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Copper content in soils and litter from fruit orchards in Central Chile and its relationship with soil microbial activity

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Abstract: This study assessed both the soil and litter copper (Cu) levels and their relationships with soil microbial activity, in fruit-tree production areas of central Chile where Cu-based pesticides are intensively sprayed. Samples of soil (0–20 cm depth) and litter from a number of selected orchards (kiwi, table grape, plum, and cherry) were collected and analysed for their Cu content and C-induced soil microbial activity. Results showed that the mean total soil Cu level was 225 mg/kg and soluble soil Cu was less than 0.01% of total soil Cu, as expected from pH values of study soils (range of 6.33 to 7.93). However, leaf litter Cu content was 3–7 times higher than in soil (mean of 681 mg/kg). Despite the soil and leaf litter Cu concentrations, no effect was observed on the C-induced soil microbial activity. We conclude that leaf litter exerts a protective role, preventing the entry of Cu into the soil and thus soil microbial toxicity.

Keywords: metals; contamination; bioavailability; MicroResp^{TM}; bioindicator

Copper (Cu)-based pesticides are used to control microbial diseases in fruit trees (Schoffer et al. 2021). Extremely high total soil Cu concentrations (> 1 000 mg/kg) have been reported worldwide as a result of the prolonged use of cupric pesticides in vineyards and fruit-tree plantations, as reviewed by Schoffer et al. (2020).

Foliar spraying of Cu-based pesticides in fruit systems may result in litter being the main sink for the atmospheric fallout of trace elements (Bergkvist et al. 1989). Therefore, the entry routes for Cu into the soil may be through the accumulation of Cu-enriched leaves and/or through the dry/wet deposition of Cu-rich aerosols. To our knowledge, only Lepp et al.' s (1984) study has reported Cu values both in leaf litter and soil of the same orchards.

In Chile, fruit production is mainly located in the Mediterranean Region of central Chile, with

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the O'Higgins Region as the most relevant, with a total surface of 52 467 ha (ODEPA-CIREN 2018). Copper-enriched soils might be expected due to the intensive use of Cu-based pesticides (Casanova et al. 2013). However, there is very little information in the literature on Cu levels from the use of Cu-based pesticides in Chilean orchard soils and their associated litter.

Due to the global scarcity of information relating to both soil and litter Cu contents in fruit-tree production systems and the relationship of Cu with soil microbial activity, the aim of the present work was to assess both the total soil Cu and litter Cu levels in fruit production systems in the O'Higgins Region of Chile where the application of Cu-based pesticides is very common and determine their relationship with soil microbial activity.

MATERIAL AND METHODS

Study area. Three orchards of kiwi, table grape, plum and cherry trees were selected from the Cachapoal River valley in the O'Higgins Region, central Chile. The cherry orchards were located in the northern part of the Rancagua county (34°10'0''S, 70°45'0''W), while the kiwi, table grape and plum orchards were located in the Peumo county (34°23'46'', 71°10'10''W). The region is characterised by a semi-arid Mediterranean climate (Luebert and Pliscoff 2017), with an average annual rainfall that varies from 450 mm in the north to 900 mm in the south, mainly concentrated in the winter months (Uribe and Catalán 2016). The average temperature ranges between 18 °C and 22 °C in the warm months, while in the cold months the temperature is around 9.6 °C (Uribe and Catalán 2016). Tables 1 and 2 summarise the cultivation practices and cupric pesticide management of selected orchards.

The plum orchards covered areas of 10–26 ha, with open-vase tree training systems and a planting density of 278–333 plants/ha. The three orchards had ridges to a height of 0.4 m and 1.0 m wide. The areas of kiwi orchards were 19.6 and 7 ha, with a pergola trellis system and ridges 0.2 m high by 1.0 m wide. Depending on the orchard, the planting density varied from 416–666 plants/ha. In table grape orchards, the areas varied from 6 to 12 ha. The planting density was 952 plants/ha. These orchards had a pergola trellis system and none had ridges. Regarding the cherry orchards, the areas varied from 4 to 6 ha, with a planting density of 694–889 plants/ha and an open-vase system. None of the cherry orchards had ridges.

Table grape's orchards were not sprayed with Cu-based pesticides and therefore used as control or-

Table 1. Cultivation characteristics of selected orchards in the O'Higgins Region, central Chile

	Plum			Kiwi			Table grape			Cherry		
Orchard	P1	P2	P3	K1	K2	K3	TG1	TG2	TG3	C1	C2	C3
Cultivar	D'Agen			Hayward			Crimson			Royal Down	Bing	Santina
Area (ha)	26	17	10	25	19	6	7	6	12	6	4	5
Between plant spacing (m)	6	5	5	3	4	3	3	3	3	2.5	2.5	3
Between row spacing (m)	6	6	6	5	6	5	3.5	3.5	3.5	4.5	4.5	4.8
Density (plants/ha)	278	333	333	666	416	666	952	952	952	889	889	694
Tree training system	open vase			pergola trellis						open vase		
Ridge height (m)	0.4	0.4	0.4	0.2	0.2	0.2	0	0	0	0	0	0
Ridge width (m)	1	1	1	1	1	1	0	0	0	0	0	0
Microtopography	plane											
Planting year	2002	2010	2005	1990	1986	1988	2007	2014	2014	2009	2009	2009
Previous use	no information			peach	wine vine	table grape	citric	citric	citric	table grape/ plum	table grape	table grape
Litter residence time (month)	6											

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Table 2. Copper-based pesticide management during 2019 of selected orchards in the O'Higgins Region, central Chile

Orchard		Plum			Kiwi			Cherry		
		P1	P2	P3	K1	K2	K3	C1	C2	C3
Cupric pesticide		Nordox Super 75 WG (Cu_2O)								
Formulation		Cu_2O (86%)[a] + Coformulant (12%)								
Application	1st	April 22	April 22	April 22	April 4	April 5	April 5	April 26	April 29	April 26
	2nd	May 3	May 3	May 3	May 14	May 13	May 22	May 16	May 16	May 16
	3rd	May 13	May 13	May 13	June 3	June 6	June 4	June 17	June 17	June 17
	4th	August 8	August 8	August 8	July 5	July 4	July 12	July 23	July 24	July 24
Dose (g 100/L)[b]		200	200	200	130	130	130	200	200	200
Application form		turbo sprayer						nebulizer		

[a]Equivalent to 75% Cu; [b]Referred to wetting of 1 500 L of water/ha for plum and cherry orchards and of 1 000 L of water/ha for kiwi orchards

chads. At the time of the soil and litter samplings, 6.8, 2.0 and 4.5 kg Cu/ha were applied in the plum, kiwi and cherry orchards, respectively. Nordox® Super 75 WG pesticide (Cu_2O) was sprayed in these orchards.

