × 70 mm x 90 mm) with 50 mm diameter holes allowing free movement into each by earthworms introduced into the central chamber.

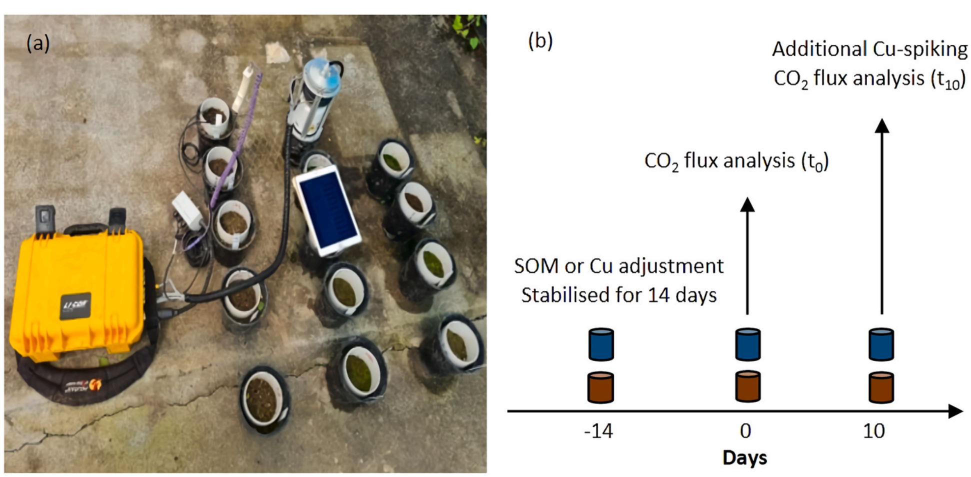

Fig. 3. Measurement of CO_2 flux in SOM and Cu adjusted soil treatments, showing IRGA, collars and chamber (n = 15). Recorded data was transferred from IRGA instrument to tablet (inserted into photograph); (b) Summary of experimental protocol: t_0 (14 days after set up) and t_{10} (10 days later).

2.2.3. *Plant growth in rhizoboxes*

Five replicate rhizoboxes (0.6 m × 0.8 m x 0.03 m) with glass panels on each side were filled with S1, S2, S3 and S4 soils in vertical strips separated by 3 mm steel mesh. They were watered until saturated and maintained in a glasshouse for three days before transplanting one hop seedling into each section. The glass panels were covered with black polythene film. Temperature range in the glasshouse was 10–33 °C with 40–60 % relative humidity. Rhizoboxes were carefully watered daily by adding 200 mL water, avoiding leaching from the apparatus. Shoot and root growth was measured and photographed every few days for 120 days. Plants were then harvested, separating roots and shoots (cone, leaf, stem, roots) which were oven-dried (65 °C) and weighed prior to Cu analysis.

2.3. Chemical analysis

At the end of all of the experiments, soils were air-dried (25–30 °C) and sieved (2.0 mm) prior to analysis following standard methodologies of Lincoln University Analytical Services. Briefly soil samples were microwave digested (65 % nitric-perchloric acid) prior to Cu determination using Atomic Absorption Spectrophotometer (AAS). QA used certified reference material ISE2021-4-1. Earthworm and plant samples were oven-dried (65 °C) individually and analyses in the same way.

2.4. Statistical analysis

Descriptive statistics were first calculated to summarize the main characteristics of the soil samples (S1-S4). To assess normality, data were inspected through visual examination of Q-Q plots and tested using the Shapiro-Wilk test. Homogeneity of variances was evaluated with Levene's test. For inferential analysis, one-way ANOVA was employed to compare means across different treatments and soil types, with post-hoc Tukey's Pairwise Comparisons used to identify specific differences among treatment groups at a significance level of $p < 0.05$. Multiple regression models were developed to account for potential confounding variables and to assess the combined effects of soil parameters on each biological outcome. Multicollinearity among predictor variables was addressed by eliminating highly correlated variables, ensuring robust model results. Principal Component Analysis (PCA) was utilized to reduce the dimensionality of the dataset and to identify key soil properties contributing to the observed biological effects in the unamended soil. All analyses used Minitab 19 software.

3. Results

3.1. Earthworms

A mortality rate of earthworms of 13 % occurred across all the orchard soils. In unamended soils there was a strong preference for the S2 soil which contained 160 mg Cu kg $^{-1}$ (Fig. 4). The preference for this soil remained when SOM was adjusted to 12 % in all soils, and when Cu was spiked to the same high concentration of 263 mg kg $^{-1}$.

