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Fertilisation with compost mitigates salt stress in tomato by affecting plant metabolomics and nutritional profiles

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Abstract

Background: Salinity is one of the major threats for crop growth and yield and its rate of expansion is expected to increase. We conducted a pot experiment to evaluate and compare the effect of a green compost addition and mineral fertilisation, on growth, nutrition and metabolites of tomato plants, exposed to increasing doses of NaCl.

Results: Although the development of stressed plants was lower than the corresponding controls, compost-treated plants performed better than mineral-amended plants watered with the same amount of salt. The different plant growth was related to an increased nutritional *status*. Namely, compost-treated plants showed a larger content of macro- and micronutrients, and a greater accumulation of osmoprotectants, such as soluble sugars and amino acids. Moreover, compost-treated plants showed a larger content of metabolites involved in modulating the response to salt stress, such as molecules related to energy transfer in plants and precursors of Reactive Oxygen Species scavenging compounds. Overall, the better performance of compost-added plants may be attributed to a greater availability of the organic forms of nutrients and to the positive bioactivity of compost-derived humic substances.

Conclusions: Compost application efficiently mitigated salt stress in tomato plants in respect to mineral fertilisation. This alleviating role was associated to the induction of a more efficient metabolic response that increased accumulation of metabolites involved in modulating the salinity stress. Therefore, fertilising with composted agricultural residue may represent a convenient alternative to mineral fertilisers to grow tomato plants in the presence of salt stress.

Keywords: Organic amendments, Salinity, Stress tolerance, Gas chromatography mass spectrometry (GC–MS), Soluble sugars and amino acids, Plant signalling, Metabolic reprogramming

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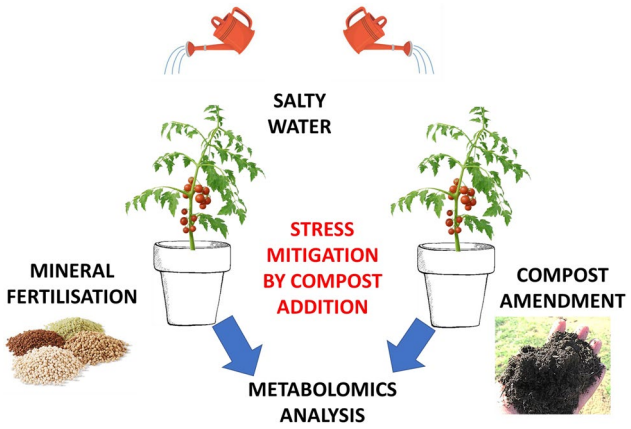
Graphical Abstract



Introduction

During their life cycle crops often face various stresses, which may significantly limit their development and thus the overall productivity. Among the most relevant stresses, salinisation induced by agricultural activity is a problem for at least 75 countries around the world [1]. Soils of Mediterranean countries are particularly prone to salinisation, as fields are frequently located close to areas with high urban and industrial concentration, wherein most of good-quality water is consumed, whereas poor-quality water (deriving from sea, urban wastewater or industrial effluents) is reserved for irrigation purposes. Furthermore, the massive use of fertilisers determines salt accumulation in surface or groundwater used for agricultural and civil purposes [1]. These pitfalls, combined with large water evaporation rates from soils and scarce rainfall in summertime, lead to a net increase of salts in soils [2]. Excessive soil salinity is one of the abiotic factors that limits plant growth and fruit production, due to a negative impact on the physical and chemical properties of soils [3, 4]. Moreover, salinity causes osmotic stress, inhibits photosynthesis and respiration and leads to nutritional imbalances [5].

The growth and yields of food crops, such as tomato (*Solanum lycopersicum* L.), are negatively impacted by soil salinisation [6, 7]. In fact, although this crop has been classified as moderately sensitive to salinity, tomato production is significantly reduced by salt accumulation in soil [8]. Various approaches were proposed to address this soil degradation, such as cultivating salinity tolerant plants or addition of improvers to restore soils [8, 9]. A promising practice is the application of composted organic matter that alleviates salt stress by increasing soil water retention and reducing the number of applications of saline irrigation water [9]. Moreover, compost fertilisation increases the amount of soil humus, thus improving the overall soil chemical and biological properties, by supporting soil microbial activity and fast cycling of plant nutrients [9].

However, there is not yet complete understanding on how compost application affects plant physiology and metabolic profile. Therefore, the aim of this work was to study, under different rates of salinity stress, the phenological and physiological impact of a green compost addition on tomato plants growth, in comparison with conventional mineral fertilisation, through a metabolomic investigation.

Materials and methods

Compost production

Green compost was produced in the composting facility of the Experimental Farm of the University of Naples, located in Castel Volturno (CE). Residues from the cultivation of artichoke were mixed with corn straw and wood chips from pruning at 70/30 (*w*/*w*). A pile of this mixture was then placed on perforated rubber tubes through which the air was periodically blown by a rotary pump, to ensure a correct biomass aeration and promote its aerobic transformation. The composting process included the thermophilic and mesophilic phases, as well as a final maturing period, and lasted a total of 100 days. The resulting compost was air-dried, sieved (2 mm mesh) and stored at 4 °C until further use [10]. The concentration of nitrogen, phosphorus, potassium and ash content in compost is reported in Table 1.

Plant growth and NaCl application

The experiments were set as it follows: 3 L pots were filled with 2.5 kg of a clay-loam soil mixed with quartz

Table 1 Elemental analysis of composted artichoke residues (g. 100 g compost^{-1})

N	2.3
P	0.34
K	2.8
Ash	48

sand in a 2:1 ratio. Then, mineral fertilisers (MIN) or artichoke compost (COM) was thoroughly mixed to the soil. The compost was mixed with the soil at the rate of 25 t ha^{-1}. The mineral fertilisers added were 600 mg pot^{-1} N as NH_4NO_3, 90 mg pot^{-1}P as KH_2PO_4 and 620 mg pot^{-1} K as K_2SO_4, to provide the same amount of nutrients added to the pots with artichoke compost. Soil pots were placed in covered plastic boxes in a greenhouse at 20 ± 2 °C for 30 days. Tomato plantlets (*Solanum lycopersicon* L., cv. Pixel) were transplanted at the fourth true leaf stage. The experiment was carried out in a randomised block design with six replicates per each treatment. Six treatments were applied, including two fertilisation regimes (mineral−−MIN; compost−−COM) combined with three rates of NaCl (0, 40 and 80 mM). After 90 days, leaves and stems were collected, frozen in liquid N_2, freeze-dried and then weighted to evaluate the dry plant biomass.