Soil and litter sampling. Litter and soil samples were collected in late fall 2019 when at least two doses of Cu-based pesticides were applied to fruit trees. Specifically, soil and litter of plum, table grape, cherry and kiwi orchards were sampled during the last week of May. Eighteen samples of soil and litter (9 from the row and 9 from the inter-row) were taken for each fruit-tree species. The leaf litter samples were collected using 0.5 × 0.5 m quadrats placed on the soil. Once all the litter had been completely removed from the quadrat, the soil sample was collected to a depth of 0.2 m using an Eijkelkamp® stainless-steel hand auger.

Physicochemical characterisation of soils. Each soil sample was passed through a 2-mm-nylon mesh sieve. An aliquot was stored at 4° C for microbiological determinations. The rest of the sample was dried at 40 °C until constant mass and then soil pH_{H_2O}, electric conductivity (EC), organic carbon (OC), total N, and available phosphorus (P) and potassium (K) were determined following Sadzawka et al. (2006). The total and soluble soil Cu were determined according to Zagal and Sadzawka (2007) and Stuckey et al. (2008), respectively. The soil texture was determined by Bouyoucos' methodology (Sandoval et al. 2011). Soil standard reference material of the Wageningen University (ISE 859) was added through the entire soil and leaf litter analysis process for the quality control (QC) and quality assurance (QA) requirements. The Cu values of the soil standard reference material were within 100 ± 20% of the certified values. Duplicates were performed on every 10th sample, to assure the quality of analyses. Blanks were always below detection limits. The limits of detection and the limit of quantification for Cu in soils were 0.009 and 0.011 mg/kg, respectively. Linearity was verified for the range of 39.6–54.5 mg/kg (R^2 = 0.99).

Leaf litter total Cu determination. The leaf litter samples were oven-dried at 65 °C for 48 h in a Binder forced-convection drying oven and then ground using a food processor with stainless steel blades (model TH-9010V, Thomas, Neunkirchen, Germany). Total Cu was determined by atomic absorption spectrometry according to Sadzawka et al. (2007). A *Solanum melongena* L. standard material (IPE 951) from Wageningen University was used for the QC and QA requirements as described above.

Soil microbial colonisation and respiration. To quantify the soil microbial growth, a dilution and plating method was performed, following Pepper and Gerba (2009). The plates were incubated at 25 °C for 3 days to determine the colony-forming unit (CFU) per gram of soil.

The soil microbiological respiration was measured using the MicroResp™ bioassay following Campbell et al. (2003). Ten C-sources were dosed to each soil stored at 4 °C. The C-sources used were α-ketoglutaric acid (AKG), glucose (GLU), fructose (FRU), malic acid (MAL), citric acid (CIT), L-arabinose (ARA), N-acetyl glucosamine (NAG), oxalic acid (OXA), L-arginine (ARG) and cysteine (CYS). The samples were dosed with water (WAT) to measure their basal

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respiration. After dosing, samples were incubated at 25 °C for 4 h with 96-well detection microplates, which had a pre-measured absorbance of λ = 570 nm (Biobase EL10 microplate spectrophotometer, Zhangqiu, China). Following the incubation, the detection microplates were read to estimate the soil microbial CO_2 emissions. Also, the average well colour development (AWCD) was calculated (the mean amount of CO_2 respired for the MicroResp test).

Statistical analyses. ANOVA and Tukey's test were applied – when the variables met normality and homoscedasticity assumptions – in order to determine the effect of the factors (species and microsite). When these assumptions were not met, log and root transformations were performed. A Kruskal-Wallis' test, was performed in cases where the assumptions were ultimately not met. Principal component analysis (PCA) was performed on the entire data set and for each species in order to explore the relationships between the physicochemical and biological soil properties. Finally, a PERMANOVA was performed to assess the multivariate effects of factors on all physicochemical and biological soil properties. Analyses were done using R software, version 3.5.1 (https://cran.r-project.org/).

RESULTS AND DISCUSSION

General soil properties. The soil pH of orchards varied from slightly acidic (6.33) to slightly alkaline (7.93), with a mean value of 7.2 (Table 3); most values were higher than those indicated by Luzio (2010) for the area (6.5). Significant differences were found (F = 68.84, P < 0.001), where kiwi had the highest (7.54; Table 4) and cherry the lowest (6.65; Table 5) soil pH values. Only table grape showed significant pH differences (F = –2.90, P = 0.011) between the row (7.3) and interrow (7.1) soils (Table 6). The mean OC value was 1.47% (Table 3), with the statistically highest (F = –4.63, P = 0.005) being for kiwi soil (1.68%) and the lowest for the table grape soil (1.28%); most values were below the OC value indicated by Luzio (2010) for the area (2%). No significant differences in OC values were found between microsites. The mean EC value was 1.14 dS/m (Table 3), considered non-saline soil (Gartley 2011). The plum orchard soil showed a statistically higher EC (1.67 dS/m; Table 7) value than the other three orchards (Tables 4–6). No significant differences were found for EC between the microsites.