3.2. Soil respiration

In the unamended soils there was significant correlation between soil respiration and soil Cu concentrations. In the SOM-amended treatments with 6 % and 18 % SOM, there a significant decrease in soil respiration. In contrast, treatments with 9 %, 12 %, and 15 % SOM maintained soil respiration rates within the anticipated normative range (3.46–6.92 μmol CO_2 m^2 s^{-1}), demonstrating no notable reduction, even following a 10-day period subsequent to the application of $CuSO_4{\cdot}5H_2O$ (Fig. 5). For the Cu-spiked treatments, soil respiration$_{60}$ dropped below the expected normal range across all initial Cu concentrations (7, 30, 100, 200, and 300 mg Cu kg^{-1}) within 10 days following the application of $CuSO_4{\cdot}5H_2O$ (Fig. 5).

3.3. Plant growth

In the rhizobox experiment there was visual evidence that the roots crossed the mesh barriers into adjacent soils (Fig. 6, Supplementary Fig. 1). Soil moisture differences were evident through preferential flow (darker regions in photograph) and this had an obvious effect on root proliferation. Some 80 % of observed roots in S1 soil (263 Cu mg kg^{-1}) foraged toward the S2 soil (160 Cu mg kg^{-1}) soil without a soil moisture influence. No roots grew from S2 soil to S1 soil, and only 20 % of roots grown in S2 soil (160 Cu mg kg^{-1}) moved toward S3 soil (91 mg kg^{-1}) without a soil moisture influence. However, no roots grew from S3 soil (91 Cu mg kg^{-1}) into S2 soil (160 Cu mg kg^{-1}). All of the roots that could be observed in S4 soil (7.7 Cu mg kg^{-1}) foraged toward S3 (91 mg kg^{-1}) soil irrespective of soil moisture influence. Total plant mass (g) was highest in S2 soil and lowest in S4 soil (Fig. 7 a). Root (Supplementary Fig. 2) and cone weight were highest in S2 soil. Roots accumulated most Cu in soils with little transference of Cu to above-ground plant components (Fig. 7 b).

4. Discussion

4.1. Differences between the four soils

Besides differing Cu concentration of the four collected and bulked soils, there were other significant differences in SOM content, pH, and CN ratio that may have affected earthworm behaviour, soil respiration, and plant growth. SOM obviously influenced how the soils responded to copper toxicity; soils with higher SOM had a greater capacity to bind copper, reducing its bioavailability and subsequent toxicity to soil biota. The interaction between SOM and soil Cu was clearly a major determinant of soil biotic responses, but other measured soil properties also varied between treatments; obviously the overall soil matrix determines the biotic impacts of Cu.

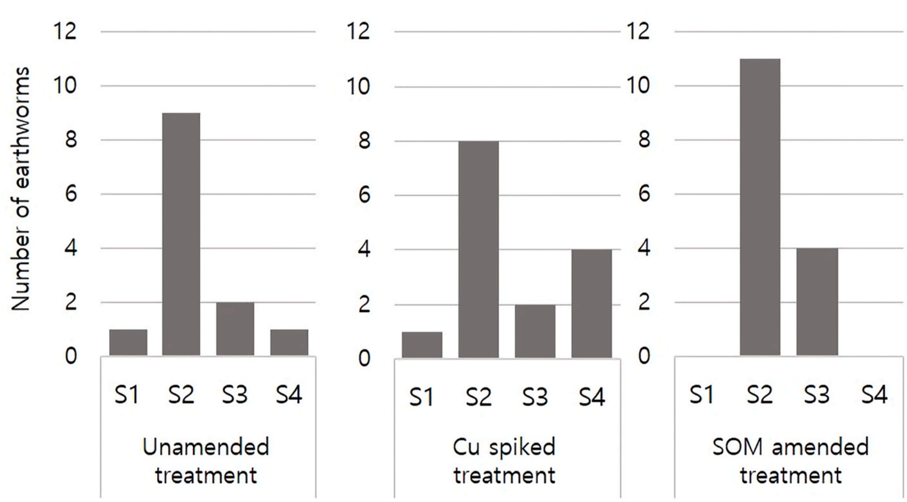

Fig. 4. Earthworm preference test after 14 days of exposure of 15 earthworms simultaneously to four unamended and amended soils in the choice chambers (n = 5). In the SOM-amended and Cu-spiked soils, SOM was adjusted to 12 % (matching S2) and Cu concentrations were adjusted to 263 mg kg^{-1} (matching S1).