Elemental analysis of plant material

Dried tissues (0.5 g) were weighted into PTFE vessels and digested in 6 mL of HNO_3 (65%), 2 mL of H_2O_2 and digested in a microwave (Milestone, Digestor/Dring Ethos 900). The volume of the solutions was then brought up to 25 mL by using Milli Q water and Ca, Mg, Zn, Mn, Fe, K and Na were analysed by atomic absorption spectrophotometry using an air-acetylene flame (Analyst 700; Perkin Elmer, Waltham, MA, USA). P content was determined applying ammonium molybdate-based reagent. The solution was then added with ascorbic acid to form a blue complex, and its absorbance read at 880 nm. Finally, leaf N concentration was measured by EA 1108 Elemental Analyzer (Fisons Instruments, Glasgow, UK).

Extraction and identification of primary metabolites

Aliquots of leaves (30 ± 0.5 mg) of plant material were weighted, and metabolites extracted in a 1 mL of water/methanol/chloroform pre-cooled solution (1:3:1 ratio), which also contained 20 µg mL^{-1} of ribitol as internal standard. Samples were incubated for 15 min at 70° in order to inhibit any enzymatic activity. The extracts were centrifuged (10 min × 9500 g at 4 °C) and the supernatants transferred into 2 mL Eppendorf tubes. Pure water (400 μL) was then added to separate methanol/water from chloroform. The solutions were centrifuged again (10 min ×9500 g at 4 °C), and an aliquot (400 µL) was collected from the water/methanol phase, transferred into 1.5 mL glass tubes for GC–MS analyses, dried under a N_2 flow and stored at − 80 °C. Sample derivatisation was carried out by dissolving the extract in 50 μL of a 20 mg mL^{-1} methoxyamine hydrochloride solution in pyridine and allowed to react at 30 °C for 90 min under gentle shaking. Then, samples were further derivatised for 30 min at 37 °C by adding 50 µL of N-methyl-N-trimethylsilyltrifluoroacetamide. Then, 2 µL of the derivatised samples were injected into the GC–MS by using a split mode. GC–MS analyses were carried out in an Agilent7683B Series Injector coupled to an Agilent HP6890 Series GC system and a 5973 Mass Selective Quadrupole Detector. The separation was achieved by an RTX-5MS WCOT capillary column (Restek, 30 m×0.25 mm; film thickness, 0.25 mm). The run comprised 2 min isothermal phase at 80 °C, followed by an increase to 310 °C by a 15 °C min^{-1} rate and a 10 min isothermal phase. Helium was used as carrier gas at 1 mL min^{-1}, while the injector temperature was set at 250 °C and the split injection mode at 25 mL min^{-1}. Mass spectra were obtained in EI mode (70 eV), scanning in the range included within 50 and 650 m/z, with a cycle time of 0.2 scan s^{-1}. Metabolite identification was carried out by comparing their retention time either with that of standard compounds or that found in molecular libraries [11].

Extraction and identification of salicylic acid (SA) and abscisic acid (ABA)

The extraction and subsequent derivatisation steps were performed as described by Rawlinson et al. [12] with some modifications. Approximately 50 mg of previously freeze-dried material was transferred into 2 mL Eppendorf tubes to which 400 µL of NaOH (1% *w*/*v*), 300 µL of methanol and 50 μL of pyridine were added. In this step, nonadecanoic acid methyl ester (20 µL of 20 µg mL^{-1}) was added as an internal standard. The tubes were vortexed for 30 s and incubated at 4 °C for 10 min. Samples were then centrifuged at 9500 g× 3 min, and 350 µL of supernatant was collected and transferred to another 1.5 mL Eppendorf tube. Methyl chloroformate (20 µL) was added to the supernatant and the solution vortexed for 30 s. Another 20 µL methyl chloroformate aliquot was added to the mixture and vortexed again for 30 s. Then, 350 µL of chloroform was added to the tubes, which was vortexed for 15 s before adding 400 µL of a 50 mM bicarbonate solution. The mixture was vortexed (15 s) and centrifuged (9500 g ×3 min) to separate the organic phase from the aqueous one. The organic phase containing the hormones of interest was transferred to new tubes containing a few crystals of anhydrous $NaSO_3$.

Finally, 100 µL of dehydrated extract was transferred to a glass vial for GC–MS analysis. The equipment used in the analysis was the gas chromatograph (Agilent 7890A), equipped with an ALS autosampler (Agilent ALS7683B) and coupled with a single quadrupole mass spectrometer (Agilent MSD5975). The chromatographic separation was obtained in a 5% Phenyl Methyl Siloxane column (Agilent HP-5, 30 m × 0.25 mm; film thickness, 0.25 µm). The injection volume was 1.5 µL while the injection temperature and transfer line were both at 250 °C. The injection pulse pressure was set at 35 psi until 1 min. The oven temperature programme had the column held at 50 °C for 3 min, then increased at a rate of 4 °C min^{-1} up to 200 °C, maintained for 1 min and increased to 300 °C with a rate of 10 °C min^{-1} and with a final 2 min isotherm (total run time 53.5 min). Helium was used as carrier gas at 1.00 mL min^{-1}. The electron ionisation (EI) source and the MS quadrupole were maintained at 230 and 150 °C, respectively. The MS was firstly run in a scan mode of acquisition in order to determine the retention time and select the appropriate EI mass fragments for each analyte. Subsequently, a Single Ion Monitoring (SIM) mode was used to monitor the specific fragment ion for the semi-quantitative analysis of each analyte. The dwell time was 30 ms and each analyte had its own SIM window. Results are expressed as relative values obtained from the ratio between the areas of the quantifying ion selected for each hormone, and the area of the quantifying ion of the internal standard, all normalised on the basis of the initial weight of the plant material.