Soil and leaf litter copper levels. The mean total soil Cu level was 225 mg/kg (range of 131–432 mg/kg) (Table 8). This value is higher than the soil threshold value (100 mg/kg) and the guideline value (150 mg/kg) proposed by the Finnish Government (MEF 2007). The mean total soil Cu values per orchard type were 243, 233, 228 and 196 mg/kg for plum, cherry, table grape and kiwi, respectively. Lower total soil Cu levels might be expected in kiwi orchards compared to plum and cherry orchards due to the lower Cu_2O doses applied. Surprisingly, total soil Cu levels in table grape orchards, where cupric pesticides were not applied, were not significantly different from the Cu levels in the soils of the cherry and plum orchards. This might be explained by the fact that selected table grape orchards used to be citrus orchards, in which Cu-based pesticides were applied.

The soluble soil Cu levels in all orchards were less than 0.01% of the total soil Cu levels, indicating low availability and thus toxicity (Ginocchio et al. 2002). Specifically, the soluble soil Cu concentrations were 0.20 mg/kg in cherry orchards and 0.14 mg/kg in plum, kiwi and table grape orchards, being statistically different (F = 3.21, P = 0.029) among the cherry and table grape soils. Only soils of cherry orchards showed significant differences (F = 3.03, P = 0.012) for soluble Cu between the microsites, being higher in the interrow (Table 5). This result is the opposite of that of Mackie et al. (2013), who

Table 3. General soil physicochemical characteristics of selected orchards in the O'Higgins Region, central Chile

Soil property	Mean ± SEM	Range
pH_{H_2O}	7.17 ± 0.05	6.33–7.93
Electrical conductivity (dS/m)	1.14 ± 0.07	0.46–3.11
Organic carbon (%)	1.47 ± 0.07	0.88–3.21
Available N (mg/kg)	18.6 ± 0.96	6.37–43.0
Available P (mg/kg)	31.2 ± 2.54	2.33–88.1
Available K (mg/kg)	354 ± 19.7	128–801
Sand (%)	26.4 ± 0.79	15.0–44.0
Clay (%)	19.1 ± 0.27	14.0–25.0
Silt (%)	54.5 ± 0.79	38.0–68.0
Total Cu – soil (mg/kg)	225 ± 6.65	131–432
Soluble Cu – soil (mg/kg)	0.16 ± 0.01	0.06–0.42
Total Cu – Leaf litter (mg/kg)	681 ± 72.90	16.3–2 290
CuT_L/S	3.17 ± 0.35	0.07–13.4

SEM – standard error of the mean; CuT_L/S – litter Cu concentration to soil Cu concentration ratio

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Table 4. Soil physicochemical characteristics and copper (Cu) leaf litter concentration of selected kiwi orchards in the O'Higgins Region, central Chile

Soil property	Site	Mean ± SEM	Range
pH_{H_2O}	between row	7.54 ± 0.07	7.32–7.93
	row	7.54 ± 0.06	7.26–7.87
	total	7.54 ± 0.05	7.26–7.93
Electrical conductivity (dS/m)	between row	1.05 ± 0.11	0.66–1.72
	row	1.09 ± 0.07	0.84–1.49
	total	1.07 ± 0.06	0.66–1.72
Organic carbon (%)	between row	1.84 ± 0.36	1.10–3.21
	row	1.46 ± 0.16	0.97–1.95
	total	1.65 ± 0.21	0.97–3.21
Available N (mg/kg)	between row	14.4 ± 1.23	9.80–21.4
	row	13.7 ± 1.34	8.12–18.9
	total	14.0 ± 0.90	8.12–21.4
Available P (mg/kg)	between row	34.5 ± 6.23	7.33–64.8
	row	16.1 ± 6.95	2.41–69.4
	total	25.3 ± 5.05	2.41–69.4
Available K (mg/kg)*	between row	354 ± 43.3	154–623
	row	158 ± 11.7	135–244
	total	256 ± 32.2	154–623
Total Cu – soil (mg/kg)	between row	176 ± 14.8	134–67.0
	row	215 ± 16.0	135–283
	total	196 ± 11.6	134–283
Soluble Cu – soil (mg/kg)	between row	0.14 ± 0.03	0.08–0.31
	row	0.15 ± 0.03	0.06–0.32
	total	0.14 ± 0.02	0.06–0.32
Sand (%)	between row	26.7 ± 1.69	20.0–33.0
	row	26.7 ± 2.20	15.0–36.0
	total	26.7 ± 1.35	15.0–36.0
Clay (%)	between row	18.6 ± 0.53	16.0–21.0
	row	18.9 ± 0.82	15.0–21.0
	total	18.7 ± 0.48	16.0–21.0
Silt (%)	between row	54.8 ± 1.70	48.0–62.0
	row	54.4 ± 1.94	45.0–64.0
	total	54.6 ± 1.25	48.0–64.0
Total Cu – litter (mg/kg)*	between row	493 ± 76.9	293–1 035
	row	268 ± 28.3	154–416
	total	381 ± 48.2	154–1 035
Litter Cu:total soil Cu[a]*	between row	2.98 ± 0.51	1.23–5.69
	row	1.28 ± 0.15	0.77–2.35
	total	2.13 ± 0.33	0.77–5.69

*significant differences at $P \leq 0.05$ among microsite; SEM – standard error of the mean

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Table 5. Soil physicochemical characteristics and copper (Cu) litter concentration of selected cherry orchards in the Libertador Bernardo O'Higgins Region, central Chile

