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Fourteen years of compost application in a commercial nectarine orchard: effect on microelements and potential harmful elements in soil and plants

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ABSTRACT

The objective of this experiment was to valuate, after 14 years, the impact of annual compost applications on micronutrient and potentially toxic trace elements on nectarine tree uptake and soil fertility. The study was performed in the Po valley, Italy, on the variety Stark RedGold (grafted on GF677). Since orchard planting, the following treatments were applied, in a randomized complete block design, with four replicates: 1. unfertilized control; 2. mineral fertilization (N was supplied as NO_3NH_4 at 70–130 kg ha^{-1} $year^{-1}$); 3. compost at 5 t DW ha^{-1} $year^{-1}$; 4. compost at 10 t DW ha^{-1} $year^{-1}$. The actual rate of application was 12.5 (LOW) and 25 (HIGH) t ha^{-1}, since compost was concentrated in the tree row. Compost was made from domestic organic wastes mixed with pruning material from urban ornamental trees and garden management and stabilized for 3 months.The supply of compost HIGH induced an enrichment of soil total Cu, Zn and Cd, and a decrease of Fe and Co concentration; with values always below the European threshold limits for heavy metals in the soil. In addition, compost (at both rates) increased availability (DTPA-extractable) of Fe, Mn and Zn, Cd, Ni, and Pb in the top soil (0–0.15 m).Total micronutrient and trace element tree content was not affected by fertilization treatments; however, the recycled fraction returned to the soil at the end of the season through abscised leaves and pruned wood of Cu, Fe, Mn and Zn was increased by mineral fertilization; Fe and Zn also by compost HIGH. Our data show that the introduction of compost at both 12.5 and 25 t ha^{-1} $year^{-1}$ in the row did not increase the risk of pollution related to potentially toxic trace elements and at the same time increased the bioavailability of Fe, Mn and Zn.

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1. Introduction

The use of organic amendments in orchards increased over the last decades (Ciavatta et al., in press), since they showed an excellent fertilization effect (Cline et al., 2011). At the same time, the recycling of agro-food related wastes increased (Nayak and Bhushan, 2019) with the result of the large availability of relatively cheap organic amendments that provide positive effects on soil chemical, physical and biological properties (Komatsuzaki and Ohta, 2007). The frequent applications of compost as a replacement of mineral fertilizers can have potential detrimental effect in term of unbalanced micronutrient supply in soil and plant tissue, and increase soil concentration of potentially harmful trace elements, such as heavy metals, that may be present in high concentration in the organic wastes. Potentially harmful trace elements such as aluminium (Al), cobalt (Co), cadmium (Cd), chromium (Cr), mercury (Hg), nickel (Ni), lead (Pb) can be toxic for tree, accumulate in soil, contaminate water and enter the human food chain (Tervahauta et al., 2014; Kelepertzis, 2014; Oliver and Gregory, 2015), with more evident detrimental effects in the case of protracted application (Tai et al., 2016). Another important factor to consider, when organic amendments are used routinely, is related to the effect that organic matter addition may have on soil mineral elements availability. The effective bioavailability of soil minerals may be reduced by dissolved organic matter, that is released as organic amendments decompose, forming complexes that sequester nutrients, reducing their availability for plants (Wright et al., 2007).

Essential micronutrients, such as iron (Fe), manganese (Mn), zinc (Zn), coppre (Cu), ~~ande coron~~ (B) are involved in all metabolic and cellular functions (Hänsch and Mendel, 2009). However, when present in elevated concentrations, their redox properties may lead to the formation of reactive oxygen species that can have detrimental consequences for cells.

Little information is available on the long-term effect of compost application on essential micronutrient and trace element accumulation and bioavailability in orchard soil and tree uptake. Consequently, the aim of the present experiment was to evaluate the impact of continuous applications (14 years) of compost on: 1) supply and availability of micronutrients for nectarine tree needs, 2) potentially harmful trace element bioavailability, and accumulation in soil and in fruit.

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2. Materials and methods

2.1. Plant material and treatments

This trial was performed in the south-east of the Po valley (44°27′ N; 12°13′ E). The detailed characteristics of the silt loam (sand 6.7%, silt 67%, clay 26.3%) Calcaric Cambisol (FAO, 2010) soil are described in Baldi et al. (2018) and include total N of 0.95‰, and organic matter of 1.7% (Table 1). The area of the experiment is characterized by a temperate climate with an annual precipitation of 670 mm (in the period 1961–2015) and an average temperature of 13.5°C (Antolini et al., 2017). The experiment was performed on an orchard of Stark RedGold nectarine [*Prunus persica*, Batsch var. *nucipersica* (Bockh.) Schn.] grafted on GF677 (hybrid *Prunus persica* × *Prunus dulcis*) rootstock. The orchard was established in August 2001 with a planting layout of 5 m between the rows and 3.8 m between trees along the row. The trees were regularly watered during the vegetative season with a drip irrigation system according to the evapotranspiration rate measured every day. Once a month, from May to September, the 2-m-wide tree row soil was tilled at 0.25 m-depth, for weed control. Spontaneous grass on the alleys was managed by mowing it 3 times a year. Phytosanitary treatments were done through the vegetative seasons according to Crop Management Guideline of the region Emilia-Romagna (www.regione.emilia-romagna.it). They included Cu at a rate of approximately 2000–4000 g ha^{-1} yr^{-1}, Zn 800–1000 g ha^{-1} yr^{-1}, and Mn 200 g ha^{-1} yr^{-1}.

The following treatments were compared according to a randomized complete block design (four replicates):

1. unfertilized control (control);
2. mineral fertilization (mineral), the rate was established according to Integrated Crop Management Guideline of the region Emilia-Romagna (www.regione.emilia-romagna.it), and included nitrogen (N) applied every year at the rate of 70 kg ha^{-1}, split in May (60% of the total dose) and September (40%). In 2004, N application rate was increased to 120 kg ha^{-1} yr^{-1} and from 2006 to 130 kg ha^{-1} yr^{-1}; phosphorous (P) at 100 kg ha^{-1}, and potassium (K) at 200 kg ha^{-1}, were applied only at planting. Nitrogen was applied as ammonium nitrate (N = 35%), while P and K were applied as a binary fertilizer (P = 10%; K = 20%); no micronutrients were used in mineral fertilization.
3. compost at a rate of 5 t DW ha^{-1} $year^{-1}$ (compost LOW); corresponding to 120 kg N ha^{-1} yr^{-1}; split as described for mineral fertilization
4. compost at a rate of 10 t DW ha^{-1} $year^{-1}$ (compost HIGH); corresponding to 240 kg N ha^{-1} $year^{-1}$; split as described for mineral fertilization. Fertilizers (both mineral and organic) were applied since orchard plantation, on the 2-m-wide tree row on a surface of 4000 m^2 ha^{-1} and tilled into the soil to a depth of 0.25 m, to allow organic amendment to exert its beneficial effects of soil fertility and avoid possible gas-N emission. This means an actual application rate of 1.25 kg m^{-2} (12.5 t ha^{-1}) and 2.5 kg m^{-2} (25 t ha^{-1}) in the treated areas for compost low and high, respectively.