Statistical analysis

Bot the phenological and metabolomics data were found to be normally distributed (according to the Shapiro–Wilk test). Significant differences between the means were determined by one-way and two-way analysis of variance, while application of LSD test to differentiate among results was given at the $P < 0.05$ probability level using the XLStat software v.9.0 (Addinsoft). The semi-quantitative evaluation of GC chromatograms was obtained by normalising the area of each peak to the area of the internal standard and further referring it to the sample fresh weight (mg). The Principal Component Analysis (PCA) was used here to reduce the dimensionality of the dataset and concomitantly preserve the useful information expressed in terms of variable variance. The XLStat software version 9.0 (Addinsoft) was used to process the PCA of the total dataset.

Results

Plant growth and nutrient uptake

Compost fertilisation significantly impacted on plant growth, regardless of the fertilisation regime applied (Table 2). Indeed, control plants added with compost showed a larger dry biomass than those amended with the mineral fertilisers. Also, despite the decrease in development observed in NaCl-treated plants, those fertilised with compost always showed a significantly larger biomass than the one amended with mineral fertiliser. Interestingly, the lower stress level of plants amended with the organic fertiliser is also pointed out by the better performance of compost-fertilised plants and watered with the largest NaCl dose (COM-80 mM) compared to those watered with the intermediate NaCl concentration and added with mineral fertiliser (MIN-40 mM) (Table 2).

Watering with saline water determined a dose-dependent increase in the concentration of N, Na, Ca, Mg, Cu and Zn, as compared to control treatments, whereas P concentration decreased (Figs. 1, 2, 3). On the contrary, K concentration was reduced only for mineral-fertilised plants and for compost-amended plants watered with 40 mM NaCl, while that of COM-80 mM did not significantly differ from the corresponding control (Fig. 2). Furthermore, Mn levels increased for plants amended with mineral fertilisers, whereas *non*-significant differences in its concentration were found for compost-added plants. Similarly, Fe concentration was not affected by of saline water in plants amended with mineral fertilisers, whereas it increased in plants added with compost (Fig. 3).

Contrary to their concentrations, nutrient content decreased with increasing NaCl doses (Figs. 2, 3). Also, the content of N, Fe and Ca was greater in control plants fertilised with conventional mineral nutrients, whereas compost-amended plants contained a larger amount of Mn. Instead, plants watered with 40 mM NaCl showed similar amount of N, P, Mg and Zn, whereas K, Na, Ca, Cu, Fe and Mn were larger in compost-treated than in mineral-treated plants. Finally, when added with 80 mM NaCl, the content of all nutrients was always larger for compost-treated plants than for those amended with mineral fertilisers (Figs. 2, 3).

Table 2 Plant dry biomass (g) of tomato plants amended with mineral fertilisation (MIN) or composted artichoke residues (COM) and watered with 0, 40 or 80 mM NaCl. Different letters indicate significant differences as evaluated by LSD post hoc test ($p < 0.05$)

	MIN-0 mM	MIN-40 mM	MIN-80 mM	COM-0 mM	COM-40 mM	COM-80 mM
Plant dry biomass	10.68a	4.03d	2.99e	11.05a	5.46b	4.61c

