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Organic fertilizers obtained from metal-free and chromium tanned leather: Evaluation of nitrogen release and the effects on the soil microbiome

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ABSTRACT

Tannery industry generates over 4 million tonnes of waste annually, with 600,000 tonnes containing 2.5–6 % chromium (Cr). Tannery wastes can be employed as fertilizers due to their N and organic matter content. However, their use raises concerns about Cr and trace element contamination. Metal-free tanning processes (wet white, WW) offer an environmentally friendly alternative to Cr-based tanning (wet blue, WB), however 90 % of leather production still relies on WB tanning. The fertilizing potential of WW tanning residues has been little investigated, while this knowledge will be essential for a rational reuse of these materials in sustainable agriculture. This study evaluates fertilizers produced from hydrolysed leather residues (WW and WB) by assessing: i) N release dynamics; ii) effects on key soil fertility indicators; iii) microbial community physiological profiling; iv) Cr and trace element bioavailability to plants. Physicochemical characterization was first performed, afterwards, a soil incubation experiment was conducted. Both fertilizers released N fitting a first-order kinetics model, releasing 40–45 % of their total N after 42 days. DTPA-extractable Cr increased but remained low (31 μg kg^{-1}) in WB-treated soils. Biochemical indicators were positively affected by WW and WB fertilizers, and microbial community profiles were influenced by their composition but not by the presence of Cr in WB. Overall, WW and WB fertilizers effectively supplied N and enhanced microbial activity, without adversely impacting soil fertility indicators. Rhizotest suggested that Cr was not bioavailable for tomato plants in both fertilizers, even though total Cr reached about 60 μg kg^{-1} in WB-fertilized soils.

1. Introduction

Environmental pollution has become a serious global issue for human health and food security due to the steady global population growth and the rapid industrialization that has occurred in recent decades [33,52]. Soil is an essential part of the ecosystems, therefore the associated potential risks are reflected in food safety via crops consumption [3]. Globally, over 5 million of soil sites are estimated to be contaminated with toxic elements and heavy metals [3,52]. Tannery industry significantly contributes to environmental risks connected to leather production in particular in developing nations [69] and is often associated with extremely high levels of soil and groundwater pollution [64]. To manage and limit environmental pollution caused by leather production and tannery industry, new industrial models have emerged in recent years, pursuing the circularity of resources employment [33].

Tannery industry produces around 4 million tons of solid wastes per year of which 600,000 tons contain trivalent chromium (Cr III) up to 2.5–6 % [55,60,68]. Solid wastes produced by tannery industry are represented by hair, dry sludge, fleshing, trimmings, buffing dusts, salts, shavings and splitting. These materials are estimated to account for 160 kg per ton [55,99]. Alternative managements of tannery wastes, different from the landfill disposal, are emerging. This has led to the successful use of tannery residues in several sectors, such as bioenergy and agriculture [26]. Tannery residues have been extensively used to produce fertilizers, because their content in organic nitrogen (N) and organic carbon (C) (i.e. organic matter) can represent a source of nutrients for crops and increase the soil microbial activity [19,30,46,81]. Fertilization with organic fertilizers produced from tannery residues have been reported to increase crop biomass yield [71,84] and the availability of nutrients such as N and phosphorus (P) [66]. In particular, leather waste are generally rich in protein (2.5–10.5 %) which can represent a precious source of organic N [38,62]. Moreover, it was reported that tannery wastes applied to the soil generally stimulate soil microbial activity [85]. On the other hand, several critical aspects are associated with the fertilization with tannery residues, such as the persistence of Cr which once accumulated into the soil can potentially

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limit the growth and functions of microorganisms [5] and be toxic for plants [24].

In the soil environment, Cr exists as trivalent and hexavalent [Cr(III) and Cr(VI)] forms. The trivalent form has a very low solubility and mobility in soil, as it tends to be retained on soil particles and organic matter [59,73]. Conversely, Cr(VI) is mobile, soluble and perhaps highly dangerous for living organisms [59,73,96]. Nevertheless, Cr(III) oxidation in soil is generally limited by the low availability of the mobile forms, and even in favourable conditions (such as in presence of Mn oxides) it is a very slow process at soil pH > 5 [59]. Plant uptake of Cr is competitive with the uptake of macro and micronutrients as it involves unspecific intake channels [50]. While plant uptake of Cr(III) is a passive mechanisms, Cr(VI) uptake is an active process which involves micronutrients carriers and energy consumption [86,97]. Normal concentrations of total Cr in plants are reported in the range of 0.02–1.0 mg kg^{-1} (dry weight), but the concentration of Cr in plants grown on contaminated soils can be between 10–190 mg kg^{-1} depending on the soil type and plant species [59]. When taken up by plants, Cr is poorly translocated and largely retained by roots, probably due to the formation of insoluble complexes, and can reach concentrations 100-times higher than shoots [19,86]. Phytotoxicity of Cr contained in the soil was observed as plant growth inhibition in relatively high ranges of 5–100 g kg^{-1} [50]. However, considering the long-term accumulation, there is still no agreement on the critical level of soil total Cr (i.e., 64, 100, 100, 150, 200 and 100 −200 mg kg^{-1} in Canada, Austria, Serbia, Poland, China and Czech Republic respectively, [25,57,86]), although literature referenced by National Instituted of Health (NIH) suggests a Maximum Allowable Toxicant Concentration (MATC) of 64 mg kg^{-1} [14].

Tanning agents that can be utilized in the tannery process and are commonly divided into i) Cr-based tanning agents, which involve the utilization of Cr salts (basic Cr sulfate); ii) natural tanning agents, which involve the use of polyphenolic molecules obtained from bark and leaves [53]. Natural – or "green", or "metal-free" – tanning agents are derived from renewable sources and potentially cause less environmental pollution for the absence of Cr and heavy metals [16,23]. Metal-free tanning agents can contain tannins, gum, oligomeric and polymeric flavan-3-ol compounds and non-tannin aromatic compounds and can be derived from diverse plant species [23]. The use of alternative tanning agents would mitigate the risk associated to the production of Cr-rich tannery wastes. Nevertheless, efficiency of natural tanning still remains much lower than Cr tanning for large-scale leather productions [53], in fact about 90 % of tannery industries worldwide still adopt the chrome tanning method [64]. However, the necessity for a correct waste management applies to every kind of tanning wastes. Moreover, a further employment of green tanning agents would create closed environmental-friendly industrial models, motivating their affordability in leather production and facilitating waste management [70]. For this reason, it is important to produce evidence of the suitability of wastes from different tanning processes to be converted into organic fertilizers.

The present work aimed to compare the effect on the soil fertility of two fertilizers produced from by-products of the Cr-tanning process (also referred to as "wet blue" tanning, [67]) and the natural tanning process (also referred to as "wet white" tanning, [89]). After physico-chemical characterization of the two products, a short-term soil incubation experiment was set up to evaluate the release of mineral N, the principal chemical and biochemical soil indicators of fertility and the changes in the microbial community functional profiling through community level physiological profiling (CLPP). Afterwards, the potential environmental bioavailability of metal contaminants, particularly of Cr, was assessed through Rhizotest (ISO 16198:2015) selecting *Lycopersicon esculentum* L. as a model plant for the test. We hypothesized that, although the tannery wastes produced through diverse tanning process can differ in their composition, the two tested materials would be similarly efficient into providing mineral N to the soil having a positive effect on the principal indicators of soil fertility and microbial functionality, and reporting very limited concentrations of metal contaminants. Although the negligible presence of the principal organic and inorganic contaminants in both products tested, the convenience of the utilization of wet white tanning agents as an alternative to the classic Cr-tanning will be emphasised, in order to increase the circularity of leather production through the re-utilization of metal-free tannery residues.