Each replication (plot) included 6 trees, with the 4 centrals used for data collection. Compost was obtained from the recycled organic fraction of municipal solid waste (50%) and urban, ornamental trees and garden management biomass (50%) stabilized for 3-months before application. The compost had on average: N 21.1 g kg^{-1} DW, organic carbon 234 g kg^{-1} DW, a C/N ratio of 10.2 and heavy metals as reported in Table 1. After pruning, wood was left into the ground and chopped.

Table 1
Selected chemical characteristics of soil at the beginning of the experiment and of the compost.

Test	Unit	Soil [a]	Compost [b]
pH		–	8.30 ± 1.15
Copper	mg kg^{-1} DW	24.8 ± 0.50	156 ± 29.9
Cadmium	mg kg^{-1} DW	<0.5	0.55 ± 0.04
Chromium $^{6+}$	mg kg^{-1} DW	35.5 ± 1.73	0.23 ± 0.18
Mercury	mg kg^{-1} DW	0.18 ± 0.04	0.22 ± 0.08
Nickel	mg kg^{-1} DW	64.3 ± 1.5	32.7 ± 7.57
Lead	mg kg^{-1} DW	33.5 ± 0.58	102 ± 1.53
Zinc	mg kg^{-1} DW	73.8 ± 0.96	339 ± 22.9

[a] Values of soil analysis represent the average of 4 replicates (blocks before compost supply) ± standard deviation.
[b] Values represent average of 3 compost analysis performed during the experiment ± standard deviation.

2.2. Soil collection and analysis

At the end of the commercial life of the orchard (December 2014), soil samples were collected with an auger at m 0–0.15, 0.16–0.25, 0.26–0.45 and 0.46–0.65 of depth in the row. Each sample was made of 4 sub-samples collected in the center of the distance between the two trees of the row and stored at 4°C. Before analysis, soil was sieved (2 mm) in order to remove all roots and visible plant residue, air-dried and used to evaluate soil total concentration of micronutrients: B, Cu, Fe, Mn, Zn and trace elements: Al, Cd, Co, Cr, Hg, Ni, and Pb. Soil was subjected to wet mineralization by treating 0.5 g of dry sample with 6 mL of hydrochloridric acid (37%), 2 mL of nitric acid (65%) and 2 mL of hydrogen peroxide (30%) in an Ethos TC microwave lab station (Milestone, Bergamo, Italy). Solutions were filtered (Whatman 42®) and the element concentration was determined by plasma spectrometer (ICP-OES; Ametek Spectro, Arcos, Kleve, Germany).

With the exception of B and Al, the DTPA-extractable (from here on called 'available') fraction was determined according to Lindsay and Norvell (1978) modified as follows: 10 g dry soil were shaken for 2 h at 60 cycles per min with 20 mL of a solution made with DTPA 1.97 g L^{-1}, triethanolamine 14.9 g L^{-1} and $CaCl_2$ 1.47 g L^{-1}, buffered to pH 7.3 with HCl (Leita and Petruzzelli, 2000). The suspension (soil + extraction solution) was filtered (Whatman 42®) and analysed as described for total nutrients.

Soil total and available mineral elements found at the end of orchard lifetime were calculated considering their concentration and the soil bulk density at the different depths and summing them up.

A suspension of 10 g of fresh sample and 25 mL of deionised water was stirred for 120 min at 25°C. After filtration, the pH was measured using a Micro TT 2022 (Crison, Spain) pH-meter.

The soil potential cation exchange capacity (CEC) was estimated using the ammonium acetate method (Sumner and Miller, 1996).

The value of the enrichment factor (EF) was calculated using the formula (Banat et al., 2005): EF = Cn/Bn

where Cn is the total concentration (mg kg^{-1}) of the examined element in the treated plots and Bn (background) is the total concentration of the examined element in the untreated control plots.

2.3. Plant analysis

In spring 2014, thinned fruits were collected, weighted and a representative sample was oven dried and milled (2 mm). In August 2014, on a representative sample of fruit collected at the main harvest, fresh and dry weight (DW) of flesh and kernel was recorded and samples were milled. In September 2014, one tree per plot (16 in total) was enclosed into a plastic net to collect abscised leaves that were dried and milled.

The same trees were harvested, divided in roots, trunk, branches (age > 2 year) and current year shoots (twigs) and weighted. A sub-sample for each organ was oven-dried, weighted and milled.

A sample (0.3 g) of thinned and mature fruits, abscised leaves, one-year-old twig, trunk, branches and roots was mineralized according

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to the US EPA Method 3052 (Kingston, 1988) in an Ethos TC microwave lab station (Milestone, Bergamo, Italy) and analysed for B, Cu, Fe, Mn, Zn, Al, Cd, Co, Cr, Hg, Ni, Pb by ICP-OES.

Mineral content in different parts of the plant (leaves, fruits, branches, trunk and roots) was calculated by multiplying each mineral concentration by DW of the specific organ. Total nutrient plant content was calculated as the sum of nutrient content in leaves, fruits, one-year-old twigs, skeleton (branches + trunk) and roots.

Total mineral elements recycled in the orchard was calculated as the sum of each nutrient content of abscised leaves, thinned fruits and pruned wood (mainly one-year-old twigs) multiplied by the number of plants per hectare (526). Total mineral elements removed yearly from the orchard was calculated as the sum of nutrient content in roots, trunk and branches divided by 13 (considering the first-year growth negligible), plus nutrient content in fruit (stone and flesh), all multiplied by the number of trees per hectare.

2.4. Statistical analysis

Soil data were analysed using the software SAS (SAS Institute Inc., Cary, North Carolina, USA), as in a factorial experimental design with treatment (4 levels: control, mineral, compost LOW and compost HIGH) and soil depth (4 levels: 0–0.15 m, 0.16–0.25 m, 0.26–0.45 m and 0.46–0.65 m) as factors. When analysis of variance showed a statistically significant ($P \leq 0.05$) effect of treatment, Student Newman-Keuls (SNK) test separated the means. On the other hand, when interaction between factors was significant, 2 times standard error of means (SEM) was used as the minimum difference between two means statistically different for $P \leq 0.05$.

Total and available mineral concentrations in soil were also analysed using the R statistical software, according to the discriminant canonical analysis (DCA).

The data of minerals in tree were analysed as in a complete randomized design with 4 treatments (control, mineral, compost LOW and compost HIGH) and 4 replications. When analysis of variance showed a statistically significant ($P \leq 0.05$) effect of treatment, Student Newman-Keuls (SNK) test separated the means.

Pearson correlation coefficient was employed to estimate the linear relationship between soil total and available nutrient concentration and total nutrient content in plants.

Table 2
Effect of fertilization treatment and depth on soil pH and CEC at the end of the experiment.

	pH H_2O	CEC ($cmol_+$ kg^{-1})
Treatment		
Control	8.42 a	12.5 bc
Mineral	8.42 a	12.2 c
Compost LOW	7.95 b	13.2 b
Compost HIGH	7.86 b	14.3 a
Significance	***	***
DEPTH (m)		
0–0.15	7.83 d	14.2 a
0.15–0.25	8.06 c	13.3 b
0.25–0.45	8.28 b	12.7 bc
0.45–0.65	8.47 a	12.0 c
Significance	***	***
*Treatment*depth*	*n.s.*	*n.s.*

n.s. ***: effect not significant or significant at $P \leq 0.001$, respectively. Within the same column, values followed by the same letter are not statistically different according to Student Neuman Keul test ($P \leq 0.05$). The effect of the main factors is reported.