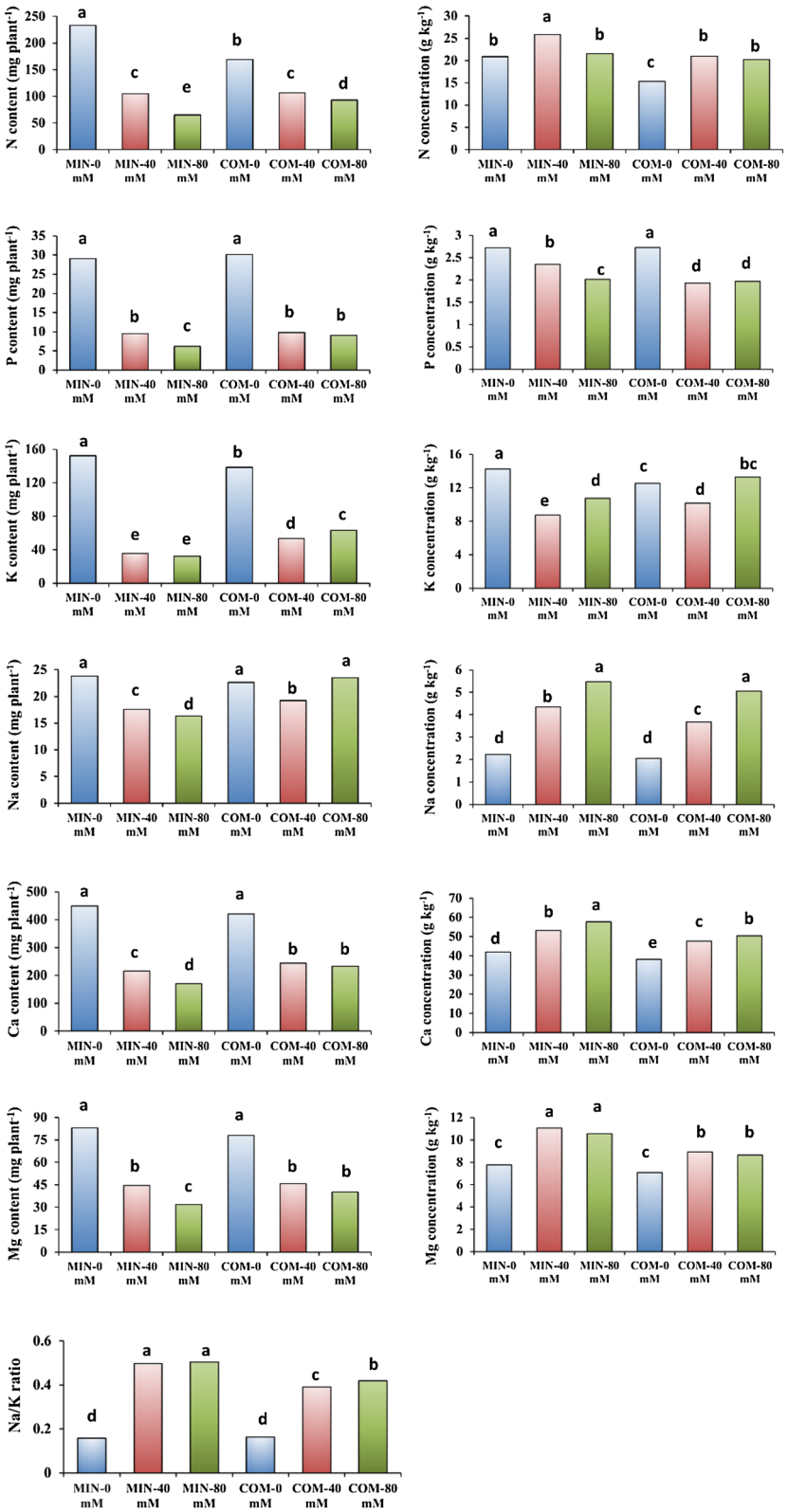


Fig. 1 Leaf nutrient concentration (dimension in brackets) and leaf nutrient content (mg plant^{-1}) of macronutrients in tomato plants amended with mineral fertilisation (MIN) or composted artichoke residues (COM) and watered with 0, 40 or 80 mM NaCl. Different letters indicate significant differences as evaluate by LSD post hoc test ($p < 0.05$)

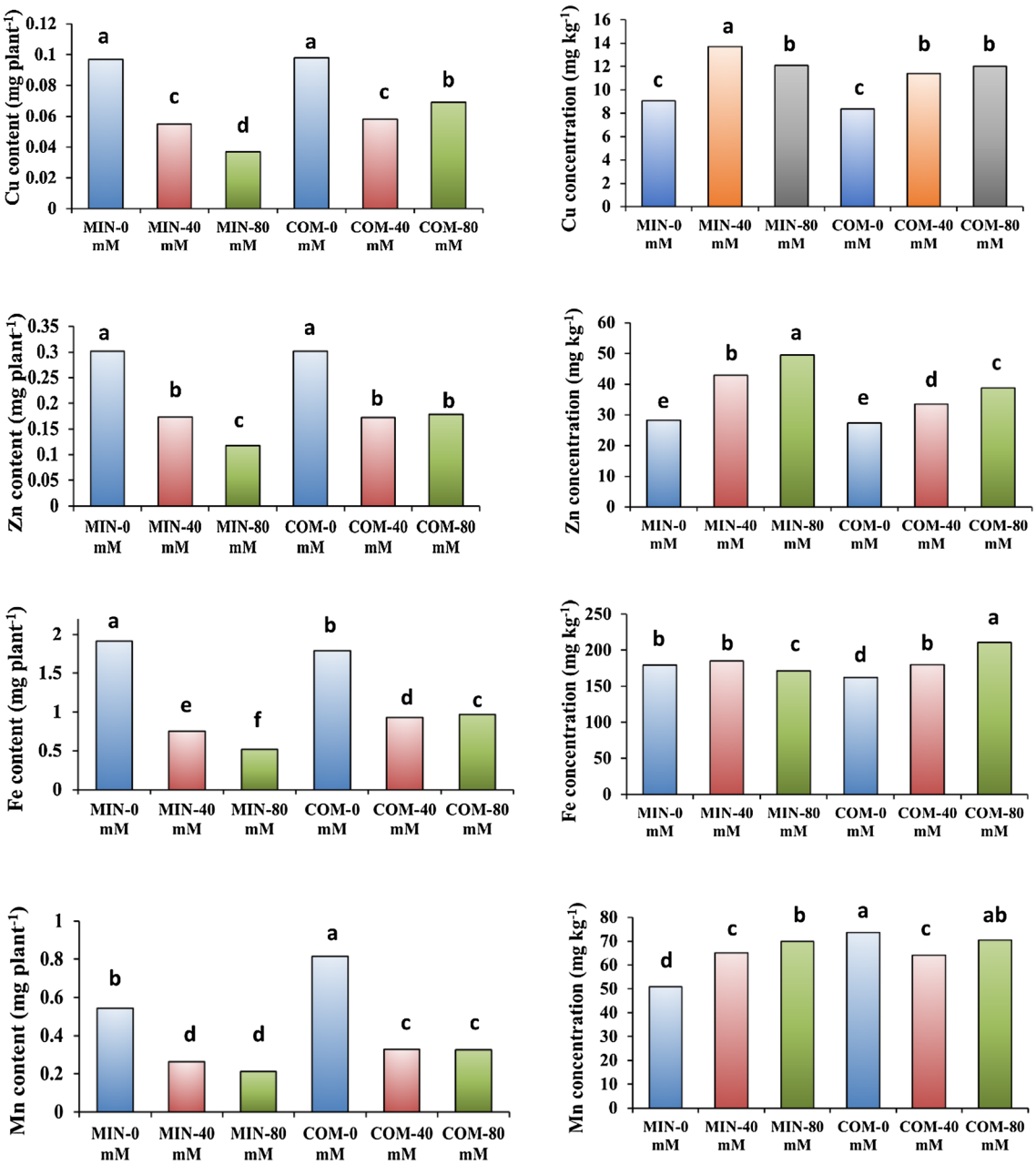


Fig. 2 Leaf nutrient concentration (dimension in brackets) and leaf nutrient content (mg plant^{-1}) of micronutrients in tomato plants amended with mineral fertilisation (MIN) or composted artichoke residues (COM) and watered with 0, 40 or 80 mM NaCl. Different letters indicate significant differences as evaluate by LSD post hoc test ($p < 0.05$)

The Na-to-K ratio in plants is known to be one of the parameters mostly characterising the salinity-induced stress [13]. In fact, we found this index to be larger for stressed plants at increasing NaCl doses. Moreover, this ratio was always greater in plants amended with mineral fertiliser and watered with NaCl-enriched water than for tomato plants fertilised with artichoke compost. An exception was the control plants, for which the different fertilisation regimes did not affect the Na-to-K ratio (Fig. 2).