Soil property	Site	Mean ± SEM	Range
pH_{H_2O}	between row	6.73 ± 0.06	6.40–6.91
	row	6.56 ± 0.05	6.33–6.82
	total	6.65 ± 0.04	6.33–6.91
Electrical conductivity (dS/m)	between row	0.74 ± 0.10	0.48–1.40
	row	1.13 ± 0.17	0.63–2.37
	total	0.94 ± 0.11	0.48–2.37
Organic carbon (%)	between row	1.39 ± 0.16	0.96–1.91
	row	1.54 ± 0.07	1.37–1.71
	total	1.46 ± 0.09	0.96–1.91
Available N (mg/kg)	between row	24.2 ± 1.83	18.6–34.2
	row	19.8 ± 2.64	12.2–38.9
	total	22.0 ± 1.65	12.2–38.9
Available P (mg/kg)	between row	49.7 ± 6.40	25.5–79.9
	row	58.9 ± 5.39	39.3–88.1
	total	54.3 ± 4.21	25.5–88.1
Available K (mg/kg)*	between row	615 ± 45.2	345–801
	row	484 ± 28.4	301–577
	total	550 ± 30.4	301–801
Total Cu – soil (mg/kg)	between row	233 ± 18.2	144–334
	row	234 ± 13.6	157–296
	total	233 ± 11.0	144–334
Soluble Cu – soil (mg/kg)*	between row	0.25 ± 0.03	0.11–0.42
	row	0.15 ± 0.01	0.09–0.20
	total	0.20 ± 0.02	0.09–0.42
Sand (%)[a]	between row	24.0 ± 1.35	18.0–31.0
	row	35.2 ± 2.05	25.0–44.0
	total	29.6 ± 1.81	18.0–44.0
Clay (%)	between row	19.3 ± 0.78	15.0–23.0
	row	19.6 ± 0.41	18.0–21.0
	total	19.1 ± 0.44	15.0–23.0
Silt (%)[a]	between row	56.8 ± 1.14	53.0–62.0
	row	45.1 ± 1.95	38.0–54.0
	total	50.9 ± 1.79	38.0–62.0
Total Cu – leaf litter (mg/kg)*	between row	1 305 ± 69.5	961–1 613
	row	1 861 ± 124	1 325–2 290
	total	1 583 ± 96.4	961–2 290
Litter Cu:total soil Cu[a]	between row	5.85 ± 0.49	3.97–8.26
	row	8.37 ± 0.95	4.48–13.4
	total	7.11 ± 0.60	3.97–13.4

*significant differences at $P \leq 0.05$ among microsite; SEM – standard error of the mean

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Table 6. Soil physicochemical characteristics and copper (Cu) leaf litter concentration of selected table grape orchards in the O'Higgins Region, central Chile

Soil property	Site	Mean ± SEM	Range
	between row	7.05 ± 0.06	6.73–7.30
pH_{H_2O}*	row	7.34 ± 0.08	7.06–7.77
	total	7.19 ± 0.06	6.73–7.77
	between row	1.12 ± 0.25	0.53–2.91
Electrical conductivity (dS/m)	row	0.63 ± 0.04	0.46–0.80
	total	0.88 ± 0.14	0.46–2.91
	between row	1.45 ± 0.10	1.18–1.72
Organic carbon (%)	row	1.14 ± 0.15	0.88–1.67
	total	1.29 ± 0.11	0.88–1.67
	between row	13.7 ± 3.18	6.37–38.0
Available N (mg/kg)	row	14.4 ± 0.48	11.60–15.8
	total	14.1 ± 1.56	6.37–38.0
	between row	25.5 ± 2.58	16.3–39.3
Available P (mg/kg)*	row	12.8 ± 2.14	4.33–24.2
	total	19.1 ± 2.24	4.33–39.3
	between row	375 ± 15.7	301–451
Available K (mg/kg)*	row	211 ± 22.8	128–335
	total	293 ± 24.0	128–451
	between row	220 ± 11.7	179–290
Total Cu – soil (mg/kg)	row	236 ± 10.9	196–290
	total	228 ± 8.01	179–290
	between row	0.15 ± 0.02	0.09–0.24
Soluble Cu – soil (mg/kg)	row	0.12 ± 0.01	0.07–0.18
	total	0.14 ± 0.01	0.07–0.24
	between row	24.7 ± 2.17	16.0–37.0
Sand (%)	row	20.3 ± 1.49	15.0–27.0
	total	22.5 ± 1.38	15.0–37.0
	between row	17.2 ± 0.66	14.0–20.0
Clay (%)	row	18.1 ± 0.59	17.0–22.0
	total	17.7 ± 0.44	14.0–22.0
	between row	58.1 ± 2.14	48.0–66.0
Silt (%)	row	61.4 ± 1.42	56.0–68.0
	total	59.8 ± 1.31	48.0–68.0
	between row	42.3 ± 4.30	29.0–61.2
Total Cu – leaf litter (mg/kg)	row	103 ± 43.0	16.3–409
	total	72.4 ± 22.2	16.3–409
	between row	0.20 ± 0.03	0.10–0.33
Litter Cu:total soil Cu	row	0.41 ± 0.15	0.07–1.41
	total	0.31 ± 0.08	0.07–1.41

*significant differences at $P \leq 0.05$ among microsite; SEM – standard error of the mean

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Table 7. Soil physicochemical characteristics and copper (Cu) litter concentration of selected plum orchards in the O'Higgins Region, central Chile

Soil property	Site	Mean ± SEM	Range
	between row	7.23 ± 0.08	6.90–7.57
pH_{H_2O}	row	7.34 ± 0.05	7.06–7.50
	total	7.29 ± 0.05	6.90–7.57
	between row	1.76 ± 0.21	0.93–3.11
Electrical conductivity (dS/m)	row	1.58 ± 0.16	1.01–2.32
	total	1.67 ± 0.13	0.93–3.11
	between row	1.51 ± 0.09	1.27–1.82
Organic carbon (%)	row	1.42 ± 0.10	1.07–1.65
	total	1.29 ± 0.11	1.07–1.82
	between row	21.6 ± 1.83	12.7–31.2
Available N (mg/kg)	row	26.8 ± 3.71	13.6–43.0
	total	24.2 ± 2.11	12.7–43.0
	between row	23.7 ± 5.21	6.50–50.3
Available P (mg/kg)	row	28.6 ± 6.18	2.33–60.5
	total	26.1 ± 3.97	2.33–60.5
	between row	285 ± 37.3	129–430
Available K (mg/kg)	row	346 ± 45.0	140–499
	total	316 ± 29.3	129–499
	between row	219 ± 24.4	131–356
Total Cu – soil (mg/kg)	row	267 ± 26.9	148–432
	total	243 ± 18.6	131–432
	between row	0.13 ± 0.02	0.07–0.20
Soluble Cu – soil (mg/kg)	row	0.15 ± 0.03	0.07–0.38
	total	0.14 ± 0.02	0.07–0.38
	between row	28.3 ± 2.03	19.0–37.0
Sand (%)	row	25.4 ± 1.99	17.0–34.0
	total	26.9 ± 1.42	17.0–37.0
	between row	20.4 ± 0.84	15.0–23.0
Clay (%)	row	20.6 ± 0.93	16.0–25.0
	total	20.5 ± 0.61	15.0–25.0
	between row	51.3 ± 1.86	45.0–61.0
Silt (%)	row	54.0 ± 1.34	48.0–60.0
	total	52.7 ± 1.16	45.0–61.0
	between row	681 ± 35.5	505–866
Total Cu – leaf litter (mg/kg)	row	694 ± 76.8	140–908
	total	687 ± 41.1	140–908
	between row	3.51 ± 0.48	1.58–5.66
Litter Cu:total soil Cu	row	2.80 ± 0.45	0.57–5.45
	total	3.16 ± 0.33	0.57–5.66