3. Results

3.1. Soil

The interaction between treatment and depth was not significant for soil pH and CEC hence Table 2 reports the effect of the main factors only. Soil pH significantly decreased from values of 8.42 (control and mineral) to values of 7.95 and 7.86 as a consequence of the supply of compost at low and high rate respectively, despite compost having average pH of 8.30. Soil pH increased with soil depth reaching the highest value in the deepest layer (Table 2).

Soil CEC was higher in compost HIGH in comparison to all other treatments, compost LOW induced values higher than mineral, but similar to control (Table 2). Soil CEC significantly decreased with depth (Table 2).

Among micronutrients, total B and Mn soil concentrations in the tree row were not affected by fertilization treatment and soil depth (Table 3). Fertilization treatment and soil depth significantly interacted with total Cu and Zn (Table S1), that in general were increased by compost application and decreased by soil depth. On the contrary, total Fe was decreased by compost application at the high rate, and increased with soil depth. Among trace elements, Al, Cr, and Pb were not affected by fertilization treatment and soil depth, although Pb lev-

Table 3
Effect of fertilization treatment and depth on soil total micronutrient and potentially harmful trace element concentration (mg kg^{-1} DW) in the tree row.

	B	Cu	Fe	Mn	Zn	Al	Co	Cr	Cd	Ni	Pb
Treatment											
Control	30.3	33.8 b	21,214 a	584	72.5 b	27,540	11.1 a	61.9	0.236 b	46.1	25.8
Mineral	30.0	32.7 b	21,081 a	582	70.6 b	27,473	11.0 a	61.6	0.237 b	45.2	19.0
Compost LOW	31.2	39.7 a	20,780 ab	576	81.4 a	26,762	10.8 ab	61.5	0.240 ab	45.4	19.6
Compost HIGH	27.9	40.1 a	20,245 b	592	84.0 a	24,121	10.5 b	58.0	0.250 a	43.7	20.1
Significance	n.s.	***	*	n.s.	***	n.s.	**	n.s.	*	n.s.	n.s.
Depth (m)											
0–0.15	29.7	50.5 a	20,155 b	584	91.4 a	25,805	10.4 b	59.4	0.251 a	43.5 b	28.4
0.16–0.25	30.4	36.8 b	20,960 a	583	78.8 b	26,756	11.0 a	61.5	0.244 a	45.4 ab	19.6
0.26–0.45	29.7	31.4 c	21,091 a	584	70.9 c	26,777	11.0 a	61.3	0.242 a	45.5 ab	18.2
0.46–0.65	29.5	27.5 d	21,113 a	583	67.3 c	26,559	11.0 a	60.9	0.225 b	46.1 a	18.4
Significance	n.s.	***	*	n.s.	***	n.s.	**	n.s.	***	**	n.s.
trt*depth	n.s.	*	n.s.	n.s.	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

n.s. *, **, ***: effect not significant, or significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, respectively. Within the same column, values followed by the same letter are not statistically different according to Student Neuman Keul test ($P \leq 0.05$). The effect of the main factors only is reported.

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els were more than 40% higher in the topsoil compared to the lower strata (Table 3). Total soil Cd concentration was increased by compost HIGH compared to mineral and control and values were significantly higher in the top 0.45 m of the soil compared to the lowest strata (Table 3). Total soil Co decreased with application of compost HIGH, and was significantly lower in the top soil (0–0.15 m) than in deeper layers (Table 3). Soil total Ni, unaffected by fertilization, increased in the deepest soil layer (0.46–0.65 m). Mercury concentrations are not reported since the values were below the detection limit.

No significant effect of fertilization strategy on the EF was observed for B, Fe and Mn as well as all trace elements investigated (Table 4). Copper and Zn EF was enhanced by compost addition, no matter the rate, in comparison to mineral fertilization. Soil depth had no effect on EF of Fe and Mn, as well as Cd, Co, Ni and Pb. Boron, Cu, Zn, Al and Cr EF decreased with soil depth (Table 4). In particular, the EF of B was higher at 0–0.15 m in comparison to 0.16–0.25 m (Table 4). In the shallowest soil layer, the EF for Cu was higher than the values calculated at 0.26–0.65 m, but similar to those at 0.16–0.25 m. The highest EF values for Zn were calculated in the shallowest soil layers, not significant differences were observed between the other layers (Table 4). Enrichment factor of Al and Cr were higher at 0–0.15 m and 0.26–0.45 m of depth in comparison to 0.16–0.25 m (Table 4).

At 0–0.15 m and 0.16–0.25 m, available Cu concentration was significantly higher in compost LOW than other treatments; in the first soil layer mineral and control treatments showed a higher Cu availability than compost HIGH, while at 0.16–0.25 m, the reverse was observed (Fig. 1a). In the deepest soil layers, fertilization treatments decreased Cu availability in comparison to control. The interaction between fertilization treatment and depth was significant, in case of Fe, Mn, and Zn concentration (Fig. 1b, c, d). In detail, Fe concentration at 0–0.15 m and 0.16–0.25 m was higher in compost HIGH, followed by compost LOW; mineral and control showed the lowest values (Fig. 1b). At 0.26–0.45 m, control and compost LOW showed similar values of available Fe (18.8 and 18.1 respectively) higher than compost HIGH (17.5) and mineral fertilization (17.1). In the deeper soil layer, the application of compost HIGH enhanced values of available Fe (17.7), in comparison to all other treatments (16.1 for control and compost LOW and 16.3 for mineral; Fig. 1b). At 0–0.15 m, available Mn concentration was higher in compost LOW, followed by compost HIGH, mineral and control. At 0.16–0.25 m the application of compost HIGH induced the highest value followed by compost LOW, mineral and control (Fig. 1c). At 0.26–0.45 m, the highest values of Mn were recorded in control and compost LOW in comparison to mineral and compost HIGH. In the deepest soil layer, organic (no matter the rate) and mineral fertilization increased available Mn concentration in comparison to control (Fig. 1c). Soil available Zn, along the entire profile, was enhanced by the application of compost at the highest rate, followed by compost at low rate, control and mineral that showed the lowest values (Fig. 1d).