Metabolomics analysis of tomato leaves

Both the salinity stress and the fertilisation regimes applied resulted in a relevant alteration of plant

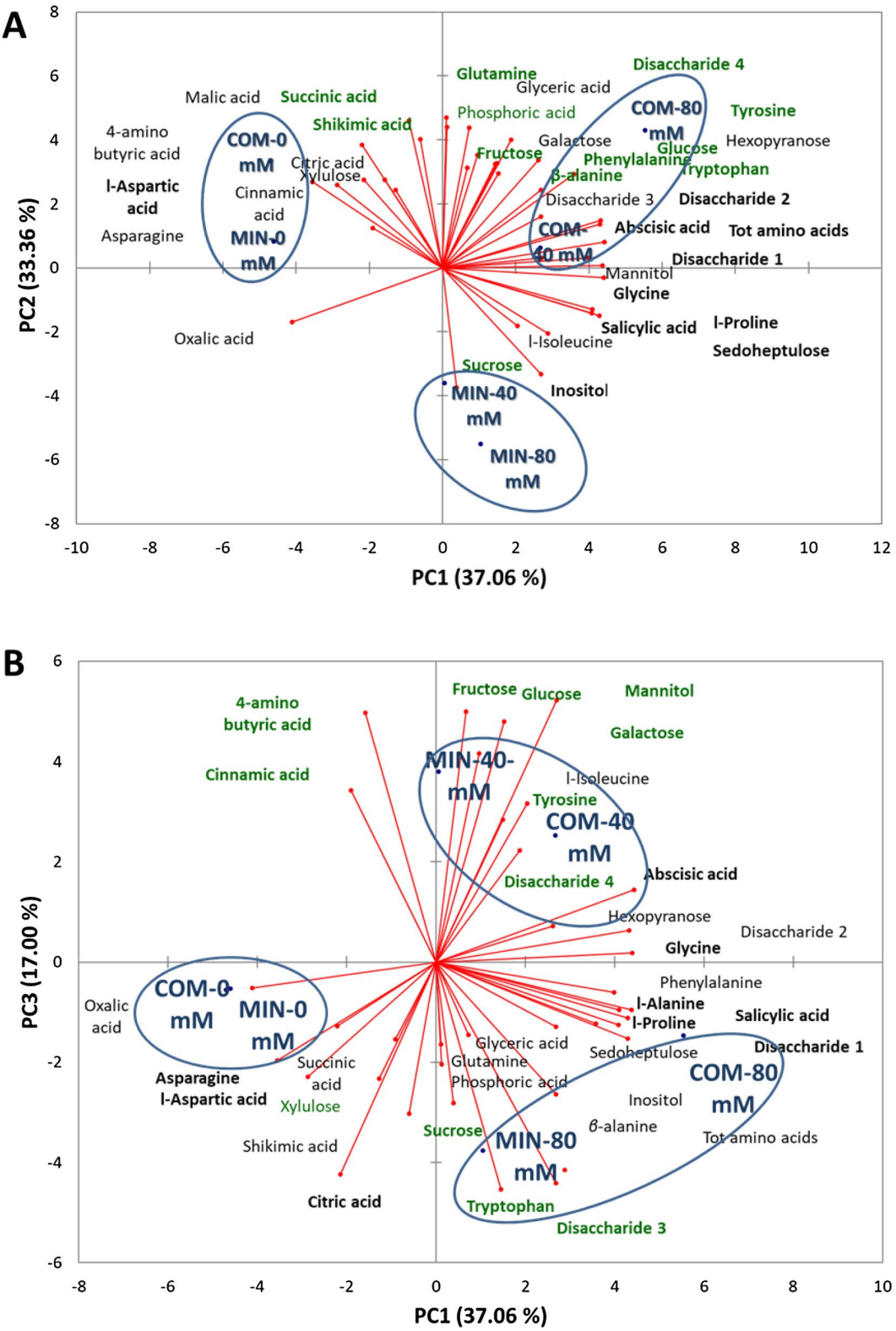


Fig. 3 PCA biplots with PC1 and C2 (**A**) and PC1 and PC3 (**B**) and loadings for the various metabolites identified in plants amended with mineral fertilisers (MIN) or compost (COM) and irrigated with different NaCl doses (0, 40 and 80 mM). Metabolites affecting the PC1 the most are indicated in black bold, whereas those influencing the PC2 or PC3 the most are indicated in green bold

metabolism and physiology. We carried out a Principal Component Analysis to unravel the effect of both factors on plant metabolic processes, and we extracted three PCs, which explained 87% of the total variance (Fig. 3A, B). In the PC1-PC2 biplot, we observed a clear separation of both control treatments from salt-added ones. Indeed, the first ones were in the left-hand quadrant, whereas the latter ones were found in the other quadrant, thus indicating a significantly different metabolic response of salt-irrigated plants compared with *non*-stressed ones (Fig. 3A). Interestingly, the treatments MIN-40 mM and MIN-80 mM were positioned in the lower quadrant, whereas COM-40 and COM-80 mM were in the upper quadrant, also pointing out the different metabolic response of salt-watered plant under diverse fertilisation regimes (Fig. 3A). Furthermore, 54% of the total variance was explained byPC1 and PC3 (Fig. 3B). Also in this case, we observed marked metabolic differences between control and NaCl-watered treatments. However, here, the main differences were highlighted between the different salt doses applied. Indeed, both MIN-40 mM and COM-40 mM were found in the upper right-hand

Table 3 List of primary metabolites and hormones from tomato leaves amended with mineral fertilisers (MIN) or composted artichoke residues (COM) and watered with 0, 40 or 80 mM NaCl[a]