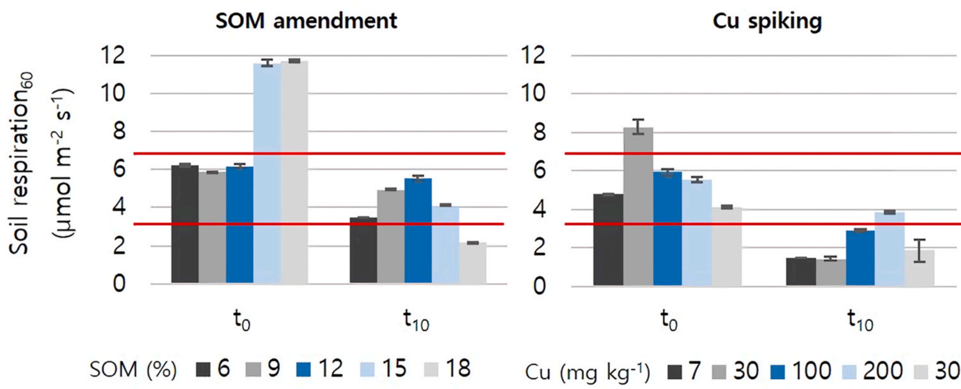

Fig. 5. Effects of adjustment of SOM and soil Cu on soil respiration$_{60}$ (μmol m^{-2} s^{-1}) after the stabilisation period (t_0) and with additional Cu added over the subsequent 10 days (t_{10}) (n = 15, means ± s.d.). Horizontal red lines indicate the expected normal range (3.46–6.92 μmol CO_2 m^2 s^{-1}) of soil respiration$_{60}$ means that "soil is in a normal state of biological activity and has adequate organic matter and active populations of microorganisms" (Woods End Research Laboratory, 1997). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

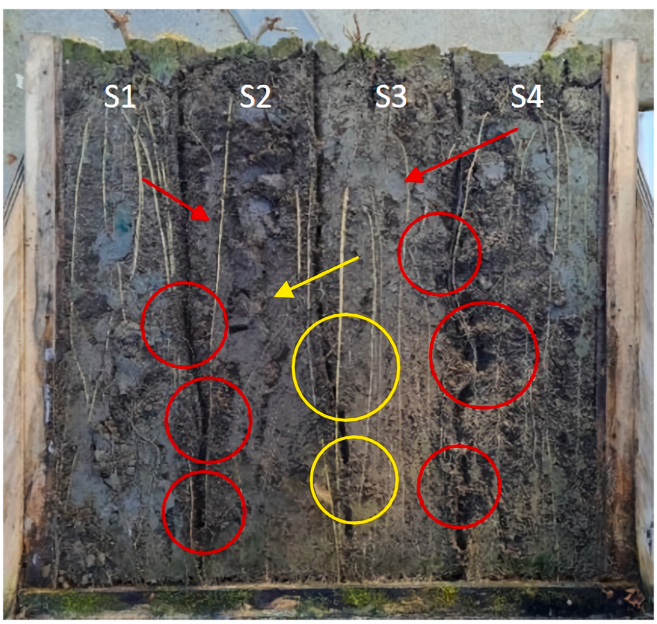

Fig. 6. Hop plant root growth in one of the rhizoboxes after 100 days. Soils from left to right represent S1 (263 Cu mg kg^{-1}), S2 (160 Cu mg kg^{-1}), S3 (91 Cu mg kg^{-1}) and S4 (7.7 Cu mg kg^{-1}) soil. Circles represent root growth into the adjacent soil. The yellow-coloured circles represent root growth that was influenced by water infiltration. Red-coloured circles represent roots' growth into the adjacent soil not associated with moisture. Arrows represent root growth directions. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4.2. Interplay between SOM, Cu and earthworm behaviour