Available Cd concentration decreased with soil depth. In the top soil layers (0–0.25 m), the application of compost (both rates) increased Cd availability in comparison to mineral and control soil (Fig. 1e). On the contrary, in the deepest soil layers the concentration of available Cd was higher in control and mineral (only at 0.26–0.45 m) than compost, no matter the rate (Fig. 1e). At 0–0.15 m, Co availability increased because of compost LOW application. Fertilization with compost HIGH and mineral decreased Co in comparison to the control (Fig. 1f). At 0.16–0.25 m, Co available concentration was higher in compost LOW and HIGH than other treatments, while in the layers between 0.26 and 0.65 m, available Co was higher in control than in fertilized soil (Fig. 1f). The interaction between fertilization treatment and depth was significant, in case of Ni and Pb. Available Ni concentration (Fig. 1g) in the shallowest soil layer was enhanced by the application of compost at the low rate, followed by compost HIGH, mineral and control. At 0.16–0.25 m, the application of compost HIGH induced the highest values followed by compost LOW, mineral and control that showed similar values (Fig. 1g). At 0.26–0.45 m, the highest values were observed in compost HIGH treated soil, compost LOW and control showed intermediate values; the lowest availability was recorded in mineral fertilized soils (Fig. 1g). In the deeper soil layer, organic amendment application (no matter the rate) increased available Ni, while control and mineral fertilization induced the lowest values (Fig. 1g). The application of 10 t ha^{-1} (25 t ha^{-1} in the row) of compost increased available Pb between 0 and 0.25 m in comparison to other treatments. Compost at 5 t ha^{-1} (12.5 t ha^{-1} in the row) induced values higher than control and mineral, with the former showing values higher than mineral fertilization (Fig. 1h). At 0.26–0.45 m the application of compost LOW reduced available soil Pb in comparison to all other treatments; control and compost showed similar values higher than mineral (Fig. 1h). No significant differences between treatments were observed in the deepest soil layer (Fig. 1h). Chromium was always below the detection limit (0.002 mg kg^{-1}), and along with B and Al is not reported.

The canonical discriminant analysis performed on soil available and total micronutrients and trace elements calculated on a hectare basis, showed that the largest gradient of variability (92.6% and 85%, respectively) of these data sets was represented by the first canonical variable; as a consequence, the distribution of objects was represented only for this axis (Figs. 2 and 3). The box plot for soil available trace elements separated compost HIGH, from other treatments and compost LOW from mineral and control that were similar (Fig. 2). Almost no effect was observed for Cu, Cd, and Co, while all other elements were influenced by compost addition (no matter the rate) with a stronger effect on Fe, Zn and Pb than others (Fig. 2). Plotting first canonical variable (Can 1) for total elements in the soil showed that the applica-

Table 4
Effect of fertilization treatment and soil depth on enrichment factor (EF) of micronutrients and trace elements in soil after 14 years of orchard cultivation.

	B	Cu	Fe	Mn	Zn	Al	Cd	Cr	Co	Ni	Pb
Treatment											
Mineral	1.08	0.971 b	0.997	0.998	0.978 b	1.06	1.01	1.02	0.996	0.984	0.990
Compost LOW	1.12	1.16 a	0.98	0.987	1.12 a	1.04	1.02	1.02	0.972	0.986	1.03
Compost high	1.02	1.17 a	0.957	1.01	1.16 a	0.937	1.06	0.961	0.945	0.951	1.07
Significance	n.s.	***	n.s.	n.s.	***	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Depth (m)											
0–0.15	1.30 a	1.20 a	0.972	1.02	1.21 a	1.14 a	1.08	1.08 a	0.959	0.962	1.06
0.16–0.25	0.830 b	1.14 ab	0.949	0.974	1.09 b	0.791 b	1.02	0.879 b	0.943	0.933	0.915
0.26–0.45	1.21 ab	1.04 bc	1.01	1.01	1.06 b	1.17 a	1.01	1.07 a	1.009	0.997	1.09
0.46–0.65	0.957 ab	1.01 c	0.981	0.994	0.98 b	0.946 ab	1.00	0.962 ab	0.971	1.002	1.05
Significance	*	**	n.s.	n.s.	***	**	n.s.	*	n.s.	n.s.	n.s.
trt x depth	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

n.s.,**, ***: effect not significant or significant at P ≤ 0.01 and P ≤ 0.001, respectively. Within the same column, values followed by the same letter are not statistically different according to Student Neuman Keul test ($P \leq 0.05$). The effect of the main factors is reported for all the elements analysed.

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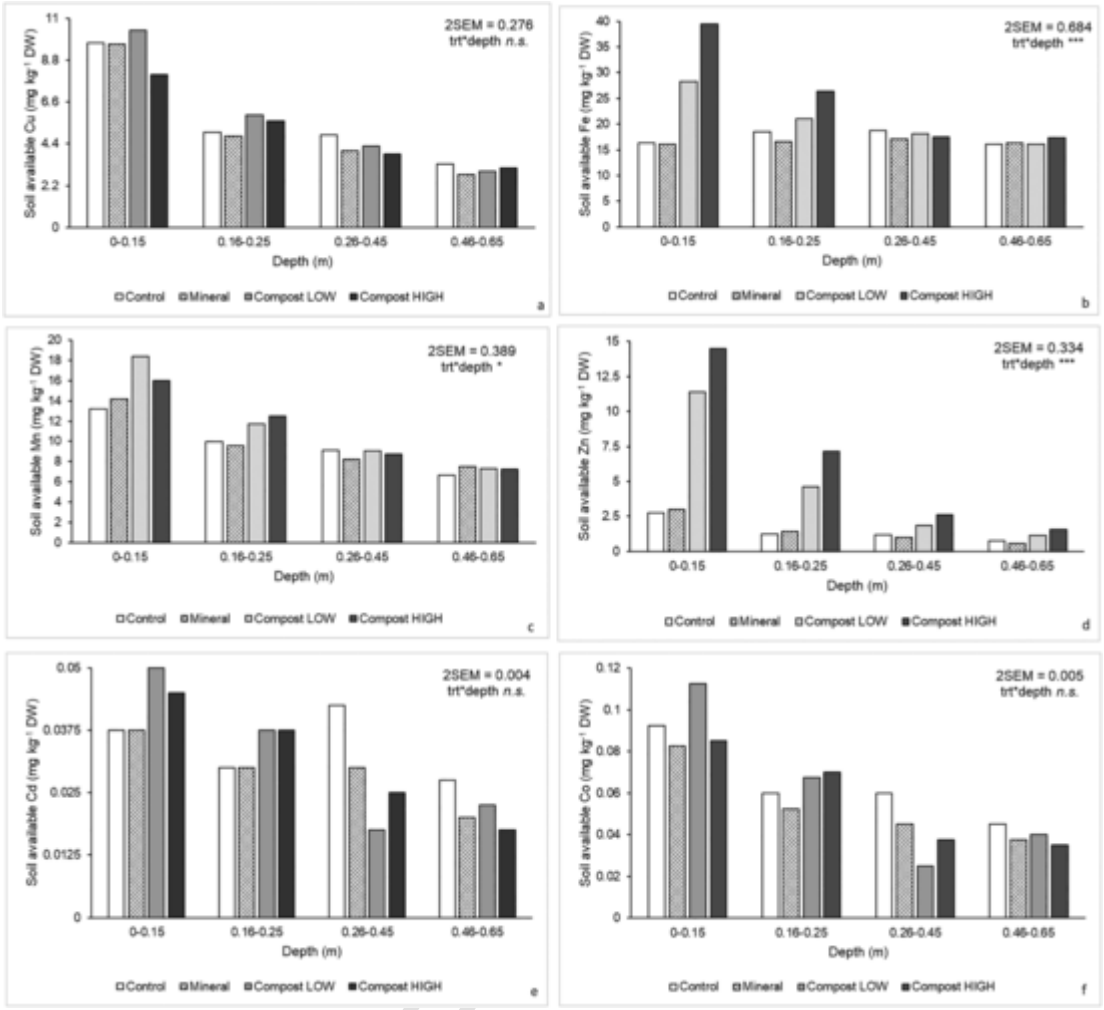

Fig. 1. Effect of fertilization treatment and depth on soil available ~~Cd (a), Co (b), Cu (c), Fe (d), Mn (e), Ni (f), Pb (g) and Zn (h)~~ concentration in the row. Values differing by 2 standard error of means (SEM) are statistically different.

tion of compost HIGH improved soil concentration of Cu, Zn and Cd (Fig. 3). The addition of 5 t ha^{-1} (12.5 t ha^{-1} in the row) of compost induced values higher than mineral and control (Fig. 3). Iron, Co, Ni and Pb total concentrations were higher in mineral fertilization and control more than compost (Fig. 3).