#	Metabolite	MIN-0 mM	MIN-40 mM	MIN-80 mM	COM-0 mM	COM-40 mM	COM-80 mM
1	Fructose	0.1744b	0.1846ab	0.1052c	0.1703b	0.2055a	0.1918ab
2	Galactose	0.1122a	0.1162a	0.0823b	0.1111a	0.1194a	0.1265a
3	Glucose	0.0330c	0.0423ab	0.0197d	0.0353bc	0.0435a	0.0459a
4	Mannitol	0.0013b	0.0028ab	0.0014b	0.0017b	0.0034a	0.0023ab
5	Inositol	0.143ab	0.1475a	0.1679a	0.1197b	0.1527a	0.1468a
6	Sedoheptulose	0.0075d	0.0101bc	0.0111ab	0.0084d	0.0098c	0.0113a
7	Sucrose	0.1377b	0.1285bc	0.1543a	0.1176c	0.1336bc	0.1234bc
8	Hexopyranose	0.0025b	0.0018b	0.0019b	0.0024b	0.0042a	0.0041a
9	Disaccharide 1	0.0016bc	0.0014c	0.0008d	0.0015c	0.0020b	0.0026a
10	Disaccharide 2	0.0052c	0.0076b	0.0082ab	0.0060bc	0.0076b	0.0105a
11	Disaccharide 3	0.0006d	0.0010c	0.0010c	0.0008 cd	0.0016b	0.0020a
12	Disaccharide 4	0.0049d	0.0050d	0.0085b	0.0076bc	0.0063 cd	0.0108a
13	Tot carbohydrates	0.6244abc	0.6492ab	0.5629c	0.5828bc	0.6901a	0.6785a
14	Alanine	0.0011b	0.0020ab	0.0028ab	0.0020ab	0.0034a	0.0034a
15	Isoleucine	0.0014a	0.0015a	0.0012a	0.0008b	0.0015a	0.0013a
16	Proline	0.0142c	0.0308b	0.0524a	0.0066d	0.0413b	0.0565a
17	Glycine	0.0049bc	0.0064b	0.0062b	0.0038c	0.0065b	0.0084a
18	*β*-Alanine	0.0003a	0.0003a	0.0005ab	0.0005b	0.0005b	0.0006b
19	Aspartic acid	0.0069a	0.0019c	0.0022c	0.0062a	0.0044b	0.0045b
20	4-amino butyric acid (GABA)	0.0194a	0.0211a	0.0116b	0.0237a	0.0226a	0.0177ab
21	Glutamine	0.0175bc	0.0062d	0.0071d	0.0208b	0.0142c	0.0260a
22	Phenylalanine	0.0021c	0.0021c	0.0022c	0.0022c	0.0026b	0.0034a
23	Asparagine	0.0038a	0.0006b	0.0011b	0.0039a	0.0010b	0.0013b
24	Tyrosine	0.0016ab	0.0008b	0.0010b	0.0013ab	0.0016ab	0.0018a
25	Tryptophan	0.0012b	0.0009b	0.0012b	0.0013b	0.0012b	0.0019a
26	Tot amino acids	0.0773bc	0.0750c	0.0900b	0.0736c	0.1007ab	0.1272a
27	Succinic acid	0.0051a	0.0017c	0.0017c	0.0058a	0.0031b	0.0059a
28	Glyceric acid	0.0057c	0.0025d	0.0041cd	0.0114a	0.0091b	0.0119ab
29	Shikimic acid	0.0049a	0.0019c	0.0028bc	0.0045ab	0.0037bc	0.0049ab
30	Cinnamic acid	0.0017a	0.0023a	0.0015a	0.0035a	0.0028a	0.0015a
31	Citric acid	0.0665a	0.0369c	0.0351c	0.0559ab	0.0418b	0.0489b
32	Salicylic acid	5.4605cd	6.6162bc	7.5379ab	3.6817d	6.6348bc	8.5836a
33	Abscisic acid	0.4803d	0.8889c	0.7541c	0.5681d	1.1442b	1.343a

[a] The concentration of the metabolites was calculated by dividing the area of each peak (corresponding to a metabolite) by that of the internal standard and then normalising it for the sample dry weight

Different letters in a row indicate significant effects as evaluated by one-way ANOVA and LSD test ($p < 0.05$)

quadrant, while the treatments irrigated with the largest salt dose were both found in the lower right-hand quadrant (Fig. 3B). The most relevant metabolites for both the biplots were aspartic acid, proline, phenylalanine, tyrosine, tryptophan, asparagine, 4-aminobutyric acid, sucrose, fructose, glucose, various unidentified disaccharides as well as salicylic acid, abscisic acid, succinic acid, citric acid and shikimic acid (Fig. 3A, B).

Generally, the two control treatments showed similar metabolic profiles, with only small differences (Table 3 and Additional file 1: Table S1). Specifically, mineral-treated plants contained more sucrose, proline and shikimic acid, whereas compost amendment resulted in larger levels of isoleucine, β-alanine, glycerate and one disaccharide (Table 3). Under increasing salinity levels, instead, compost fertilisation induced a relevant redirection plant metabolism towards an increased accumulation of several metabolites, especially at the largest salt dose (Table 3, Additional file 1: Table S1). Indeed, the concentration of eleven metabolites was significantly larger in compost-amended plants under irrigation with 40 mM NaCl. In detail, the amount of β-alanine, aspartic acid, glycine, phenylalanine and succinic, glyceric, citric acids and the hormone abscisic acid (ABA), as well as that of various unidentified carbohydrates, increased significantly upon compost fertilisation in plants watered with 40 mM NaCl (Table 3). Moreover, even nineteen metabolites significantly differed between the two fertilisation regimes under 80 mM NaCl irrigation (Table 3). Here, the organic amendment induced an accumulation of several carbohydrates, including fructose, galactose and glucose, various amino acids (glycine, aspartic acid, phenylalanine, tyrosine and tryptophan), organic acids (succinic, glyceric, citric, shikimic acids) and ABA. On the contrary, sucrose concentration was significantly larger in MIN-80 mM (Table 3). Altogether, these results indicated that the biosynthesis and transformation of amino acids, carbohydrates, ABA and that of compounds involved in the respiration are the most affected metabolic pathways by the irrigation with NaCl-added water under compost fertilisation (Table 3).