SEM – standard error of the mean

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Table 8. Copper (Cu) concentrations in study soils and leaf litter of selected orchards in the O'Higgins Region, central Chile

	Plum	Kiwi	Table grape	Cherry
	mean ± SEM			
Total Cu-soil (mg/kg)	243 ± 18.6	196 ± 11.6	228 ± 8.01	233 ± 11.0
Soluble Cu-soil (mg/kg)	0.14 ± 0.02	0.14 ± 0.02	0.14 ± 0.01	0.20 ± 0.02
Total Cu-leaf litter (mg/kg)	687 ± 41.1	381 ± 48.2	72.4 ± 22.2	1 583 ± 96.4
Litter Cu:total soil Cu	3.16 ± 0.33	2.13 ± 0.33	0.31 ± 0.08	7.11 ± 0.60

SEM – standard error of the mean

showed significant differences in soluble Cu in vineyard soil. Komárek et al. (2008), however, reported no significant differences between the microsites in a vineyard, similarly to the kiwi, plum and table grape in the present study. In the kiwi orchard, we expected this behaviour as being in a pergola trellis system, it loses the microsite effect, as it has complete coverage of the soil through the canopy. The same occurs with table grape orchards that also do not apply Cu-based pesticides. Nevertheless, due to the tree training system of plum orchards, we expected differences between the row and the interrow.

The leaf litter Cu levels of the cherry, plum and kiwi orchards were statistically higher (F = 213.02, P < 0.001) than the table grape leaf litter (Table 8), as expected, as no Cu-based pesticides were applied to the table grape orchard. Furthermore, the cherry (1 583 mg/kg) and plum (687 mg/kg) leaf litter showed higher Cu values than the kiwi (380 mg/kg) leaf litter due to the higher doses of Cu-based pesticide applied. With the exception of table grape orchards, Cu levels in leaf litters were much higher than total soil Cu levels (Table 8). Specifically, the CuT_L/S ratio values were 0.3, 2.1, 3.2 and 7.1 for table grape, kiwi, plum and cherry, respectively. A significant difference (F = 122.25, P < 0.001) were found between table grapes and the other species in terms of the CuT_L/S ratio. This result supports the hypothesis proposed by Schoffer et al. (2020), that the leaf litter exerts a protective role against the incorporation of Cu into the soil of orchards where this element is applied as a Cu-based pesticide. Lepp et al. (1984) reported Cu litter levels of 884 and 320 mg/kg in coffee orchards being of 24 and 14 years old, respectively, which our finding also supports. These Cu levels correspond to approximately twice the total soil Cu concentration also reported by these researchers.

Soil microbial colonisation and carbon-induced soil microbial respiration. There was no significant difference between the fruit-tree species (F = 2.37, P = 0.08) and the soil microsites (F = 0.78, P = 0.38) in terms of soil microbial colonisation. This may be the result of the high variability in the data. In this context, all the orchards are drip irrigated, which causes the soils to be constantly mixing. Hence, the changes in the soil are very noticeable under the dripper, but not in the rest of the soil (Osorio and Césped 2000). In the kiwi orchards (Figure 1A), although slightly higher use of C sources in the interrow, no effect of the microsite was observed for any C source, nor for WAT and AWCD. In the table grape orchards (Figure 1B), higher microbial activity was observed in the row than in the interrow for ARG (F = –2.46, P = 0.027), NAG (F = –2.93, P = 0.010) and AWCD (F = –2.256, P = 0.023). As mentioned above, Cu was not applied in the table grape orchards, and so the concentrations of total and soluble Cu in the soil do not differ between the rows and inter rows. Similarly, in the plum orchards (Figure 1C), greater activity in the rows in terms of AKG (F = –2.37, P = 0.033), NAG (F = –2.28, P = 0.044) and AWCD (F = –2.67, P = 0.017) was found. None of the parameters related to Cu, either in the soil or litter, differed between the soils of the rows and inter rows (Table 7). In the cherry orchards (Figure 1D) the microsite effect affected most sources of C, as well as WAT and AWCD. In this case OXA (F = 4.68, P < 0.001), CYS (F = 6.44, P < 0.001), ARG (F = 5.81, P < 0.001), GLU (F = 3.42, P = 0.004), NAG (F = 3.78, P = 0.002), FRU (F = 2.86, P = 0.016), WAT (F = 3.41, P = 0.004) and AWCD (F = 3.08, P = 0.007) showed greater activity in the interrow than the rows. Even though the available Cu fraction was low, it was significantly higher in the inter rows in the cherry orchards (Table 5), where a decrease in soil microbial activity might be expected; nevertheless, the opposite was the case. According to Aponte et al. (2021), differences between the rows