2. Materials and methods

2.1. Organic fertilizers characterization

The organic fertilizers tested were provided by Organazoto Fertilizzanti SPA (https://www.organazoto.it/) and were obtained from leather by-products after: i) green (Wet White, WW) tanning process, in which glutaraldehyde was used as tanning agent; ii) basic chromium sulphate (Wet Blue, WB) tanning process. By-products were subjected to thermobaric hydrolysis (1.5 h at ~160°C and pressure of 3–4 bar) and dried according to the common processes used by the company in compliance with the Reg. (EU) 2021/1372. A commercial manure-based organic fertilizer (OF) was introduced as control in the characterization. The dried sampled fertilizers were analysed for their physico-chemical characteristics. Humidity of samples was determined after oven-drying at 105°C, according to EU standard 12880:2000 method. Ashes and volatile solids were determined in muffle at 550°C according to EU standard 12879:2000 method. Reaction (pH) and electrical conductivity (EC) were determined following the EU standards methods 13037:2012 and 13038:2012, in 3:50 and 1:10 mass-to-water ratios, respectively. Total organic C (TOC) was determined with dichromate acid oxidation method according to Ciavatta et al. [18]. Total Kjeldahl nitrogen (TKN) was determined using a Kjeldahl automatic instrument (KjelFlex K360, BUCHI Labortechnik AG, Flawil, Switzerland) after acid digestion with sulfuric acid and selenium-potassium persulfate as catalyser. The ammonium and nitrate N were determined after extraction with 1 M KCl (1:10 w:v) and steam distillation with Kjeldahl automatic instrument after adding magnesium oxide for ammonium-N (NH_4^+-N) and Devarda's alloy for nitrate-N (NO_3^--N). Organic N (Norg) was calculated by the difference between TKN and inorganic N (NH_4^+-N + NO_3^--N). The other macronutrients, micronutrients and metals were determined after microwave wet acid digestion (Start-E, Milestone, U.S.A.) by elemental analysis using inductively coupled plasma optical emission spectrometer (Spectro Arcos ICP-OES Analyzer, Spectro Analytical Instrument GmbH, Kleve, Germany). Hexavalent Cr was determined according to ISO 17075–2:2017 method. The principal organic contaminants were determined and are reported in Table S1.

2.2. Soil characteristics

The soil incubation experiment was carried out onto an agricultural soil sampled in an area of common utilization of these fertilizers, located in Northern Italy (Quinto Vicentino, VI, 45.15° N, 11.15° E, 35 m a.s.l., annual average temperature 13.3 °C, annual average precipitation 1103 mm). The topsoil (0–0.3 m) of a clay-loam cambisol soil (WRB, 2014) was collected. Soil was spread in a thin layer (1–2 cm) then air dried for 2–3 weeks at room temperature (25 ± 3 °C, with relative humidity 40–60 %) to constant weight. Roots, stones and visible organic matter were manually removed. Afterwards, the soil clods were gently milled in mortar and the soil was sieved at 2 mm in a stainless-steel sieve. After the pre-treatment, the sieved soil was analysed for their physic-chemical main characteristics in agreement with SSSA methods [87], whose results of are reported in Table S2. Also, oxidising capacity for Cr(VI) was determined following the method described by Bartlett & James [87].

2.3. Soil incubation

Soil subsamples were placed in pots (200 g of dry soil per pot, 3 replicate-pots for each condition) and preincubated at 20 ± 2 °C and 30 % of full water holding capacity for 42 days. Moisture was kept approximately constant during the incubation by weighing the pot weekly and adding deionized water when necessary. The three organic fertilizers WW, WB and OF and a mineral one (MF) constituted by diammonium phosphate (DAP) were added to the soil at 100 mg N kg^{-1}, corresponding approximately to 240 kg ha^{-1} of N and enters the ranges suggested by the fertilizers producers. Treatments were compared to untreated soils used as control (CK). At 0, 3, 7, 14, 21, 28, 35 and 42 days the pots were sampled and analysed for NH_4^+-N and NO_3^--N. The pots were then sampled at the end of the incubation for biochemical indicators and community level physiological profile (CLPP). During these analyses, soil samples were kept in a cold room at 4 °C for one week. Once completed the analyses of biochemical indicators, the soil samples were air dried and analysed for chemical indicators.

2.3.1. Mineral nitrogen release

Ammonium-N (NH_4^+-N) and nitrate–N (NO_3^--N) were determined according to ISO 14238: 2012 method on soil suspension extracted in 1 M KCl (2:10, w/v) shaking for 2 h at room temperature, then colorimetrical determination was done using a flow autoanalyzer (AA3, Bran Lubbe, Germany).

2.3.2. Chemical indicators

Soil pH, electrical conductivity (EC) and DTPA (Diethylenetriaminepentaacetic acid) extractable metals were determined in agreement with SSSA methods [87]. Exchangeable magnesium (Mg), calcium (Ca), potassium (K) and sodium (Na) were extracted from 1 g of soil in 20 mL of hexamine cobalt chloride 0.017 M saturated with $CaCO_3$ according to ISO 23470:2018 method and determined by ICP-OES.

2.3.3. Biochemical indicators

Soil extractable organic C and N (C_{ext} and N_{ext}) were extracted in 0.5 M K_2SO_4 in a ratio of (1:4 w/v) from fresh soil, shaking for 30 min and determined by OC-VCPH/CPN (Shimadzu, Japan). Soil microbial biomass C (MBC) was determined through the fumigation-extraction method [90] and OC-VCPH/CPN. Dehydrogenase activity was determined according to von Mersi and Schinner [91] and the activity was expressed as mmol INTF kg_{dw}^{-1} h^{-1}.

2.3.4. Community level physiological profiling (CLPP)

Functional analysis of soil microbial community (CLPP) was performed in 96-well EcoPlates™ containing 31 carbon-based substrates and a water control colorimetrically read by Biolog® device (OmniLog, U.S.A.). Fresh soil samples were prepared according to Rutgers et al. [82]: briefly, 2 ± 0.05 g of dry soil were extracted in 20 mL NaCl 0.85 % (w/v), gently agitated for 2 h in and decanted for other 2 h. A dilution factor of 3.16 was used to dilute soil suspension with the extracting solution. EcoPlates™ were incubated with 100 µL suspension per well, each plate containing 3 replicates for each soil treatment. OmniLog device was set at 25 °C and plates were incubated for 120 h [39], measuring absorbance at 590 nm (OD_{590}) every 30 min. Values obtained after 120 h were used to perform the statistical analyses.

2.4. Trace elements translocation in Rhizotest

An independent experiment was conducted with Rhizotest (ISO 16198:2015) to assess the bioavailability of heavy metal contaminants and trace elements from soil fertilized with WB and WW to plants. Rhizotest was conducted in a growth chamber with 200–400 µE photosynthetically active radiation (PAR), 12:12 light:day photoperiod, temperature of 25 ± 4 °C. Forty seeds of *Solanum lycopersicum* L. cv Marmande per each pot were hydroponically germinated for 7 days in nutrient solution A) composed by 600 µmol L^{-1} $CaCl_2$ and 2 µmol L^{-1} H_3BO_3, covered with an aluminium foil for the first 2–3 days until radicle emerged. The seedlings were then grown for 7 days in solution B) with the following composition: 500 µmol L^{-1} KH_2PO_4; 2000 µmol $L^{-1}KNO_3$; 2000 µmol L^{-1} $Ca(NO_3)_2$; 1000 µmol L^{-1} $MgSO_4$; 0,2 µmol L^{-1} $CuCl_2$;10 µmol L^{-1} H_3BO_3; 2 µmol L^{-1} $MnCl_2$ (6.12); 1 µmol L^{-1} $ZnSO_4$; 0,05 µmol L^{-1} Na_2MoO_4 L^{-1}; and 100 µmol L^{-1} NaFe(III)EDTA; solution B) was replaced every third day. During the same two weeks, soil subsamples were placed in other pots, fertilized with WW and WB in a dose corresponding to 100 mg N kg^{-1} of dry soil (i.e. the same dose used in soil incubation test) and preincubated in the same growth chamber at 30 % of full water holding capacity for two weeks. Then, five replicates for each condition to test (WW, WB) and an untreated reference (REF) were established in the Rhizotest. Five pots with seedlings previously grown in hydroponics were then transferred in the soil test apparatus with root mat physically separated from the soil by a nylon mesh with 30 µm pores diameters [11]. An aliquot of the incubated fresh soil corresponding to 9 g of dry soil per pot was placed in the Rhizotest apparatus after the incubation with the tested products. Soil was continuously supplied by capillarity through a filter paper with solution C) composed by 50 µmol L^{-1} KH_2PO_4; 2000 µmol L^{-1} KNO_3; 2000 µmol L^{-1} $Ca(NO_3)_2$; and 1000 µmol L^{-1} $MgSO_4$, to avoid nutrient limitations during plant growth and ensure the highest potential uptake of heavy metals. The test with soil and solution C) lasted 8 days. The plant material was then rinsed with milliQ water and shoot and root tissues collected together. Plant samples were then frozen in liquid N_2 and immediately lyophilized for 3 days and finely ground with mortar. Soil was removed from the apparatus and dried at 105°C and ground in fine powder. Soil and plant tissues (~150–250 mg) were subjected to microwave wet acid digestion (Start-E, Milestone, U.S.A.) in 65 % HNO_3 + 37 %HCl + 3–5 drops of H_2O_2 for soil samples and in 65 % HNO_3 + 3–5 drops of H_2O_2 for plant tissues. Total trace elements were determined by ICP-OES. Trace elements concentrations in plants were corrected subtracting the concentrations in a set of plants sampled and analysed after hydroponic growth and before the phase of contact with soil.