The increasing NaCl doses in irrigation water showed significant effect on plant metabolism (Table 3 and Additional file 1: Table S1). Accumulation of almost all detected carbohydrates, such as fructose, glucose, inositol, sedoheptulose and mannitol, as well as several other unidentified saccharides, was observed in salt-stressed plants fertilised with compost in respect to control. On the contrary, only the levels of sedoheptulose, sucrose and three disaccharides were significantly increased in NaCl-watered plants amended with mineral fertiliser (Table 3, Additional file 1: Table S1). Conversely, fructose, galactose and unidentified carbohydrates significantly decreased by watering mineral-fertilised plants with saline water (Table 3).

A larger concentration of almost all free amino acids progressively was found in compost-amended plants undergone salinity stress, while only proline levels increased in mineral-fertilised plants (Table 3). Moreover, lower contents of glutamine, 4-aminobutyric acid (GABA) and asparagine were reported for MIN-40 mM and MIN-80 mM. Conversely, GABA concentration was not affected by salt irrigation in compost treatments, which instead determined a significant increase in glutamine and asparagine levels (Table 3 and Additional file 1: Table S1). Additionally, succinic, citric and shikimic acids decreased with increasing NaCl doses in mineral-fertilised plants, whereas the effect of salt addition on their levels was not statistically relevant in compost-treated plants (Table 3). Cinnamic acid was not affected neither by the type of fertilisation nor by the level of salt stress (Table 3 and Additional file 1: Table S1). Finally, the concentrations of both salicylic and abscisic acids increased in a dose-dependent manner for both type of fertilisations, although these metabolites were generally more abundant in plants amended with compost (Table 3 and Additional file 1: Table S1).

Overall, both the PCA and the ANOVA suggested that watering with saline water and amending with artichoke-derived compost most affected the metabolism of amino acids, carbohydrates and Kelvin cycle, as well as that associated to the shikimate-derived compounds and the abscisic acid biosynthesis.

Discussion

Soil salinisation is one of the major threats impairing plant development, and results in relevant loss of crop quality and production worldwide [14]. Indeed, the growth of salt-stressed plants was significantly reduced as compared to controls regardless of the fertilisation used (Table 2). However, plants amended with compost tolerated the imposed stress better than those added with mineral fertilisers (Table 2). This outcome was indicated not only by the larger biomass in COM-40 mM and COM-80 mM compared with MIN-40 mM and MIN-80 mM but also by their greater nutrient content, which enabled compost-treated plants to face the salinity stress more efficiently. For example, the larger P content in compost-added plants may have improved the Na compartmentalisation within vacuole, therefore alleviating the stress undergone by compost-treated plants [15]. Similarly, Na noxious effects on plant development were reduced due to the larger K uptake, which is involved in hindering Na accumulation [16, 17]. Indeed, the Na/K ratio, a well-known salt stress indicator, is significantly lower in treatments added with compost (Fig. 2). The

larger concentration of K in compost-fertilised plants can be further related to both an enhanced N use efficiency and an improved plant response to salinity [18]. Finally, the greater tolerance to the damages induced by salt-added watering was conferred by the increasing content of micronutrients, such as Fe, Mn and Cu, which are constituents of enzymes involved in scavenging the Reactive Oxygen Species (ROS) produced under stress [19].

Due to the relevance of both macro- and micronutrients in affecting plant physiological processes, the better nutritional profiles of compost-treated plants watered with NaCl-enriched water were likely responsible for the reported metabolic reprogramming induced by the organic fertilisation. Indeed, compost-amended plants accumulated more carbohydrates than mineral-amended plants (Table 3). These metabolites, such as fructose, glucose and several unknown saccharides acted as osmolytes, lowered the cell water potential, thus sustaining water and nutrient absorption [19]. Also, some amino acids, whose levels are greater in compost-amended plants, are known to be involved in salinity-induced response. Indeed, proline was indicated as one of the most relevant osmolytes accumulated under drought or salinity conditions, whereas β-alanine is the precursor of β-alanine betaine, another well-known osmolyte whose synthesis is boosted under salt stress [20]. Finally, the larger K^+ levels in compost-treated plants may have played a role in increasing cell osmolarity, as its accumulation may significantly contribute to salinity stress alleviation [21, 22]. Interestingly, Argiolas et al. [23] reported that the combined action of the above-mentioned osmolytes, rather than their single effect on cell turgor, was responsible for an increased capacity to maintain an optimal cell water potential by reducing K^+ loss from plant cells. In fact, although K efflux from plant cells is inherently high, the occurrence of large concentrations of organic osmolytes may reduce its loss by complexing K, ultimately keeping cellular K at large levels, ultimately supporting water uptake from the soil.

The biosynthesis of aromatic and phenolic compounds also contributed to stress adaptation in compost-treated plants, especially in COM-80 mM, where significantly larger concentrations of all the aromatic amino acids detected was observed (Table 3). This outcome is further corroborated by the accumulation of shikimic acid (Table 3). In fact, this compound is precursor of aromatic amino acids, as well as of salicylic acid, which play a significant role in mitigating the salt-derived detrimental effects (Fig. 4) [24]. Salicylic acid was indicated to improve salinity tolerance by preventing salt-induced K^+ efflux via a guard cell outward rectifying K (+) (GORK) channel [25]. Moreover, the protective effect of salicylic acid could be attributed to its role in increasing proline