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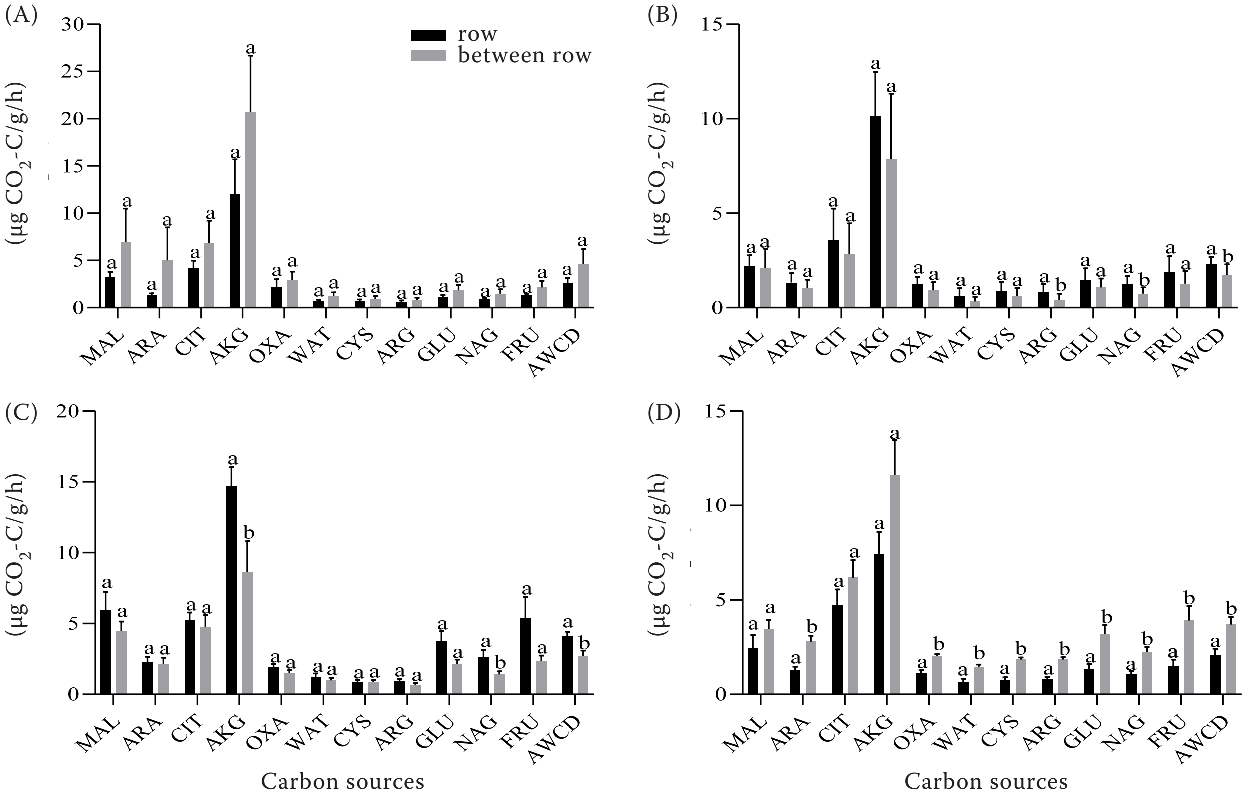

Figure 1. Carbon (C)-induced soil microbial activity of soil samples taken in the row and in the interrow in (A) kiwi; (B) table grape; (C) plum, and (D) cherry orchards of selected orchards of O'Higgins Region, central Chile. Different letters denote significant differences ($P \leq 0.05$) among microsites. MAL – malic acid; ARA – L-arabinose; CIT – citric acid; AKG – α-ketoglutaric acid; OXA – oxalic acid; WAT – microbial basal respiration; CYS – cysteine; ARG – L-arginine; GLU – glucose; NAG – N-acetyl glucosamine; FRU – fructose; AWCD – average well color development

and inter rows can be due to a shift in the utilisation of the C substrate by microbial communities that may be associated with soil Cu contamination. However, we found no relation to the soil total Cu, soil soluble Cu, litter Cu content or CuT_L/S ratio values. Therefore, it is possible there other variables are responsible for the shift in C-source utilisation by the soil microbial community that are not Cu related, such as soil tilling practices (Mackie et al. 2013) or dissolved organic matter (DOM), which is available as a source of energy and nutrients to the soil microorganisms, accelerating the success of the soil microbial community after a period of stress caused by Cu toxicity (Brandt et al. 2010). The AWCD values were highest in the kiwi orchard soils (3.59 μg CO_2-C/g/h), followed by plum (3.40 μg CO_2-C/g/h) and cherry (2.89 μg CO_2-C/g/h). The lowest AWCD value was observed in the table grape soils (2.03 μg CO_2-C/g/h). Significant differences in the AWCD ($F = 3.44$, $P < 0.022$) were only found between the plum and table grape soils. As explained above, the plum orchard soils had high total Cu concentrations and AWCD values. In this context, PCA showed that AWCD explained the highest variability proportion ($r = 0.87$; Table 9) in the principal component PC1 explained 29% of the variability. In this line, almost all C sources represented the positive highest loadings (after AWCD) in PC1 compared to CFU and soil physicochemical properties (Table 9), which showed the highest eigenvectors in the PC2 (19%) for pH ($r = -0.79$; Table 9), P ($r = 0.62$; Table 9), K1 ($r = 0.67$; Table 9), CuT_L ($r = 0.72$; Table 9) and CuT_L/S ($r = 0.65$; Table 9). These results agree with Aponte et al. (2021) who reported that most C sources represented the highest positive loadings in the PC1 (51.2%) compared to soil physicochemical properties, which showed positive loadings in the PC2 (14.4%) in metal contaminated soils near to a Cu smelter. Additionally, Aponte et al. (2021) found positive relationships between pH and some C sources and

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Table 9. Results of principal component analysis for all orchards data