2.5. Statistical analyses

Data from soil incubation experiment and Rhizotest followed a completely randomised experimental design and were analysed in the R environment using R studio version 4.4.2 [75]. One-way ANOVA followed by Post hoc HSD Tukey's test were performed on the data to investigate differences between treatments. The assumptions of ANOVA were verified through Shapiro–Wilk's test for the normality of the distributions and Bartlett's test for homogeneity of variances. The significance of tests was assessed at *p*-value less than or equal to 0.05. Nitrogen release fitting was performed on net mineral N which was calculated by subtracting the control (CK) at each sampling time to each treatment condition. According to their trend, WB and WW were fitted to a first order kinetic model having the following equation:

$$N_{min} = N_0 \cdot (1 - e^{-kt})$$

where N_{min} is net mineral N, N_0 is the potentially mineralizable organic N, k is the first order kinetic constant, and t is the time. MF and OF did not follow this trend (p-$R^2 < 0.5$) and therefore were not fitted.

Data obtained from CLPP were analysed as average well colour development (AWCD), on which Shannon Diversity and Simpson Evenness indexes were calculated, according to Ge et al. [35]. Obtained indexes were then analysed through one-way ANOVA. Principal Component Analysis (PCA) was performed on chemical parameters, biochemical parameters and AWCD for each substrate group (carbohydrates, carboxylic and ketonic acids, polymers, amino acids and amines and amides) using the *prcomp* function in the R environment. Prior to perform PCA, a correlation matrix of all the variables was produced through the function *corrplot* and the dataset was filtered for highly

correlated variables ($r > 0.9$). Moreover, variables reporting no significant differences between treatments ($p > 0.05$) were removed from the final dataset. On principal components one-way ANOVA was conducted to assess the significance of the observed separation in the scatterplot.

3. Results

3.1. Organic fertilizers characterization

Table 1 shows the main characteristics of WW and WB beside the characteristics of the commercial organic fertilizer (OF) tested as a control in the characterization. The three materials reported comparable levels of volatile solids (79–81 % in WB and WW, respectively), although slightly higher than OF (71 %). Reaction (pH) of WW and WB was lower (3.8–4.8, respectively) in comparison with OF in which it was neutral (7.1), as well as electrical conductivity (EC) which reported lower values in WB and WW (4.7, 3.5 dS m^{-1}) than OF (9.4 dS m^{-1}). Total organic carbon (TOC) was 39 and 42 % on dry weight basis in WB and WW, respectively, slightly higher than in OF which was 34 %. Total Kjeldahl-N (TKN) was considerably higher in WB and WW (11 and 14 %) than OF (3.2 %), of which 1.5, 2.2 and 13.8 %, respectively was $NH4^{+}$-N. Nitrate-N was instead generally negligible (<0.02 mg kg^{-1}). Most of the N was in the organic form (98, 98 and 88 % of the total N in WB, WW and OF, respectively). C/N ratio resulted to be > 10 only in OF, whereas WB and WW reported values < 4. Total phosphorus (P) was the highest in OF, followed by WB and WW (1.2, 0.61 and 0.06 g kg^{-1} respectively). Total K and magnesium Mg were always < 1 g kg^{-1} instead of 19 and 7 g kg^{-1}, respectively in OF. Total Ca was 0.3 and 2.1 g kg^{-1} in WB and WW, respectively, and was not determined in OF. Total sulphur (S) reported comparable values ranging around 3–6 g kg^{-1} in the three products. Micronutrients concentrations were very low in WB and WW in respect to OF, with copper (Cu) and manganese (Mn) ranging from 5 to 21 mg kg^{-1} and Zn from 22 to 103 mg kg^{-1}, whereas in OF were found 91, 402 and 326 mg kg^{-1} of Cu, Mn and Zinc (Zn), respectively. Iron (Fe) was almost the double in WB in respect to WW and reported intermediate value in OF, ranging from 290 to 530 mg kg^{-1}. Among the principal trace elements analysed, Cr showed the highest values and was above 2 % in WB, 376 mg kg^{-1} in WW and 34 mg kg^{-1} in OF. However, hexavalent Cr [Cr(VI)] was below the limits of quantification (<0.5 mg kg^{-1}); cadmium (Cd) was below 0.01 mg kg^{-1} in all the three products; nichel (Ni) was 1.9 and 0.3 mg kg^{-1} in WB and WW, respectively; lead (Pb) was 1.4 mg kg^{-1} in WB and 0.6 mg kg^{-1} in WW. Conversely, OF reported values of around 10–1 magnitude order greater than WW and WB (13 and 5 mg kg^{-1} of Ni and Pb, respectively).

Table 1
Main properties of fertilizers obtained from chromium tanned leather (WB), wet white tanned leather (WW), and organic fertilizer from cattle manure (OF).

Properties	Unit	WB	WW	OF
Humidity (water)	% fw	11.1	11.7	7.9
Volatile solids	% dw	79.2	80.8	71.2
pH (water)		3.8	4.8	7.1
Electr. Conductivity (EC)	dS m^{-1}	4.7	3.5	9.4
Total organic C (TOC)	%C dw	39	41.6	33.9
Total Kjeldahl N (TKN)	%N dw	11.3	14.4	3.2
Ammonium-N (NH_4^+-N)	%N dw	0.17	0.31	0.44
Nitrate-N (NO_3^--N)	%N dw	< 0.01	< 0.01	0.02
Organic N (Norg)	%N dw	11.1	14.1	2.8
C/N ratio		3.5	2.9	10.6
Total P	g kg^{-1} dw	0.61	0.06	1.2
Total K	g kg^{-1} dw	0.8	0.8	19
Toal Mg	g kg^{-1} dw	0.03	0.02	7
Total Ca	g kg^{-1} dw	0.3	2.1	n.d.
Total S	g kg^{-1} dw	6	3	6
Total Fe	mg kg^{-1} dw	529	292	391
Total Cu	mg kg^{-1} dw	8.7	5.2	91
Total Mn	mg kg^{-1} dw	21	4.8	402
Total Zn	mg kg^{-1} dw	103	22	326
Total Cr	mg kg^{-1} dw	20884	376	34
Hexavalent Cr (VI)	mg kg^{-1} dw	< 0.5	< 0.5	< 0.5
Total Cd	mg kg^{-1} dw	< 0.01	< 0.01	< 0.1
Total Ni	mg kg^{-1} dw	1.9	0.3	13
Total Pb	mg kg^{-1} dw	1.4	0.6	5

The principal organic contaminants were analysed in WW and WB (Table S1) and mostly reported values under the limits of quantification (<LoQ) of the method, or they were always considerably lower than the thresholds indicated in Reg. (EU) 2019/1009.

3.2. Soil Incubation

3.2.1. Nitrogen release

Fig. 1 shows the results of NH_4^+- and NO_3^--N released during 42 days of the incubation in the soil treated with the different fertilizers WW, WB, OF and MF. Ammonium-N (Fig. 1A) was released into the soil within 21 days. At day 0 higher significant values were found in MF and OF accounting for 91 and 16 % of their TKN content, respectively ($p < 0.001$). Conversely, WW and WB reported not significantly different values in respect to CK. After 7 days, among organic fertilizers WW reported highest values of released NH_4^+-N ($p < 0.001$), however already after 14 days differences between organic fertilizers became negligible and around 60 % of NH_4^+-N in MF resulted converted in NO_3^--N. Nitrate-N (Fig. 1B) showed the first differences among treatments after 14 days, with highest values in MF, WW and ($p < 0.01$). The same trend was maintained until day 42 ($p = 0.001$) at which MF, WW and WB released on average 61, 50 and 36 % of their TKN content, respectively, in comparison with OF (14 %). Fig. 1C reports net mineral-N (NH_4^+-N + NO_3^--N – CK values) release, which were fitted for WW and WB to a 1st order kinetic model described by the equation described in par 2.5. Fitting reported appreciable sensitivity (p-R^2 0.90 and 0.87, respectively, Table S3) although showing not significantly different N_0 values among WW and WB (44.6 ± 3.4 and 39.7 ± 4.5, respectively, Table S3). The estimation of the model is in accordance with the observation at day 42: WW and WB were able to release 40–45 % of their N content with no significant differences ($p > 0.05$).