After 14 years, Cu and Zn total soil concentration increased in comparison to the beginning of the experiment, while Cd, Ni and Pb decreased (Fig. 4 – top). The second canonical variable, Can2 (Fig. 4 – bottom) showed that compost (both LOW and HIGH) increased Cu and Zn in soil, while mineral and control lead to a higher Ni and Pb; no effect was observed for Cd (Fig. 4 – bottom).

3.2. Plant

In summer, leaf B concentration was higher in control than in mineral; compost LOW and compost HIGH evidenced intermediate values not different from other treatments (Table 5). Copper, Fe and Zn leaf concentrations were not significantly influenced by fertilization; while Mn increased in mineral in comparison to all other treatments (Table 5). Potentially toxic trace element leaf concentration was not affected by fertilization treatments.

At harvest, B, Fe, Mn, Zn, Al, Cd, Cr and Ni concentrations in fruit flesh were not influenced by fertilization strategy (Table 6). Copper was higher in fertilized plots (both mineral and organic) than in control (Table 6). Lead fruit flesh concentration was decreased by the application of compost HIGH. The concentration of micronutrient and potentially harmful trace element in other tree organs is reported in supplemental materials (Table S2).

Cobalt and Hg were below detection limits; consequently, no data are reported.

Thinned fruit content of B, Cu, Mn, Zn, Al, Ni and Pb was unaffected by fertilization treatment; Fe content was increased by compost HIGH (Table 7).

The amount of B, Cu, Fe, and Zn in fruit at harvest was increased by fertilization, while Mn and all trace elements were unaffected. The amount of B, Zn, Cr and Pb in abscised leaves was not affected by fertilization; while Cu, Fe, Mn, Al were increased by mineral and compost HIGH, compared to control and compost LOW (Table 7).

Fertilizers did not modify the amount of micronutrients and trace elements in pruned wood, roots, and skeleton (Table 7).

The fertilization treatments did not modify the micronutrient and trace element tree total content in the last (fourteenth) year of cultivation, also the fraction of mineral element removed by trees was not affected. However, the fraction of Cu, Fe, Mn, Zn, Al, and Ni that was cycled internally within the orchard was increased by mineral fertilizer. While the supply of compost at high rate increased recycled Fe, Mn, Zn and Al compared with the unfertilized control (Table 8).

Total Fe plant content was positively correlated to soil Fe available in the two shallowest soil layers (Fig. 5); no significant correlation was found for other nutrient and depths (data not reported).

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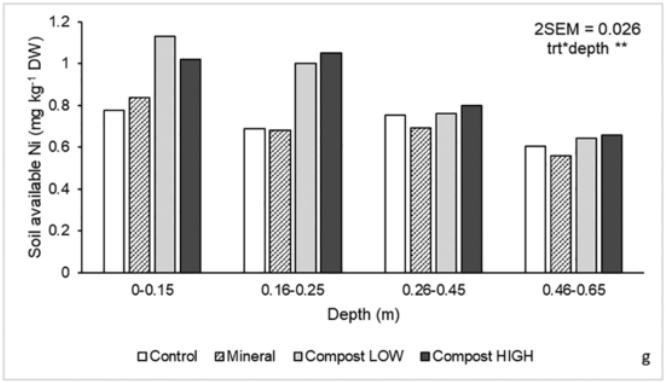

Fig. 1. *Continued*

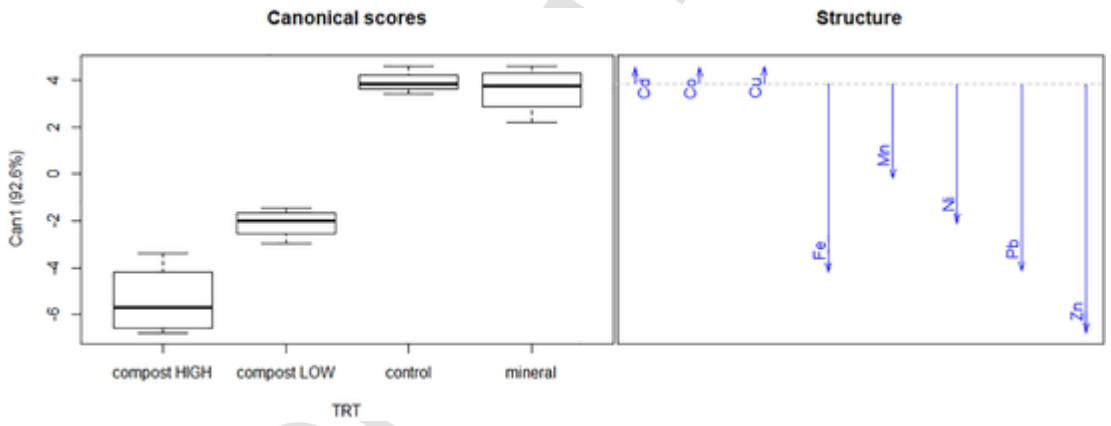

Fig. 2. Discriminant canonical analysis of the effect of fertilization treatment on soil available nutrients in the orchard at the end of the experiment.

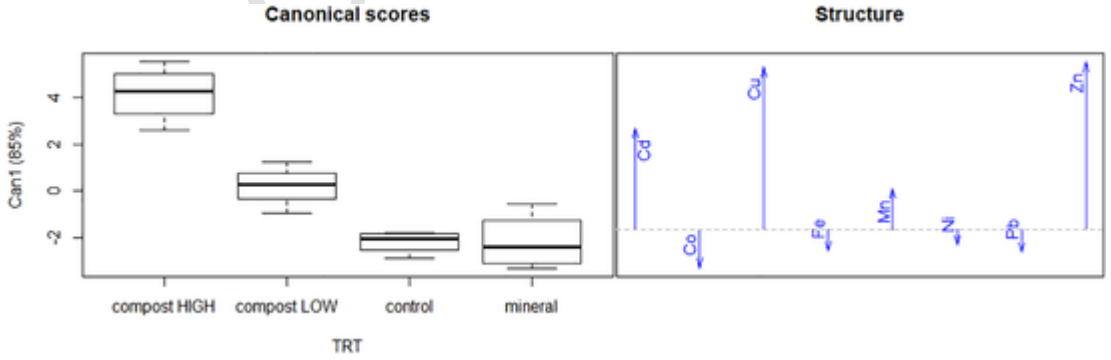

Fig. 3. Discriminant canonical analysis of the effect of fertilization treatment on soil total nutrients in the orchard at the end of the experiment.