After addressing multicollinearity by eliminating highly correlated variables, the multiple regression model was simplified to focus on key variables that could best explain the biotic impacts of soil amendments on earthworm preference. The simplified multiple regression analysis revealed that SOM (2.21) had the most significant positive effect on earthworm preference, confirming its strong influence compared to other soil variables (Table 2). While CN ratio (−0.703), pH (−0.212), and Total Cu (−0.023) exhibited negative effects on earthworm populations, their impacts were relatively minor in comparison to SOM. This underscores the crucial role of SOM in supporting earthworm activity. The preference of earthworms for soils with higher SOM content, irrespective of the presence of Cu, indicated a protective role of SOM against Cu toxicity (Chelinho et al., 2011; Natal-Da-Luz et al., 2008). Our findings showed resilience of earthworms to Cu concentrations up to 160 mg Cu kg^{-1}, with no evidence they were sensitive to or avoided Cu-rich environments below this concentration. Regression model revealed a non-linear tolerance to Cu, with a threshold effect of around 135 mg Cu kg^{-1} (not shown), above which earthworm activity significantly reduced, indicating the limits of SOM protection from Cu toxicity. These findings suggest a threshold of tolerance influenced by organic matter, which can mitigate the toxic effects of Cu and support earthworm survival. However, the results also showed that earthworms avoided soils with higher of extremely low Cu concentrations; notably in soil S4 (with 7.7 mg Cu kg^{-1}) and in S1 (with 263 mg Cu kg^{-1}). In these cases, impacts were evident on earthworms and, by extension, to soil health (Brady and Weil, 2005; Hatten and Liles, 2019). These nuanced responses to different combinations of Cu and SOM indicate the requirement for careful soil management practices to support the ecological functions of soil biota.

4.3. Dynamics of soil organic matter and Cu on microbial activity

The multiple regression model for respiration demonstrated that SOM (0.0708) had the largest positive effect, suggesting its critical role in promoting soil respiration (Table 2). Total Cu (−0.019) exhibited a small negative effect on respiration, indicating that higher Cu concentrations may slightly inhibit microbial and biological activity. However, the overall impact of Total Cu was much smaller than that of SOM, and the positive effect of SOM appeared to counterbalance any negative effects of Cu on respiration. This highlights the importance of SOM in maintaining soil biological functions, even in the presence of Cu. Soil respiration was also influenced by the interplay between SOM and Cu; decreasing sharply beyond 265 mg Cu kg^{-1} regardless of initial SOM or Cu levels. This illustrates the suppressive impact of Cu on soil biological processes, similar to research findings elsewhere (Sereni et al., 2021). The buffering capacity of SOM against the adverse effects of Cu was evident. In the highest SOM-amended treatment (15 % and 18 %), increased respiration may be explained by SOM mineralisation increasing microbial activity. Linear regression analysis showed a positive correlation between increased SOM and pH levels with soil respiration, concurrently with a decline in Cu concentration. SOM probably has a dual role, enhancing microbial activity and limiting Cu bioavailability, thus protecting against Cu toxicity. This supports findings elsewhere (Amery et al., 2008; Degryse et al., 2009; Groenenberg et al., 2010; Ren et al., 2015). Our study also highlights the critical balance required in SOM levels to avoid adverse conditions that inhibit microbial