3.2.2. Chemical indicators

After the soil incubation, an aliquot of each sample was air dried and analysed for the principal chemical indicators. Soil reaction (pH) (Fig. 2 A) was significantly decreased ($p < 0.001$) by all the treatments from 7.9 of the CK soil to around 7.75. Electrical conductivity (EC, Fig. 2B) increased the most (+27 %) after MF treatment, followed by the treatments with WB and WW (+24 %) and OF (+20 %, $p < 0.001$). Extractable metals in DTPA are reported in Table 2. Among the analysed elements, Cu, Fe and Pb did not report differences either among the treatments nor with CK; Zn was increased of 1.5 times only by OF ($p > 0.01$); Cd reported slight significant increases only after OF and WW treatments ($p = 0.008$); Co was increased by OF, WW and WB but not by MF ($p < 0.001$); Cr was increased ~7 times after WB treatment whereas reported intermediate values in the other treatments in respect to CK ($p < 0.001$); Mn was increased by OF and WW treatment of about 30 %; Ni was mostly increased by OF (+20 %) followed by WW and WB (+10 %, $p < 0.001$).

Exchangeable cations are reported in Table 3. Sodium was not affected by the treatments; Ca did not report significant increase (p~0.05) and K was only increased around 18 % by OF ($p < 0.001$); Mg was significantly but slightly increased by MF (+7 %) whereas WW and WB reported intermediate values between CK and MF ($p = 0.002$).

3.2.3. Biochemical indicators

After the soil incubation, some of the principal biochemical indicators were analysed on fresh soil samples. Extractable N (N_{ext}, Fig. 2 C) was significantly increased by all the fertilizers ($p < 0.001$), in particular it was twofold increased by MF in respect to CK and increased of 24, 73 and 59 % by OF, WW and WB, respectively, however with no significant differences among WW and WB. Extractable C (C_{ext}, Fig. 2 D)

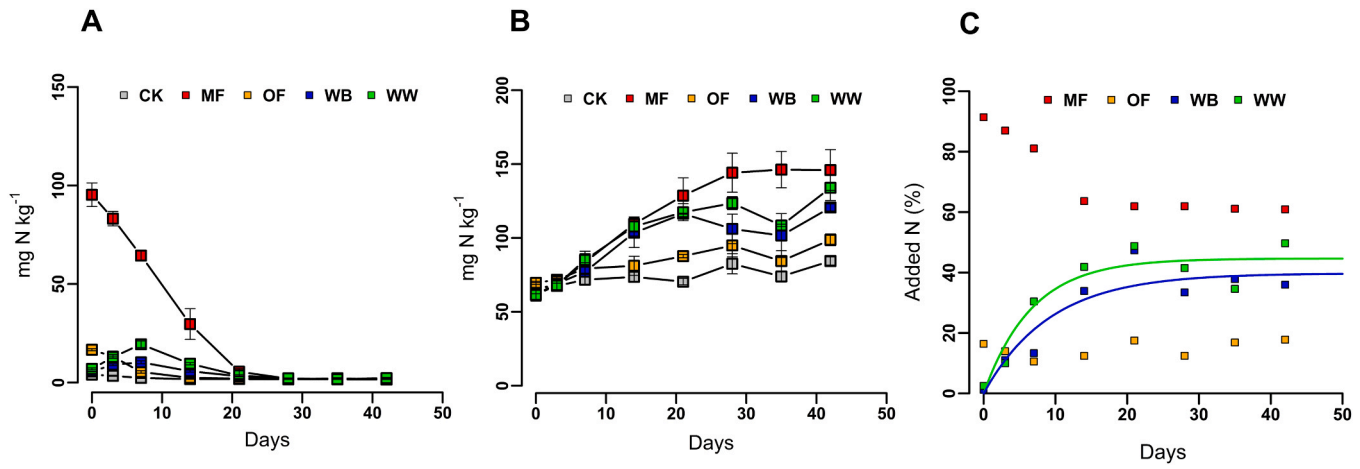

Fig. 1. Mineral-N release as ammonium-N (A), nitrate-N (B) and net mineral N (C) during the incubation period of 42 days. Data are expressed in mg of N per kg of dry soil. CK: control; MF: mineral fertilizer; OF: organic fertilizer; WB: fertilizer from Cr tanned leather; WW: fertilizer from wet white tanned leather. Data are means ± standard error.

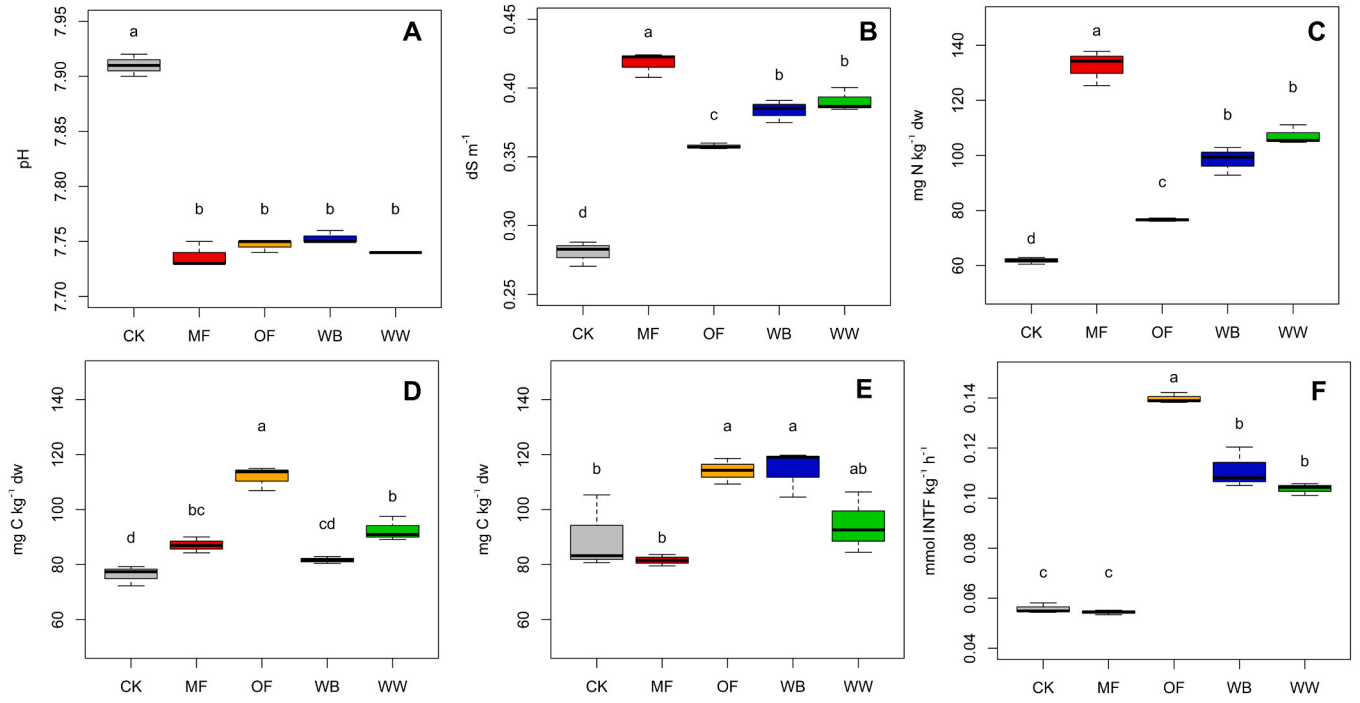

Fig. 2. Chemical indicators: pH (A), electrical conductivity (EC, B); and biochemical indicators: extractable N (N_{ext}, C), extractable C (C_{ext}, D), microbial biomass C (MBC, E) and dehydrogenase enzymatic activity (F) analysed after 42 days of incubation in soil. Data are expressed on dry soil basis. CK: control; MF: mineral fertilizer; OF: organic fertilizer; WB: fertilizer from Cr tanned leather; WW: fertilizer from wet white tanned leather. Data are means ± standard error and different letters indicate significant differences at post-hoc Tukey test (p = 0.05).