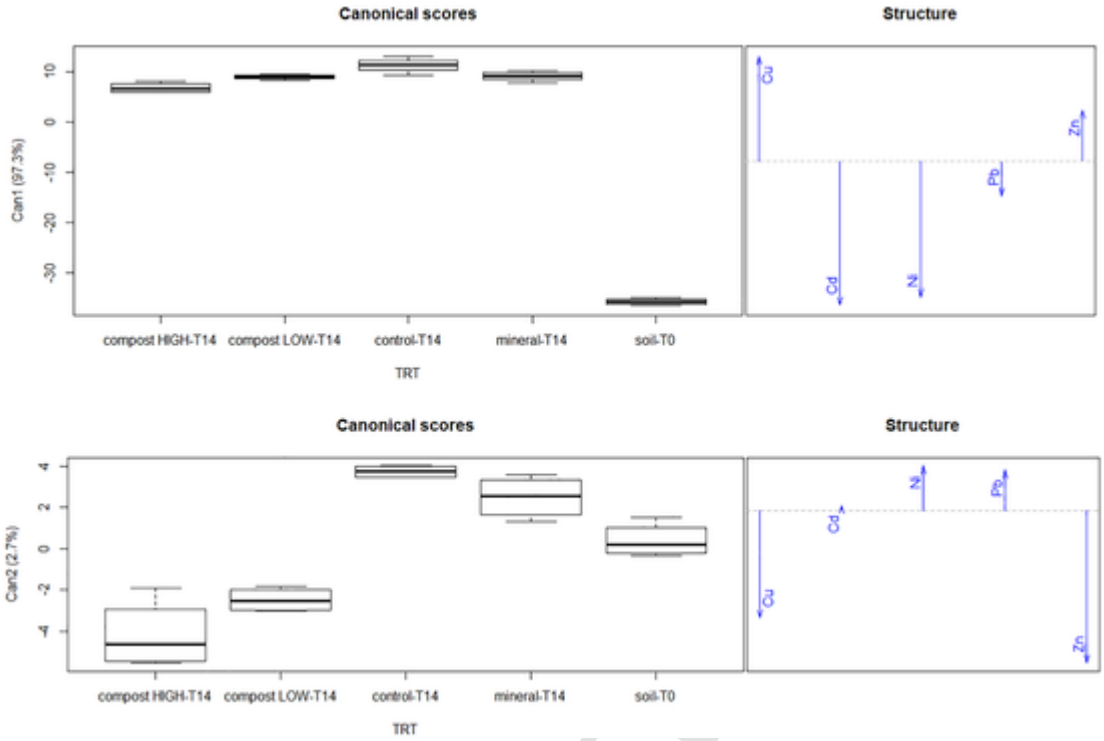

Fig. 4. Discriminant canonical analysis of the comparison between soil total trace element concentration at the beginning of the experiment (0) and at the end.

Table 5
Effect of fertilization treatment on micronutrient and trace element concentration (mg kg^{-1} DW) of leaves sampled in summer.

Treatment	B	Cu	Fe	Mn	Zn	Al	Cr	Cd	NI	Pb
Control	34.4 a	9.75	59.0	38.9 b	35.7	37.8	1.09	0.227	1.69	1.71
Mineral	31.2 b	10.5	62.0	49.3 a	33.4	27.8	0.882	0.217	2.21	2.19
Compost LOW	33.6 ab	9.17	66.4	35.3 b	39.7	41.8	1.17	0.178	1.83	1.87
Compost HIGH	32.7 ab	10.0	64.4	38.0 b	36.2	24.5	1.19	0.224	1.98	1.81
Significance	*	*n.s.*	*n.s.*	***	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*

n.s., *, ***: effect not significant or significant at P ≤ 0.05 and P ≤ 0.001, respectively. Within the same column, values followed by the same letter are not statistically different according to Student Neuman Keul test ($P \leq 0.05$).

Table 6
Effect of fertilization treatment on micronutrient and trace element concentration (mg kg^{-1} DW) of fruit flesh at harvest.

Treatment	B	Cu	Fe	Mn	Zn	Al	Cd	Cr	Ni	Pb
Control	21.6	6.39 b	11.6	5.73	8.07	0.519	0.096	0.121	1.81	1.15 a
Mineral	20.6	8.07 a	13.2	5.91	9.20	0.359	0.09	0.090	1.72	1.18 a
Compost LOW	22.0	7.76 a	12.8	5.42	8.89	1.61	0.098	0.123	1.52	1.20 a
Compost HIGH	21.8	8.07 a	12.6	5.70	9.08	0.715	0.099	0.148	1.66	1.01 b
Significance	*n.s.*	*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*

n.s., *: effect not significant or significant at P ≤ 0.05, respectively. Within the same column, values followed by the same letter are not statistically different according to Student Neuman Keul test (P ≤ 0.05).

4. Discussion

Fourteen years of consecutive applications of compost resulted in an enrichment of soil total Cu, Zn and Cd, and a decrease of soil total concentration of Fe and Co; while B, Mn, Al, Cr, Ni and Pb were unaffected by fertilization treatments. The average values measured in the soil profile of 0–0.65 m were far below the threshold limits established by the European community (E.U., 1986) that are (in mg kg^{-1}): Cd 3; Cu 140; Ni 75; Pb 300; Zn 300; evidencing the low heavy metal contamination of the compost supplied.

Considering the variation of total mineral element concentrations with soil depth, different behaviors can be observed. Cadmium, Cu and Zn decreased with increasing soil depth, B, Mn, Al, Cr and Pb were stable, while Fe, Co and N increased. These responses, along with the trend of accumulation induced by fertilizer applications indicate that, in our experimental conditions, Fe and Co may have been diluted by the addition of organic amendments, as their concentration in compost was lower than in soil. To complete the picture we have to stress that Fe concentration in compost was reported to range between 2000 and 3000 mg kg^{-1} (Baldi personal communication), 10-time lower than

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Table 7
Effect of fertilization treatment on micronutrient and trace elements content (mg plant $^{-1}$) in different plant organs.