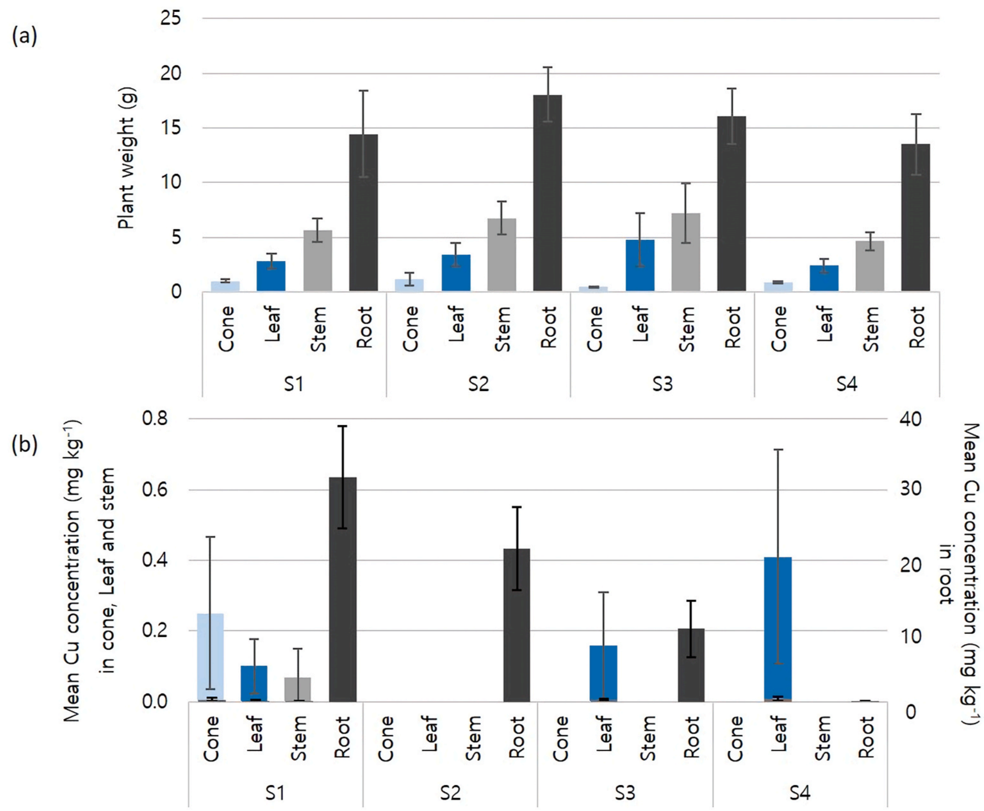

Fig. 7. (a) Plant mass and (b) Cu concentrations in plant components after 120-day rhizobox experiment. Soil types labelled as S1 (SOM: 6.1 %, Cu: 263 mg kg^{-1}), S2 (SOM: 12.0 %, Cu: 160 mg kg^{-1}), S3 (SOM: 7.30 %, Cu: 91.0 mg kg^{-1}), and S4 (SOM: 4.00 %, Cu: 7.70 mg kg^{-1}) (*see* Table 1). Results are from 5 replicate rhizoboxes. Error bars are standard deviations (n = 5).

Table 2
Simplified multiple regression results (Multicollinearity was eliminated).

Biotic indicators	Soil parameters	Coefficient value	p-value
Earthworm preference	SOM	2.21	<0.001
	Total Cu	−0.023	<0.05
	CN ratio	−0.703	<0.01
	pH	−0.212	<0.01
	Intercept	0.094	
	R^2	0.872	<0.001
Respiration	SOM	0.071	<0.001
	Total Cu	−0.019	<0.01
	Intercept	0.008	
	R^2	0.783	<0.01
Root growth	SOM	0.635	<0.01
	Total Cu	−0.024	<0.05
	Intercept	11.0	
	R^2	0.712	<0.01

activity or lead to SOM loss. The findings show that excessively low (<6 %) or high (>18 %) SOM is not conducive to soil health in production soils, underscoring the necessity for balanced soil management that considers the interconnected roles of chemical elements and biological functions. Relying solely on soil respiration as a singular indicator of microbial activity in response to Cu contamination appears to be unsatisfactory.

4.4. *Complex interactions between soil copper concentration, nutrient availability, and plant growth*

In the case of root growth, the simplified regression model showed that SOM (0.635) again had a substantial positive impact, suggesting its importance in promoting healthy root development (Table 2). Total Cu (−0.024), on the other hand, had a slight negative effect on root weight, indicating that elevated copper levels may hinder root growth, albeit to a minimal extent. The fact that SOM had a stronger effect than Total Cu supports the hypothesis that SOM is a key factor in enhancing root development, likely by improving nutrient availability and soil structure. Earlier studies have found that plant roots avoid high Cu zones of soils (Comas et al., 2013; Hodge et al., 2009). However, the present study indicates that root growth dynamics are not solely affected by high Cu levels. Instead, root foraging is largely influenced by the availability of moisture and SOM, the latter likely to be associated with fertility. Contrary to our hypothesis, there was no significant root migration from soil with moderately high Cu (91 mg Cu kg^{-1}, S3) to lower Cu levels (7.7 mg Cu kg^{-1}, S4), underscoring the complexities of root growth preferences that extend beyond Cu concentration alone. Root growth was adversely affected where Cu concentration was highest (263 mg Cu kg^{-1}, S1). Symptom of Cu toxicity have been widely reported to disrupt root morphology, structural integrity and root biomass (Lombi et al., 2004; Sheldon and Menzies, 2005; Cruz et al., 2022). Rhodes grass roots were extremely stunted with root cuticle thickened (Sheldon and Menzies, 2005).

Soil nutrients were a more significant factor influencing root growth directionality than Cu levels across the range of orchard soils (Supplementary Fig. 3). The PCA first component highlighted the association between increased presence of magnesium (Mg), total nitrogen (TN), calcium (Ca), cation exchange capacity (CEC), phosphorus (P), soil organic matter (SOM), and potassium (K). Hop roots foraged for nutrient-rich environments, even in the presence of moderately high Cu concentrations. This interplay of different factors suggests that increasing Cu concentrations in orchard soils is just one component of plant health and productivity.