Table 2
Extractable metals extracted in DTPA after soil incubation. Data are means ± standard error and different letters indicate significant differences at post-hoc Tukey test (p = 0.05).

	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
	μg kg^{-1}	μg kg^{-1}	μg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
CK	70.8 b	38.9 b	4.5 bc	2.2	10.6	5.54 bc	0.45c	1.49	0.86 b
MF	72.7 ab	36.1 b	2.1 c	2.2	10.8	5.32 c	0.47 bc	1.56	0.99 b
OF	76.4 a	54.4 a	5.2 bc	2.2	11.6	7.36 a	0.55 a	1.59	1.35 a
WW	74.7 a	48.7 a	6.6 b	2.2	11.2	6.61 a	0.50 b	1.59	0.86 b
WB	74.4 ab	47.5 a	31.4 a	2.2	11.1	6.43 ab	0.50 b	1.59	0.96 b

Table 3
Exchangeable cations extracted in hexamine cobalt after soil incubation. Data are means ± standard error and different letters indicate significant differences at post-hoc Tukey test (p = 0.05).

	K	Ca	Mg	Na
	mg kg^{-1}	g kg^{-1}	mg kg^{-1}	mg kg^{-1}
CK	208 b	3,61 b	373 b	0.51
MF	214 b	3,75 a	401 a	0.51
OF	246 a	3,65 ab	383 b	0.52
WW	208 b	3,70 ab	387 ab	0.51
WB	207 b	3,68 ab	386 ab	0.50

was increased the most (+46 %) by OF, followed by WW (+21 %) and MF (+14 %, $p < 0.001$). Microbial biomass C (MBC, Fig. 2 E) was significantly increased both by WB and OF ($P = 0.004$) of about 27 % whereas it was not significantly increased by MF and WW. Dehydrogenase enzymatic activity (Fig. 2 F) reported the highest increase (+150 %) after OF treatment, followed by both WB and WW (+95 % on average, $p < 0.001$).

3.2.4. Community level physiological profiling (CLPP)

After the soil incubation, fresh aliquots of soil samples were extracted to perform CLPP analysis through Biolog® Eco-plates, whose incubation at 25°C lasted 120 h. Data related to microbial growth at the endpoints (120 h) are reported per each group of substrates [31] after 120 h of incubation in Fig. 3.

Carbohydrates consumption (Fig. 3 A) was the highest in WW in respect to CK (+26 %, $p = 0.003$), whereas no differences were detected in MF and WB; OF instead reported intermediate values. Organic acids i.e. carboxylic and ketonic acids substrates consumption (Fig. 3 B) increased in OF and WW (+140 and +160 %, respectively, $p < 0.001$), whereas WB and MF did not report significant differences in comparison with CK. Amino acids consumption (Fig. 3 C) did not report significant differences among the treatments ($p = 0.11$). Polymers consumption (Fig. 3 D) reported significant differences only among MF (-30 % in respect to CK) and WW (+60 % in respect to CK). Amines and amides consumption (Fig. 3 E) was significantly inhibited by MF, OF and WB (-99 % on average). In Fig. 4 are reported data regarding the total average well colour development (AWCD) values of all the substrates grouped together. Total AWCD (Fig. 4 A) revealed that the highest significant increase was observed in WW treatment (+61 %, $P < 0.001$), whereas the other treatments reported no significant differences with CK. However, OF showed 50 % increase in respect to MF. On the AWCD values, microbial diversity indexes (Shannon diversity, Fig. 4 B and Simpson evenness, Fig. 4 C) were calculated. The two indexes followed the same trend, with the only significant differences between WW and MF which reported changes in respect to CK of + 9 % and −5 %, respectively for both indexes ($p = 0.0178$ in Shannon diversity and $p = 0.0183$ in Simpson evenness).

3.2.5. Principal Components Analysis (PCA)

Data obtained after the soil incubation experiment and after the CLPP were subjected to Principal Component Analysis (PCA, Fig. 5) to investigate the differences between the different treatments on 16 variables. A clear separation was observed among the treatments along the two principal components and was confirmed by the one-way ANOVA (Table S4) performed on PC1 ($p < 0.001$), PC2 ($p < 0.001$) and PC3. Strong separation was observed between CK, MF and OF, whereas WW and WB reported the closest relationship in the plot. The direction of the arrows shows the relationships among the different variables and revealed positive correlation among the different substrates after CLPP analysis as well as between exchangeable cations and DTPA extractable metals (Figure S1). CK segregates along PC1 and PC2, particularly toward pH after soil incubation and amines/amides substrates after CLPP; MF treatment segregates the most along PC2 toward exchangeable Mg, extractable N and EC; OF segregates the most along PC1 toward DTPA extractable metals, dehydrogenase activity, extractable C and MBC; WB and WW remained centred in the plot, with WB particularly directed toward extractable Cr in DTPA. One way ANOVA (Table S4) confirms

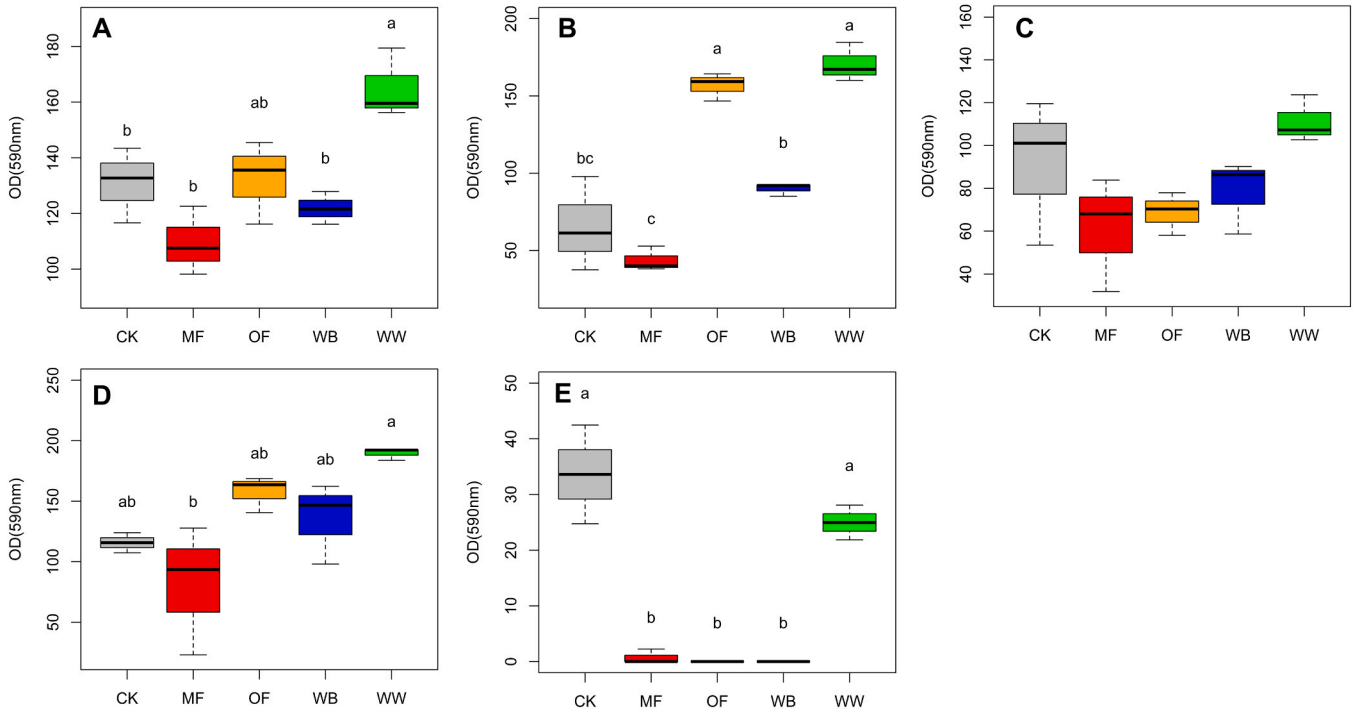

Fig. 3. Results of Community Level Physiological Profiling (CLPP) analysis after 120 h of incubation in Biolog® Eco-Plates divided by group of substrates: A) carbohydrates; B) carboxylic and ketonic acids; C) amino acids; D) polymers; E) amines and amides. CK: control; MF: mineral fertilizer; OF: organic fertilizer; WB: fertilizer from Cr tanned leather; WW: fertilizer from wet white tanned leather. Data are means ± standard error and different letters indicate significant differences at post-hoc Tukey test (p = 0.05).