Treatment	B	Cu	Fe	Mn	Zn	Al	Cr	Ni	Pb
Thinned fruits									
Control	1.68	4.90	8.13 b	2.37	6.99	16.9	–	0.112	0.112
Mineral	3.81	4.13	6.10 b	2.03	5.99	5.32	–	0.094	0.072
Compost LOW	4.45	5.07	8.51 b	2.56	8.03	13.6	–	0	0.134
Compost HIGH	7.28	7.13	12.4 a	3.43	10.1	11.3	–	0.033	0.215
Significance	*n.s.*	*n.s.*	*	*n.s.*	*n.s.*	*n.s.*	–	*n.s.*	*n.s.*
Fruit at harvest									
Control	143 b	52.8 b	291 b	56.0	59.9 b	23.2	41.2	56.0	10.2
Mineral	163 ab	76.3 a	453 a	70.4	82.9 a	25.7	75.7	89.0	11.6
Compost LOW	177 a	76.4 a	480 a	70.1	79.6 a	31.2	82.0	93.7	12.7
Compost HIGH	181 a	80.1 a	444 a	71.6	82.0 a	16.9	69.6	87.2	12.0
Significance	*	*	*	*n.s.*	**	*n.s.*	*n.s.*	*n.s.*	*n.s.*
Abscised leaves									
Control	34.2	23.0 b	334 b	45.9 b	116	295 b	0.113	–	2.30
Mineral	66.6	36.3 a	476 a	123 a	129	405 a	0	–	1.88
Compost LOW	39.1	24.1 b	344 b	46.4 b	102	290 b	0	–	2.84
Compost HIGH	86.1	38.7 a	506 a	73.2 a	145	410 a	0	–	3.02
Significance	*n.s.*	*	*	***	*n.s.*	*	*n.s.*	–	*n.s.*
Pruned wood									
Control	–	514	137	24.8	95.5	90.7	2.70	1.33	2.38
Mineral	–	613	212	36.7	156	141	4.49	3.24	2.14
Compost LOW	–	530	155	28.9	112	102	4.23	2.35	1.78
Compost HIGH	–	466	153	29.9	127	98.7	4.33	2.20	2.46
Significance	–	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
Roots									
Control	25.0	9.92	2529	71.8	34.3	2925	13.2	9.07	3.01
Mineral	12.8	16.4	2568	74.9	35.5	3198	17.3	11.7	3.18
Compost LOW	22.7	10.3	2221	66.9	29.4	2353	11.8	8.90	2.70
Compost HIGH	13.3	12.6	3279	96.2	34.2	3529	12.3	9.56	3.77
Significance	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
Skeleton									
Control	217	117	1240	52.5	171	1534	4.81	5.37	3.34
Mineral	287	128	1254	52.9	241	1491	4.48	6.53	3.84
Compost LOW	215	125	1241	51.7	205	1976	4.94	9.15	4.22
Compost HIGH	292	125	1003	49.8	276	1184	9.38	5.89	3.62
Significance	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*

n.s.,*, ***: effect not significant or significant at $P \leq 0.05$ and $P \leq 0.001$, respectively. Within the same column, values followed by the same letter are not statistically different according to Student Neuman Keul test ($P \leq 0.05$).

the Fe soil concentration. The same response was observed for soil total Ni that, at the beginning of the trial, was 64.4 mg kg^{-1} while at the end it was 43–46 mg kg^{-1}, showing a probable dilution induced by the addition of compost. However, unlike Fe and Co, Ni soil total concentration was not affected by fertilization treatments, at the same time it increased with soil depth, indicating a relative mobility and migration throughout the soil profile with a potential for leaving the soil volume explored by root system. These results are confirmed by the analysis of the Enrichment Factor (EF), an indicator used to assess metals presence and intensity of anthropogenic contaminant deposition on surface soil (Barbieri, 2016). According to several authors (Zhang and Liu, 2002; Gil et al., 2018; Brtnický et al., 2019) values of EF lower than 2 indicate minimal enrichment with no risk of pollution; in our experiment, after 14 years only Cu and Zn EF increased as a consequence of compost addition, but the values calculated were well below 2. It is noticeable that Cu and Zn were used abundantly in disease control; however, no count was made.

Considering the total amount of element (soil concentrations by soil volume) at the beginning and at the end of the trial, the canonical analysis showed that the routinely application of compost in the orchard was responsible, even at the low rate (12.5 t ha^{-1} in the row), for the increase of both total (Cu and Zn), and available (Fe and Zn) micronutrients, with low impact on potentially harmful heavy metal (limited to Cd and Pb). Our data showed a fertilization effect of compost at low rate higher than mineral fertilizer. It has to be pointed out, however that mineral fertilization, in this trial, did not include micronutrients.

Considering the DTPA-extractable fraction of elements, that probably has been the most affected by application of organic amendments, metal availability is expected to be low in calcareous soils due to the prevailing alkaline pH; whereas, lower pH values result in higher cation mobility and availability (Lee et al., 2009; Brokbartold et al., 2012). The increase of availability of most of the elements studied, with the exception of Cu and Co, in the amended plots can be partially attributed to a significant decrease of soil pH (Rieuwerts et al., 1998).

Literature reports both increases and decreases of the fractions of trace elements available for tree uptake following compost amendments (Shuman et al., 2001; Lee et al., 2004), depending on soil properties, chemical form of the metals and compost quality (Baldantoni et al., 2010). The decreased availability is mainly related to complexation and adsorption by organic matter, because the active component in metal binding by organic matter is negatively-charged functional groups (Rieuwerts et al., 1998).

On the other hand, a large body of literature reports an increase of metal availability as a consequence of organic fertilizers supply (Harg-

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Table 8
Effect of fertilization treatment on micronutrient and trace element, removed and recycled and total content (mg plant $^{-1}$ year $^{-1}$) of nectarine tree the fourteenth year of orchard life. Recycled fraction is the sum of the content in abscised leaves, thinned fruits and pruned wood; removed fraction is the sum of nutrients in fruits at harvest, root and skeleton; total content is the sum of removed and recycled.

Treatment	B	Cu	Fe	Mn	Zn	Al	Cr	Ni	Pb
Recycled									
Control	35.9	542 b	479 b	73.1 c	218 b	403 c	2.81	1.44 b	4.79
Mineral	70.4	653 a	694 a	162 a	291 a	551 a	4.49	3.33 a	4.09
Compost low	43.5	559 b	508 b	77.9 c	222 b	406 c	4.23	2.35 ab	4.75
Compost high	93.4	502 b	671 a	107 b	282 a	520 b	4.33	2.23 ab	5.69
Significance	n.s.	*	**	***	**	**	n.s.	*	n.s.
Removed									
Control	385	180	4060	180	265	4482	59.2	70.4	16.6
Mineral	463	221	4275	198	359	4715	97.5	107	18.6
Compost low	415	212	3942	189	314	4360	98.7	112	19.6
Compost high	486	218	4726	218	392	4730	91.3	103	19.4
Significance	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
Total content									
Control	421	722	4539	253	484	4885	62.0	71.9	21.4
Mineral	533	874	4969	360	650	5266	102	111	22.7
Compost low	458	771	4450	267	536	4766	103	114	24.4
Compost high	580	720	5397	325	674	5250	95.6	105	25.1
Significance	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*

n.s.,*, **, ***: effect not significant or significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively. Within the same column, values followed by the same letter are not statistically different according to Student Neuman Keul test ($P \leq 0.05$).

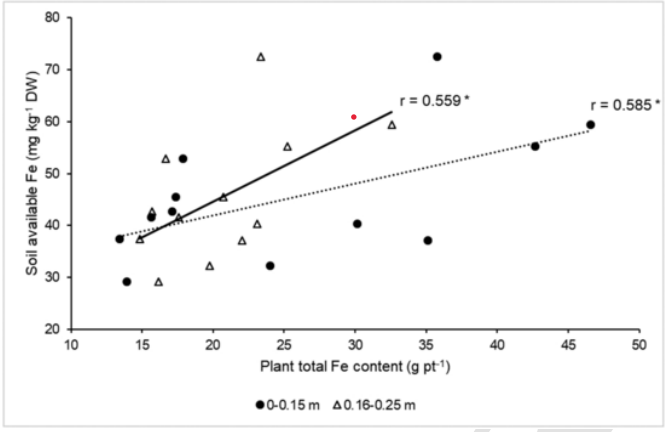

Fig. 5. Correlation between total Fe content in plants and soil available Fe concentration at 0–0.15 and 0.16–0.25 m of depth (r = Pearson correlation coefficient).

reaves et al., 2008), particularly in the shallower soil layers compared to the deeper ones (Cambier et al., 2014). The effect is similar for the different organic fertilizers, evidencing a negligible effect of the kind of amendment on the mobility of these metals (Businelli et al., 1996; Korboulewski et al., 2002).