Principal components	7.61	4.83	2.98
Eigenvalues	29.3	18.6	11.5
Variance (%)	29.3	47.8	59.3
Cummulative variance (%)	7.61	4.83	2.98
Eigenvectors			
pH_{H_2O}	–0.05	–0.79	–0.09
EC	0.16	0.14	0.08
TOC	0.27	0.14	0.08
N	0.37	0.44	–0.09
P	0.33	0.62	0.30
K	0.48	0.67	0.08
CuT_S	0.26	0.15	–0.45
Cu_S	0.47	0.33	–0.30
Sand	0.23	0.42	0.50
Clay	0.21	0.03	0.10
Silt	–0.31	–0.43	–0.54
CFU	–0.07	–0.33	0.34
MAL	0.70	–0.51	0.32
ARA	0.68	–0.49	0.39
CIT	0.73	–0.41	0.36
AKG	0.67	–0.55	0.15
OXA	0.75	–0.42	0.09
WAT	0.76	–0.21	0.15
CYS	0.55	0.32	–0.45
ARG	0.74	0.21	–0.29
GLU	0.78	0.12	–0.50
NAG	0.81	0.08	–0.45
FRU	0.65	0.13	–0.54
AWCD	0.87	–0.45	0.12
CuT_L	0.32	0.72	0.40
CuT_L/S	0.26	0.65	0.51

EC – electrical conductivity; TOC – organic carbon; N – available nitrogen; P – available phosphorous; K – available potassium; CuT_S – total soil copper; Cu_S – soil soluble copper; CFU – colony-forming unit; MAL – malic acid; ARA – L-arabinose; CIT – citric acid; AKG – α-ketoglutaric acid; OXA – oxalic acid; WAT – microbial basal respiration; CYS – cysteine; ARG – L-arginine; GLU – glucose; NAG – N-acetyl glucosamine; FRU – fructose; AWCD – average well color development; CuT_L – litter total copper; CuT_L/S – litter Cu concentration to soil Cu concentration ratio

enzyme activities when decreasing total and available Cu contents, which was expected since organic matter can increase soil pH with further decreasing of metal availability (Xian and In Shokohifard 1989), as probably occurred in soils of the present study. On the other hand, Donoso et al. (2016) stated that AWCD is not a good bioindicator for Cu soil toxicity; however, these authors studied soils with 20 ppm of Cu and used Biolog Ecoplates™ to assess the AWCD. Therefore, a protective role of organic matter against Cu that litter exert should be evaluated since organic substances can mitigate the metal effects (Medina et al. 2017). Thus, the evaluation of litter protective effect in Cu contaminated soils can be performed by using the better indicator for Cu toxicity such as earthworms (Delgadillo et al. 2017) and some soil enzyme activities (Aponte et al. 2020).

The PCA's ellipsoids showed discrimination between the orchards (Figure 2A) based on the two first principal components, which explained 48% of the overall variability, resulting in the following order: kiwi = table grape ≠ plum ≠ cherry. Nevertheless, samples showed a considerable dispersion out of the 95% confidence ellipsoids, with important overlapping between soil samples when classifying by orchard species and the presence of Cu-based pesticides (Figure 2). This reflects the high data variability and potential low effect of factors, especially on physicochemical properties that showed the lowest contribution compared to biological properties in the PCA (Figure 2 and Table 9). In this sense, PERMANOVA showed that factors did not exert multivariate effects on soil properties ($P > 0.05$). On the other hand, the PCA and PERMANOVA did not show any differences by soil microsite (Figure 3). It is important to note that PC1 and PC2 only explained 48% of the total variance (Figure 2), but with PC3, the explanation of the variance increased to 59% (Table 9). Thus, low univariate and multivariate effects of factors on soil properties can be associated with the protective role of leaves on soil; however, soil biological properties (except for CFU) explained the most variability proportion.

In conclusion, as a result of the large annual amount (4.0 to 9.2 kg/ha/year) of Cu-based pesticide applied to selected orchards, we can conclude that:

- copper has been accumulated mainly in the litter, which exerted a protective role regarding copper incorporation into the soil;
- although soil has been enriched in copper, its bioavailability and ecotoxicity were relatively low; and

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(A)

(B)