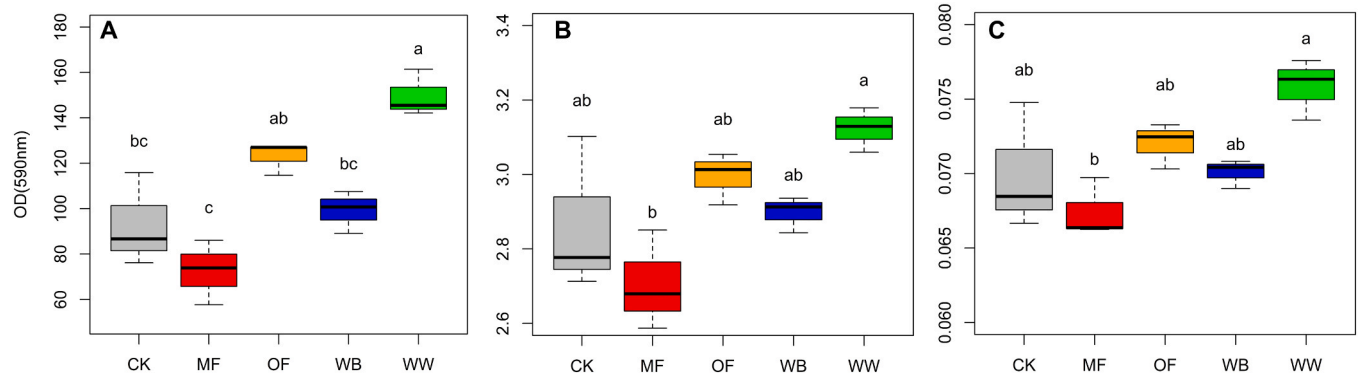

Fig. 4. Results of Community Level Physiological Profiling (CLPP) analysis after 120 h of incubation in Biolog® Eco-Plates: A) total average well colour development (AWCD) from the consumption of the different groups of substrates; B) Shannon diversity index; C) Simpson evenness index. CK: control; MF: mineral fertilizer; OF: organic fertilizer; WB: fertilizer from Cr tanned leather; WW: fertilizer from wet white tanned leather. Data are means ± standard error and different letters indicate significant differences at post-hoc Tukey test (p = 0.05).

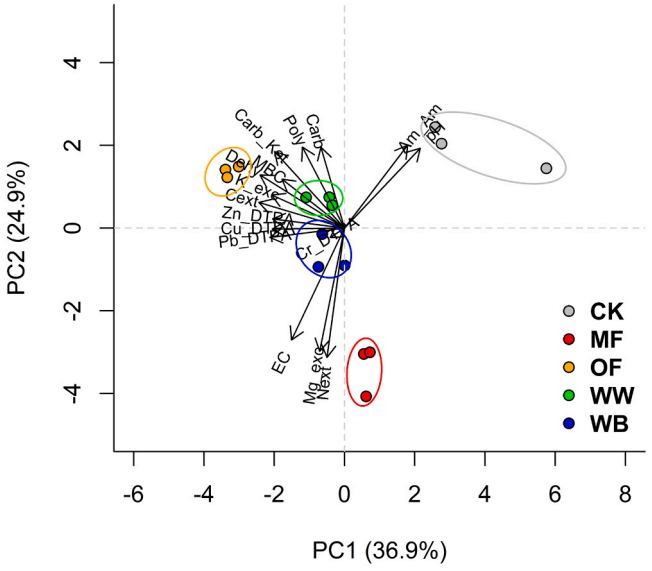

Fig. 5. Results of Principal Component Analysis (PCA) performed on 16 variables from data after soil incubation and after CLPP. CK: control; MF: mineral fertilizer; OF: organic fertilizer; WB: fertilizer from Cr tanned leather; WW: fertilizer from wet white tanned leather.

that the greatest significant separation among the treatments occurred along PC2 ($p < 0.001$). Nevertheless, WW and WB did not report significant differences either in PC1 or PC2 whereas they did only in PC3 (14.3 % of the explained variance) which is not showed in the graph (Table S4).

3.3. Trace elements bioavailability in Rhizotest

After Rhizotest, plant material and soil were independently sampled and analysed through ICP-OES for total trace elements. Among the trace elements analysed (Table 4), Cr showed significant differences ($p < 0.001$) in the soil with mean values around 40 % higher in WB than WW and REF, the latter not reporting differences with reference untreated soil (REF). Conversely, Cr did not report significant differences in plant samples, indicating that no absorption from soil to plant occurred either in WB or in WW. In the soil samples, WW reported a slight increase of Zn (<10 %) in respect to REF even with low significance ($p = 0.03$). However, in plant samples, Zn was decreased of ~55 % in WB and WW in respect to REF ($p = 0.001$), indicating a decreased bioavailability. Lower values of Mn in plants were also reported in WB in respect to REF (-40 %) even though with low significance ($p = 0.03$).

4. Discussion

4.1. Organic fertilizers characterization

The two fertilizers from tannery residues, WW and WB, reported similar physic-chemical characteristics (Table 1), particularly in the case of pH and EC that exhibited lower values than OF. The pH of tannery

Table 4
Results of elemental analysis through ICP-OES in soil and in plant tissues after the exposure in the Rhizotest with the two products. REF: unfertilized soil reference; WB: soil with fertilizer from Cr tanned leather; WW: soil with fertilizer from wet white tanned leather. Data are means and different lowercase letters indicate significance at one-way ANOVA after post hoc Tukey test ($p < 0.05$). Negative values in Mo refers to lower concentrations in plants after the contact with soil than in plants after hydroponic growth.

Plant	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Mo**	**Ni**	**Pb**	**Zn**
	μg kg^{-1}	μg kg^{-1}	μg kg^{-1}	μg kg^{-1}	μg kg^{-1}	μg kg^{-1}	μg kg^{-1}	μg kg^{-1}	μg kg^{-1}	μg kg^{-1}
REF	0.61	1.3	2.2	6.7	359	28.3 a	−0.4	2.8	0.42	26.1 a
WW	0.60	1.2	1.2	8.1	288	22.7 ab	−0.4	3.5	0.40	12.6 b
WB	0.56	1.1	1.6	5.9	198	17.7 b	−0.4	2.9	0.36	11.0 b
p-value	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	0.03	> 0.05	> 0.05	> 0.05	0.001
Soil	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Mo**	**Ni**	**Pb**	**Zn**
	μg kg^{-1}	μg kg^{-1}	μg kg^{-1}	μg kg^{-1}	mg kg^{-1}	μg kg^{-1}	μg kg^{-1}	μg kg^{-1}	μg kg^{-1}	μg kg^{-1}
REF	0.24	12.2	42.7 b	22.4	19.3	489	1.0	33.8	16.6	67.7 b
WW	0.23	12.1	42.5 b	22.3	19.1	487	1.0	33.3	16.0	74.5 a
WB	0.24	12.4	60.7 a	22.7	19.5	500	1.0	34.0	16.8	69.3 ab
p-value	> 0.05	> 0.05	< 0.001	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	0.03

residues can vary depending on the raw material composition, but pH below 7 was often reported in tannery residues [96,100], probably due to the use of acids in the tanning process [19], whereas EC is generally reported to be much higher than what found in WW and WB [100]. Nevertheless, the values found in WW and WB for pH and EC were in the range of other commercial organic fertilizers and fertilizers from recycled materials [43,63,79,96].

Total organic carbon (TOC) content strongly depends on the raw material chemical characteristics and on the stability reached by the final product [72]. Total organic C ranged from 34 % to 42 % in the three organic fertilizers tested, similar to what reported by other authors for commercial organic fertilizers [63]. In WW and WB, TOC was very similar (42–39 % respectively), in agreement with other findings for these kind of organic fertilizers (14–40 %, [79]).