Using chemical extractants or soil pore water to reflect the bioavailability of copper (Cu) in soil is well documented in literature.

When soluble Cu is introduced to soils, various reactions reduce its bioavailability as Cu binds to soil particles (Ma et al., 2006). Cu bioavailability and toxicity decreases due to aging effects that reduce extractability of Cu (Hogg et al., 1993; McLaren and Ritchie, 1993). Sequential extraction techniques also reveal the complexity of the partitioning between extractable and reducible fractions (Nielsen et al., 2015), that are further influenced by anthropogenic inputs (Zimmerman and Weindorf, 2010). In the limited scope of the present study we considered that understanding the biological response to Cu, irrespective of its chemical mobility or speciation, was most significant.

4.5. Understanding the biotic impact of Cu

This integrated approach of the present study focused on the simultaneous effect of Cu on earthworm behaviour, soil respiration, and plant growth within the same soil system in an attempt to understand the impact of this metal on soil health. *Ex-situ* assays using soils from orchard blocks allowed better control of treatment conditions and comparisons that were difficult to measure in the field. The findings showed that a soil concentration of 91 mg Cu kg^{-1} did not significantly impact these biotic variables. At 263 mg kg^{-1}, there was potential for Cu to be mobilised from the soil and transferred to the aboveground parts of hops, with obvious agricultural and environmental implications. When total Cu in soil reached 500 mg kg^{-1} (10 days' $CuSO_4$ application), soil respiration decreased significantly, indicating an adverse impact on soil microbial activity. Additional spiking of the soils with either SOM or inorganic Cu showed that organic matter is a more important driver of biological activity, also playing an important role in amelioration of the impact of Cu.

Toxic impacts of Cu were only evident at soil concentrations above existing published thresholds (100 mg kg^{-1}), although lack of solubility of elevated Cu in soil may not be temporally stable; dissolution and mobility could be altered by changed environmental conditions and biological activity. The extent and limits of biological resilience to Cu toxicity through adaptation or acclimation is uncertain. Clearly there are interconnections between biological processes and integration of measures of plants, microbial and faunal impacts in the present study is likely to be more useful than a focus on single receptors. However, future limits to biological resilience may only be evident from longer-term studies when critical parameters are exceeded, or key ecological processes are disrupted. Of particular concern is the extremely slow disappearance rates of Cu from soils, once they become polluted.

5. Conclusions

Earthworms had a marked preference for the orchard soil with highest SOM (12 %) and 160 mg Cu kg^{-1}. The preference for this soil persisted when other soils were spiked with Cu. There was no evidence of Cu sensitivity in the earthworms or adverse effects up to this Cu concentration. Impacts of Cu on soil respiration were found in spiked soils where other variables were controlled, although the addition of SOM appeared to be beneficial in addition to mitigating the detrimental effects of Cu. High soil Cu adversely affected root foraging and plant growth, particularly in soils with the highest Cu concentrations, but soil organic matter was also influential, likely to be explained by its role in water retention and fertility. These findings reveal the complex interplay between soil Cu, SOM, and with the biological indicators that may accurately reflect the concept of soil health.

The resilience of biological determinants of soil health to Cu-contamination in terms of SOM appears somewhat incongruous, since SOM is also the primary substrate required for biological activity and fertility in soil. Decomposition of SOM would be expected to increase the mobility of Cu and resultant exposure of biota to Cu. However, to an unexplained degree, SOM alleviates copper toxicity due to its influence on soil physicochemistry, particularly moisture and nutrition. An additional variable is likely to be adaptation or acclimation of the soil biota to elevated Cu.

Continued use of Cu for disease control is not viable in the longer term. There is clearly an impact on structural and functional aspects of soil ecology at higher soil Cu loadings. Maintaining or enhancing SOM levels, as well as maintaining high soil pH, has been a crucial strategy in managing the impact of Cu on soil health in the short term awaiting a switch to alternatives to Cu usage. Despite the apparent resilience of biota to increasing Cu, this metal has exceptionally long residence times in soil and continued contamination of soils is not compatible with increasing attention being given to the clean green credentials of horticulture and agriculture.

CRediT authorship contribution statement

Dasom Jeon: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Brett Robinson:** Writing – review & editing, Supervision, Investigation. **Nicholas Dickinson:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2024.125145.

Data availability

Data will be made available on request.

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