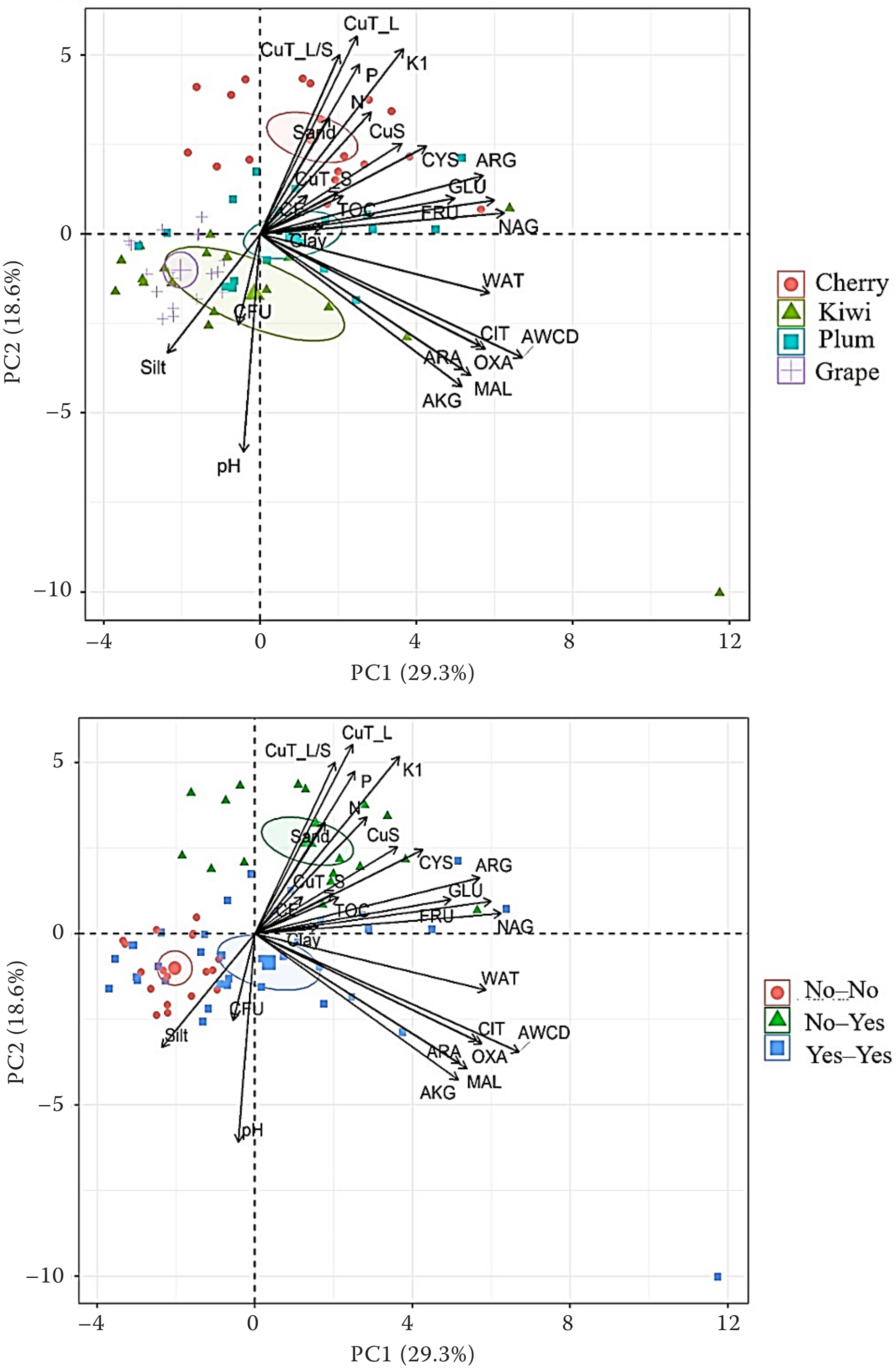

Figure 2. Principal component analysis labeled by orchard species (A) and by ridge presence/absence and the use or not of copper (Cu)-based pesticides. EC – electrical conductivity; TOC – total organic carbon; N – available nitrogen; P – available phosphorous; K – available potassium; CuT_S – total copper; CuS – soluble copper; CFU – colony-forming units; MAL – malic acid; ARA – L-arabinose; CIT – citric acid; AKG – α-ketoglutaric acid; OXA – oxalic acid; WAT – microbial basal respiration; CYS – cysteine; ARG – L-arginine; GLU – glucose; NAG – N-acetyl glucosamine; FRU – fructose; AWCD – average well color development; CuT_L – litter total copper; CuT_L/S – litter Cu concentration to soil Cu concentration ratio; No–No – no ridge and no pesticide use; No–Yes – no ridge and pesticide use; Yes–Yes – ridge and pesticide use

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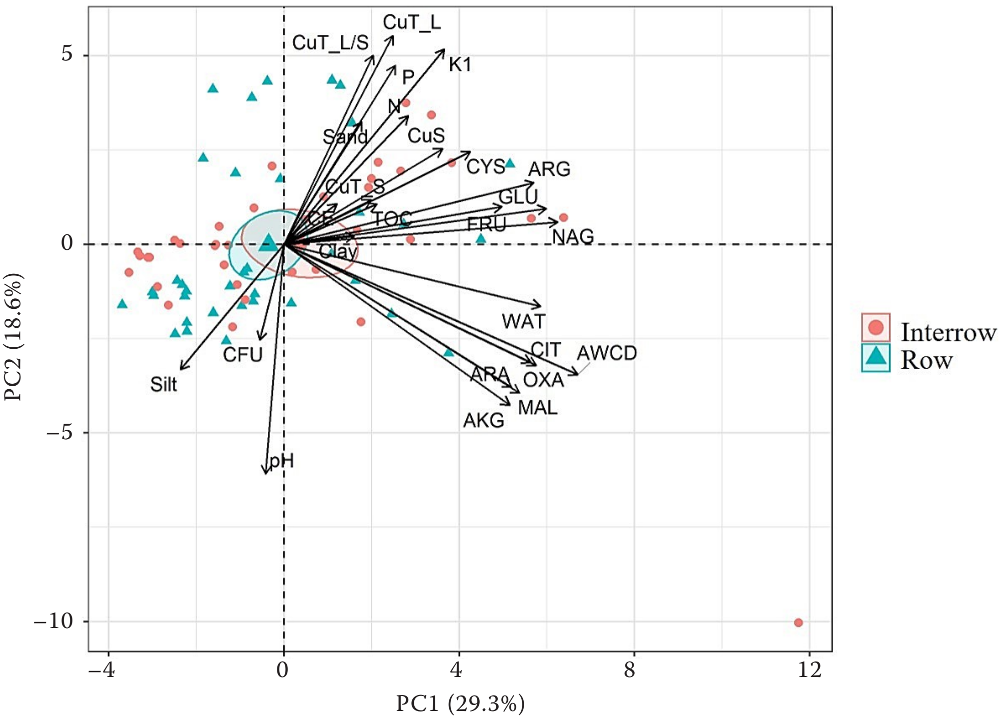

Figure 3. Principal component analysis labeled by soil sampling site. EC – electrical conductivity; TOC – total organic carbon; N – available nitrogen; P – available phosphorous; K – available potassium; CuT_S – total soil copper; CuS – soil soluble copper; CuT_L – litter total copper; CuT_L/S – litter Cu concentration to soil Cu concentration ratio; CFU – colony-forming units; MAL – malic acid; ARA – L-arabinose; CIT – citric acid; AKG – α-ketoglutaric acid; OXA – oxalic acid; WAT – microbial basal respiration; CYS – cysteine; ARG – L-arginine; GLU – glucose; NAG – N-acetyl glucosamine; FRU – fructose; AWCD – average well color development

– it is important to study the adsorption capacity and degradability of Cu-enriched litter since this could be a way of incorporating the element into the soil.

To date, this is the first work that describes the Cu content in soils and leaf litter of orchards in central Chile. It is also one of the few studies, worldwide, carried out on fruit trees other than vineyards. The study has highlighted the importance of litter as a protective layer against the incorporation of Cu in the soil.

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