Total N (TKN) of WW and WB was of 11–14 % on dry weight (dw), which was higher than OF (3 % dw) and other organic fertilizers reported in literature (2–11 % dw, [63]), and among the highest values reported for tannery residues (0.9–14 % dw, [43,67,96,100]). The higher amount of N in respect to the other fertilizers is due to the massive protein content of leather wastes such as hair and skin that for this reason have an intrinsic potential to be used as fertilizers in circular-business models [17]. Total P content was always lower than OF (1.2 g kg^{-1} dw) and was 0.6 and 0.06 g kg^{-1} dw in WB and WW, respectively, 10–100 times lower than what reported by Isalm et al. [43]. However, WB showed total P in the same ranges reported by Mikula et al. [62]. The other macronutrients reported the same range of other tannery wastes [62], with K and Mg lower than OF, whereas S was in the same range. Micronutrients and heavy metals are the most occurring contaminants in tannery residues [48,96]. In WB and WW, Cu, Mn, Zn, Ni and Pb were 5–500 times lower than OF and other commercial organic fertilizers [56,63,96], whereas Cd was below the detection limit (<0.01 mg kg^{-1} dw) and Fe in the same range of OF and other organic fertilizers [63]. Chromium was around 2 % in WB coherently with Cr-tannery wastes [62], whereas in WW it was 0.04 % i.e. ten times higher than OF, which might be due to residues on the plant. Critical concentrations of Cr content are reported only in WB, coherently with the raw material represented by Cr-tanned leather residues. On total Cr, the most present forms in tanned leather residues are Cr(III) and Cr(VI) [19]. The Cr(III) form is not mobile and insoluble, and is present into complex with organic matter or other compounds which tend to precipitate, particularly in alkaline medium; Cr(VI) instead is soluble and mobile [19,73]. The most toxic form is represented by Cr(VI), whereas Cr(III) does not possess high intrinsically environmental risks [73,96]. Nevertheless, a high amount of Cr(III) in the soil environment (>100 mg kg^{-1}) is not recommended as it can oxidise and form Cr(VI) in soil, particularly in presence of Mn oxides that may function as catalysts [4,78], and because of pH or redox-potential changes [41]. Hexavalent Cr has a much higher soil−plant transfer index in respect to Cr(III) [42,58], therefore the accumulation of Cr should be as less as possible to avoid possible phytotoxicity and accumulation in crop plants [97]. Hexavalent Cr in WB and WW was always below the detection limits (0.5 mg kg^{-1} dw), moreover the soil used in this study reported a redox potential for Cr of 0.024 μmol Cr(VI) (Table S2). A soil warranting caution would report values > 1 μmol Cr(VI), as reported in Italian law 99/92 [22] implementing Council Directive 86/278/EEC [27], therefore our results discouraged the diffusion of Cr(VI). However, further investigations regarding plant uptake of Cr were performed through Rhizotest. Organic contaminants were mostly under the detection limits (Table S1) preventing the contamination after their application to the soil.

4.2. Soil incubation

4.2.1. Nitrogen release

The fertilizers WB and WW were incubated in the soil for 42 days to monitor the release of mineral N. As positive controls, an organic (OF) and mineral fertilizers (MF) were included. Ammonium-N was released within the first 21 days of incubation with a trend specular to NO_3^--N release, indicating that nitrification was occurring [37,83]. MF always reported higher values of mineral forms of released mineral-N as expected since it was constituted by DAP, but among the other fertilizers higher ammonium-N release at day 0 was observed in OF in respect to WW and WB, reflecting their ammonium content (13.8 %, 2.2 % and 1.5 % on total N, respectively, Table 1). Conversely, nitrate-N was the highest in WW and WB already at 14 days, and the trend was maintained until day 42. Trend of mineral-N release of WW and WB followed a I order kinetics model and reported not different values of N_0 and k, indicating that they have the same mineralization rate [95]. These values are in the same range of other organic fertilizers fitted to similar models, such as poultry manure (3–75 %) and poultry manure compost (27–54 % [36]) and fertilizers derived from leather and tannery residues (11–44 % [79]). Net mineral N-released by MF and OF did not follow the I order kinetics model, however in MF tended to decrease during the first 14 days of incubation whereas in OF it remained stable. Volatilization of ammonia in MF fertilized soil samples cannot be excluded to occur during the first two weeks, as soil type, soil nutrient imbalance (the soil used was very poor in available P content, Table S2), high pH and high ammonium-N content in the fertilizer can contribute to this phenomenon [93]. Despite the higher content of NH_4^+-N in OF, the steady net mineral-N release is typical of slow release fertilizers [13], therefore 42 days in the soil used could not be enough to observe the I order kinetics.

4.2.2. Chemical indicators

At the end of the incubation, chemical indicators such as pH, EC, extractable metals in DTPA and exchangeable cations were analysed. pH was decreased by all the treatments according to the lower pH of the fertilizers in respect to the soil and can be further due to the organic acids released by the decomposition of organic materials [2]. EC was instead increased by all the fertilizers, but mostly by MF, according to their content of soluble forms of N (Fig. 2B, C) [12,28,49]. However, according to the characterization, EC values in soil are always below the toxicity limits in range of 0.75–3.49 dS m^{-1} [1], minimizing the risks for soil salinization.

Environmental risks associated to fertilizers produced from tannery residues are mostly related to the presence of heavy metals such as Co, Cu, Zn, Ni, Pb, Cd and Cr; these contaminants can be leached into the soil and groundwater, being absorbed by organisms inhabiting soil, causing toxicity and eventually representing risks for health consequent to crops consumption [44]. Chromium is considered as one of the main concerns associated to these kind of fertilizers [19,50,69]. In both WW and WB, the only critical element concentration was represented by Cr, therefore we focused on this element along the study. After the incubation, DTPA-extractable Cr significantly increased in the soil treated with WB (Table 2), and this was expected, considering the presence of 2 % of total chromium in this fertilizer whose soluble forms can be released within 40 days to the soil [20]. Conversely, WW reported a 10-times higher Cr content than OF, probably due to contamination related to the production plant. However, the release of DTPA-extractable Cr in the soil was not significant for WW.

These observations are in accordance with the presence of Cr in soil treated with WB after Rhizotest (Table 4). However, even though total Cr in soil after Rhizotest was increase up to ~60 μg kg^{-1} by WB treatment, after the incubation DTPA-extractable Cr was only 31 μg kg^{-1}(Table 2). Since DTPA-extractable fraction would represent a good predictor for phytoavailable Cr [74] it is reasonable to assume that is still very low [34] and however do not present risks for accumulation in plants, as evidenced by Rhizotest (Table 4, [80]). Concerning other DTPA-extractable metals, Co, Cd and Ni are generally increased after the three organic fertilizers application, as it was reported for many organic fertilizers [56,63]. Threshold concentrations of DTPA-extractable metals reported in literature for contaminated soils were 0.1 and 1.0 mg kg^{-1} for Cd and Ni, respectively [98], and 0.5 g kg^{-1} for Co [65]. Therefore,

we can assess that at agronomic doses, none of the tested fertilizers reported an accumulation in the phytoavailable pool of heavy metals. However, it cannot be excluded that organic matter content of organic fertilizers could globally mobilize heavy metals contained in soil even without provide any significant addition of heavy metals [76], so that metal accumulation remains an important concern to evaluate when substituting mineral fertilizers with organic ones. Exchangeable cations measured after the incubation were poorly affected by the treatment with organic fertilizers (Table 3), as previously reported [21]. In fact, significant increases were reported after MF treatment for Ca and Mg, as expected for an inorganic fertilizer, whereas only OF increased K levels. No significant variations were also observed in K/Mg ratio, which however in the soil used were ranging from 0.5 to 0.7, deviating from the value of 2, considered ideal for the fertility of the soil [54,7].

4.2.3. Biochemical indicators

After the incubation, aliquots of fresh soil samples were analysed for the principal biochemical indicators of soil fertility. The fertilization of the soil with mineral N generally increase soluble N forms in the soil (N_{ext}, Fig. 2 C) whose data are coherent of what observe at the day 42 after the incubation (Fig. 1). Representing the soluble N forms, values of N_{ext} are highly correlated with EC values ($r = 0.92$, $p < 0.05$, Figure S1), whereas no significant correlation with soluble C (C_{ext}) and MBC were observed. In fact, the effect on C_{ext} depends on the organic matter origin of the fertilizer, the applied C dose and on the amount of soluble C forms [32,61]. Increase of C_{ext} is evident after organic amendments application [15] as OF, which mostly increase C_{ext} in respect to CK, whereas WW and WB reported values similar to MF, suggesting them to act more as organo-mineral fertilizers [77]. Microbial biomass carbon (MBC) is generally increased by organic fertilizers but not by mineral ones [51]. After the incubation, MBC was only significantly increased by OF and WB, with intermediate values reported by WW. This could be related to the different amount of soluble C sources [51], as observed for values of C_{ext}. However, overall provided organic C was different for each fertilizer, since doses of application were calculated on total N. Dehydrogenase enzymatic activity represents an index of an overall microbial activity [10] and its increase is normally associated with an increase in C_{ext} [8]. Dehydrogenase resulted to be positively correlated with MBC and C_{ext} ($r = 0.75$ and 0.72, respectively, $p < 0.05$, Figure S1) as previously reported [15], indicating that the addition of organic fertilizers OF, WB and WW increased the overall microbial activity, whereas MF did not. These findings revealed that both WW and WB produced stimulant effects on the biochemical indicators in line with OF and other organic fertilizers.