In our study, the only exception to the general increase of soil metal availability was the ambiguous behavior of Cu availability with a decrease after application of compost at 10 t ha^{-1} (25 t ha^{-1} in the row) at 0–0.15 m, and 0.26–0.45 m compared to control, that disagrees with previous reports (Hargreaves et al., 2008; Cambier et al., 2014). Our results can possibly be explained by considering that Cu binds to organic molecules (Donner et al., 2012) reducing its availability for root uptake, depending on the characteristics of organic matter functional groups (Aiken et al., 2011), soil CEC and retention capacity (Elfoughi et al., 2012; Shaheen et al., 2017). This result contrasts with increase of total Cu in the soil amended with high rate of compost. Copper was abundant in the applied compost (156 mg kg dw^{-1}, making an annual application rate of approximately 1.560 g ha^{-1}) consequently, the increase observed in the present experiment could be due to the routinely application of organic amendments. At the same time, the high affinity of Cu with soil organic matter (Calace et al., 2001) and humic substances (Leita et al., 1999) induced the formation of stable complexes that could have decreased its availability (Cavani et al., 2016). Also the declining of total Cu concentrations with increasing soil depth supports the evidence that Cu availability and mobility is low particularly in clay soil (Toselli et al., 2009).

Similarly, also Cd tightly bound to the organic matter (Tills and Alloway, 1983), but in this case, an increase of both total and available soil concentrations (0–0.25 m) were found after compost application.

In line with results reported earlier (Maftoun et al., 2005; Warman et al., 2004), we observed a positive effect of compost on Fe availability in soil and in plants as already reported in the same area for pears (Sorrenti et al., 2012). Organic amendments can provide available Fe to plants as a result of Fe complexation by humic molecules (Chen et al., 2004; Bocanegra et al., 2006; Zanin et al., 2019). Humic acids in soil are the result of the partial degradation and re-synthesis of organic material and they are able to form stable complexes with metal micronutrients, due to the presence in their structure of oxygen-, nitrogen- and sulfur-containing functional groups (Zanin et al., 2019). In compost-amended soil, Fe availability was enhanced, and probably root uptake was more efficient because of the presence of organic matter decomposition material, and this may have contributed to lowering total Fe concentration in the root zone, in comparison with untreated control and mineral. In addition, the soluble complex between humic substances and Fe can be directly used by the plants (Pandeya et al., 1998) as also confirmed by the positive correlation between Fe in soil and in plants. Significantly increased Fe availability in compost-amended top soil (0–0.25 m) compared to control and mineral treatments, despite the lower total Fe concentrations, support the above concepts. Iron availability is particularly important for orchards grown in alkaline calcareous soil, as those of the Mediterranean area (Rombolà and Tagliavini, 2006), where iron chlorosis affects many fruit species including peach.

DTPA-extractable Pb concentrations in soil (at 0–0.25 m) increased with compost additions while no significant effect was reported for soil total Pb concentration. This result is in contrast with previous reports from Spanish experiment (Walter et al., 2006) where the application of increasing rate of compost induced an increase of both total

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and available Pb. The increased availability of Pb in soil, however, did not affect plant Pb concentration and particularly the content in fruits that was actually decreased by the highest compost rate.

Available and total soil Zn concentration increased as a consequence of compost addition, no matter the application rate as also reported by other authors (Zhang et al., 2006; Montemurro et al., 2010).

In the present experiment, increased availability of Fe, Mn and Zn in soil due to compost supply did not modify the total tree content, however it increased their fraction that re-cycled with leaves and pruned wood, meaning that Fe, Mn and Zn are mainly partitioned in tree new growth. In general, micronutrient leaf concentration was optimal for the species in the investigated area (Baldi et al., 2004).

Increased soil availability of Cu, Cd, Co, Ni and Pb showed no effect on plant total content; this can be explained by the fact that plant roots have a natural barrier for heavy metals translocation (Montemurro et al., 2010). This tree self-control mechanism prevented negative accumulation of heavy metal in fruit flesh that over 14 years did not show any increase of potentially toxic metals, confirming data previously showed in the same trial (Baldi et al., 2016).

Results from a study on different Spanish peach varieties assessed that, calculated on a tree basis, total annual micro nutrient requirements are (in g plant^{-1}): Fe 0.9–4.1; Mn and Cu 0.2–0.8; Zn 0.2–0.9 (El-Jendoubi et al., 2013). Considering that our tree density was 526 plant ha^{-1}, and assuming that peaches have similar nutrient requirements to nectarines, the micronutrients actually removed (total tree content) accounted for (in g ha^{-1}): B: 200–250; Cu: 90–115; Fe 2100–2400; Mn: 90–115; Zn: 130–120. In disagreement with El-Jendoubi et al. (2013), trace elements requirements were not satisfied by their recycling, that in our experiment accounted for 10–20% for B, 10–15% for Fe, 40–50% for Mn, 70–80% for Zn. An exception is the recycling of Cu, that was 2.3–3 fold higher the actual net demand. This response can be the result of the use of Cu-based fungicides for an approximate total of 2000–4000 kg ha^{-1} year^{-1}, much higher than tree demand. In fact Cu soil concentration at the beginning of the study accounted for 24.8 mg kg^{-1} and finished at 33–40 mg kg^{-1}.

It was possible to calculate an annual total nutrient demand (in g) per plant: B 0.4–0.5; Cu 0.7–0.9; Fe 4.5–5.4; Mn 0.2–0.4; Zn 0.5–0.7. These values are the sum of nutrients found in fruits and in skeleton, abscised leaves, thinned fruits and pruned wood and are in the range defined by El-Jendoubi et al. (2013), in a similar Mediterranean environment.

In conclusion, annual compost application of 10 t ha^{-1} (corresponding to 25 t ha^{-1}, in the row) increased soil total Cu, Zn and Cd, and decreased Fe and Co concentration. Particularly it was effective in increasing the DTPA-extractable fraction of Fe, Mn, Zn, Cu, Cd, Ni, Pb in the cultivated top-soil layer (0–0.25 m). The available soil fraction of the metals appeared to be more affected by compost applications than the total concentrations, evidencing that the former is more effective in estimating soil fertility. The effect of compost application was clear in the top-soil layer (0–0.25 m of depth), interested by continuous applications. Total amount of micronutrient removed from the orchard were not affected by compost application. Our data show that the use of compost at both rates (12.5 and 25 t ha^{-1} year^{-1} in the row) is an excellent source of micronutrient for nectarine tree and at the same time does not represent a risk of pollution related to heavy metal distribution in the environment.

CRediT authorship contribution statement

Baldi: Conceptualization, methodology, formal analysis, data curation, writing, review and editing

Toselli: writing, review and editing, supervision

Marzadori: supervision

Cavani: Methodology, formal analysis, data curation, review and editing

Mazzon: methodology, data analysis

Quartieri: methodology

Uncited references

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Regione Emilia Romagna, n.d

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.141894.

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