4.2.4. Community level physiological profiling (CLPP)

Community level physiological profiling (CLPP, Fig. 3) can detect changes in the soil microbial community metabolic functions based on the consumption profiling of different carbon sources [9]. Generally, organic acids and carbohydrates were reported to be the most utilized carbon sources by soil microorganisms, whereas amines and amides were the least utilized carbon sources [45,88]. However, in field studies plant activity can dramatically change microbial substrate consumption profiles [45]. Globally, a tendency for most substrates groups is to be decreased by conventional practices i.e. mineral fertilization in respect to organic fertilization [88]. Our data partially agree with this tendency, particularly in this direction for organic acids degradation. Most of the evidence of changes in microbial community metabolic profiling are dependent on environmental factors [82] rather than on a fertilizer type [31]. Therefore, one must be cautious when using CLPP in evaluating microbial community metabolic changes. However, considered the relative convenience of this technique in terms of rapidity and costs it remains a sensitive analysis to detect important metabolic shift in main heterotrophic bacteria groups in soil [40,82,92]. Total AWCD values, as well as Shannon Diversity and Simpson Evenness indexes (Fig. 4), remark the pattern observed by carbohydrates, organic acids and polymers substrates degradation, suggesting that these three groups of substrates could be the main predictors of metabolic shifts in CLPP. In diversity and evenness indexes the only difference detected was between MF and WW, which probably better represents mineral and organic fertilizers, respectively, in this trial [88]. Finally, Cr accumulation in soil is known to select microbial biomass species [5]. We observed significant shifts between WB and WW but not between WB and CK soil, suggesting that shifts of principal metabolic pathways were not related to Cr content in WB.

4.2.5. Principal component analysis (PCA)

To summarize the data, principal component analysis (PCA) was performed on data produced from chemical, biochemical indicators and on degradation of substrates groups after CLPP (Fig. 5). Together, the two principal components explain up to 62 % of the observed variance, which represents an acceptable approximation of the total variance [47]. Major diversity among treatments was observed in PC2, in which a clear separation of each treatment is observed except amongst WW and WB, as showed after one-way ANOVA performed on PCA scores (Table S4). Most contribution in PC2 were given by exchangeable Mg (-0.43), Next (-0.45), EC (-0.39) and amines and amides substrates (0.29, Table S5). In fact, these parameters resulted mostly impacted by MF and are typically affected by mineral fertilization. Therefore, a minor separation in PC2 of OF and WW can be related to their organic nature. Conversely, major separation on PC1 was observed among CK and OF and most contributors of PC1 were dehydrogenase activity (-0.34), extractable C (-0.35), DTPA extractable Pb (-0.30) as they are parameters typically related to organic fertilizers. In PCA, WW and WB reported the highest similarity among the treatments in our experiments. Our findings suggest that, after their application to the soil, fertilizers produced from hydrolysed animal epithelium as a tannery by-product would act similarly with no substantial differences depending on the tanning agent used during leather production.

4.3. Chromium uptake in Rhizotest

Rhizotest bioassay (ISO1698:2015) allows the estimation of the bioavailability of micro- and trace elements in a device in which plant roots are separated from soil through a 30 μm polyamide mesh, ensuring the collection of plant roots without contamination from soil particles [11]. After Rhizotest, trace elements were not considerably accumulated in the soil and not absorbed by plants, event tough an accumulation in the soil was observed for total Cr in WB (Table 4). No Cr uptake in plants was observed, since plant levels of Cr did not vary among the treatment and the reference (Table 4). Studies demonstrated the low solubility and mobility of Cr III in the soil environment [29,59]. Its poor mobility leads to a very reduced uptake of Cr in plant tissues [50,94], of which however the preferred absorbed form is Cr VI through transporters of other essential elements [97]. Nevertheless, even though in these conditions Cr can be taken up by plant roots, it is poorly translocated and largely retained in roots [19,86]. The majority of soils hold Cr naturally in the range of 10–100 mg kg^{-1} [78], whereas plant Cr toxicity in most higher plants occurs around 5 mg kg^{-1} plant dry weight [24], which is roughly 10^3 times higher than what observed in our experiment. Our data revealed that, at agronomical doses of application, accumulation of Cr in plants and soil using WW and WB as fertilizers is negligible. However, application rates of fertilizers from tannery and leather residues must be controlled, since continuous application to agricultural soils could lead to critical Cr concentrations over the years. It was observed that a significant reduction of the microbial biomass growth and activity would occur after 10 years at rates of 10–20 tons ha^{-1} [5,6], that would be 3–7 times higher than the dose suggested by the fertilizer producers and used in this study. Beside our positive findings concerning the not significant bioavailability of Cr for tomato plants after the utilization of WW and WB, inherent limitations connected to the utilization of Rhizotest are: i) the very short exposure time (8-days) cannot capture chronic exposure

effects that could eventually trigger the uptake of Cr even at low concentrations; ii) different plant species, particularly graminaceous plants, can respond differently to the same treatments at the same dosage. Concerning the utilization of Rhizotest to evaluate organic fertilizers produced from tannery and leather residues, further investigations should contribute to clarify these aspects.

5. Conclusions

The two fertilizers tested were constituted by hydrolysed animal epithelium produced from wet blue (WB) and wet white (WW) tanning residues. Their physico-chemical characteristics were in range of organic fertilizers and revealed that they were compatible with their application to the soil. In fact, TOC was around 40 % for both products, whereas TKN was 11 and 14 % in WB and WW, respectively, of which 98 % was in the organic form due to their protein content. The heavy metal content was negligible, except for total Cr which was $> 20{,}000$ mg kg^{-1} in WB. However, Cr (VI) was below the detection limits. The soil incubation experiment revealed that WW and WB released up to 40–45 % of their TKN fitting a first order kinetics model. At the end of the incubation, pH was significantly affected in respect to the untreated soil, decreasing from 7.90 to 7.75; however, chemical parameters did not report great variations after the addition of the fertilizers. Extractable Cr in DTPA was increased by WB up to 31 μg kg^{-1}, which represents a fraction of the added Cr through the fertilization. Biochemical indicators revealed a stimulant effect on dehydrogenase activity, N_{ext} and MBC. Moreover, CLPP revealed that WW and WB shown similar functional profiling and the variations occurred among the two fertilizers were not caused by the presence of Cr in WB but mostly by their C content and soluble forms. Rhizotest suggested that the bioavailability of Cr was extremely low, discouraging tomato plants to absorb it from the soil. In the soil after Rhizotest, total Cr reached ~ 60 μg kg^{-1} which can be considered a low concentration in the range of total Cr naturally present in soils. We can assess that the two fertilizers produced by tannery residues which used different tanning agents were equally effective in providing N to the soil and stimulating the microbial community activity, without any critical concerns represented by Cr bioavailability. However, the short-term nature of the study cannot rule out the mechanisms underlining the effects of chronic exposure to Cr containing fertilizers on plant Cr-accumulation and microbial community shifts after continuous application. Future perspectives should include experiments designed to extend the results obtained in this study on long-term pot, greenhouse and field experiments, using different plant species from a broad range of taxas. Moreover, the sole bioavailability of total Cr in the whole plant cannot outline the potential risks associated to Cr accumulation in plant tissues. Therefore, even at low Cr bioavailability, further experiments should also focus on the speciation of Cr in different plant tissues after the contact with soil fertilized with these kinds of fertilizers, other than investigating root exclusion and sequestration mechanisms in soils, possibly under different pedo-climatic conditions.

CRediT authorship contribution statement

Giampaolo Di Biase: Investigation, Data curation. **Andrea Ciurli:** Writing – original draft, Investigation, Formal analysis, Data curation. **Luciano Cavani:** Writing – review & editing, Investigation, Data curation, Conceptualization. **Claudio Ciavatta:** Funding acquisition, 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. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jece.2025.118990.

Data availability

Data will be made available on request.

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