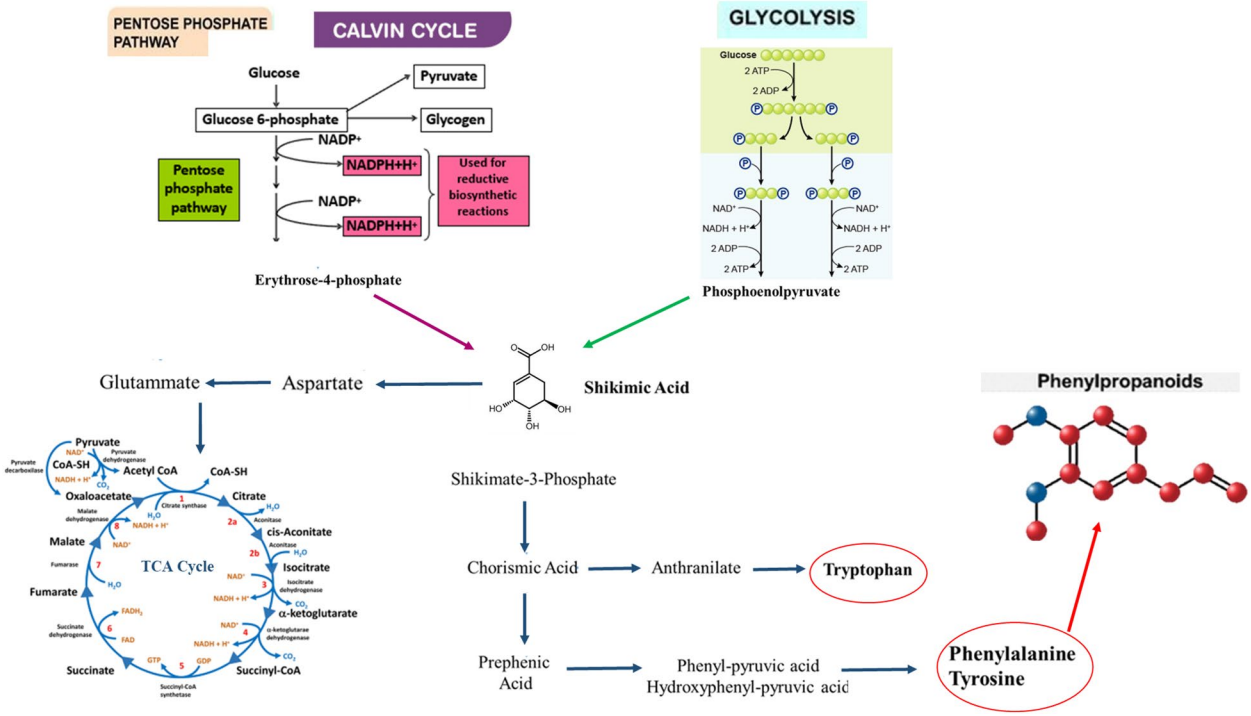


Fig. 4 Metabolic pathways elicited in tomato plants amended with composted artichoke residues

metabolism under stress by enhancing the activity of proline biosynthetic enzymes [26]. Instead, tyrosine is used to synthesise the well-known ROS scavenger plastoquinone, whereas tryptophan is the precursor of melatonin, commonly accumulated during salinity due to its antioxidant capacity [27, 28]. Finally, phenylalanine may have been being exploited for producing other protective compounds, such as caffeic acid and flavonoids (Fig. 4) [29, 30]. Finally, as already mentioned, the better ability of compost-amended plants grown under salinity to counteract the detrimental effects of ROS was further supported by their larger levels of micronutrients, which are constituents of enzymes required for ROS scavenging, and involved in various essential physiological processes [31]. For example, Mn is needed for CO_2 assimilation and the activation of TCA-related enzymes and the electron transport chain [19]. Instead, Fe and Cu are contained in enzymes needed in redox reactions, such as plastocyanin and the Fe-S complex contained in plastids [19, 32], while Zn is crucial in tryptophan biosynthesis [19]. Therefore, their larger content in compost-treated plants may have enhanced the efficiency of ROS quenching, thus avoiding an impairment of essential plant biochemical routes. The metabolism redirection towards accumulation of aromatic molecules is also corroborated by the progressively increasing concentrations of fructose in compost-treated plants, whereas it steadily decreased in mineral-fertilised treatments (Table 3). Fructose is a precursor of ehythrose-4-phosphate, which is in turn exploited to produce a shikimic acid, and then aromatic amino acids [33].

Another key biochemical route involved is that related to aspartic acid metabolism, this compound being another precursor of the aromatic amino acids via the production of prephenate (Fig. 4) [34]. Aspartic acid is however also consumed to obtain GABA, which is then exploited to produce succinic acid to fuel the tricarboxylic acid cycle (TCA) via the GABA shunt [35]. In this pathway, GABA is metabolised to synthesise succinic semialdehyde, which is in turn transformed into succinic acid, then entering the TCA. This mechanism therefore allowed to reduce the negative effects of salinity on cell respiration [36]. Our hypothesis is in line not only with the decreasing levels of succinic and citric acids in MIN-fertilised treatments, which instead were not significantly affected by NaCl irrigation in compost-fertilised treatments, but also with the increasing Ca contents in COM treatments (Tables 2, 3). In fact, Ca is part of the Ca^{2+}/calmodulin-dependent glutamate decarboxylase, whose activation stimulates the GABA synthesis. Therefore, the larger Ca^{2+}content in compost-amended plants may have enhanced the production of this *non*-proteogenic metabolite. Thus, the constant levels of GABA may have granted an unceasing synthesis of succinic acid at sufficient levels to continuously fuel the TCA cycle. On the contrary, the reduced Ca concentration in mineral-amended plants may have limited GABA biosynthesis, therefore resulting in an inadequate production of succinic acid and lower respiration efficiency (Table 3).

The mitigation of the applied salinity stress in compost-amended plants should be finally attributed to the increase of carbohydrates and amino acids with signalling role. Glucose, for example, is involved in several signalling processes in salt-stressed tissues, such as in modulating key antioxidant enzymes, thus reducing both lipid peroxidation and membrane permeability [22]. Furthermore, it is involved in reducing Na^+ toxic effects by enhancing Na^+ transport in the vacuole and helping reduce Na^+ levels in the cytoplasm [37, 38]. Moreover, glucose favourably modulates the biosynthesis and regulation of ABA production, which is recognised as a key molecule involved in plant defence mechanisms under salinity [39] and a well-known signalling compound. As an example, it is involved in proline accumulation upon NaCl addition [40] and in the crosstalk with the hormone ethylene. Specifically, ABA may modulate the expression of several genes associated with ethylene biosynthesis and signalling [41]. It has been previously reported that large levels of ABA may negatively regulate ethylene production, which is in turn associated to a lesser leaf necrosis and abscission [42]. Overall, compost application may have triggered the accumulation of larger ABA amount in plants that may have in turn inhibited ethylene biosynthesis, thus finally preventing leaf abscission and reducing plant stress levels.

The larger nutrient uptake efficiency shown by compost-treated plants had already been reported and may be attributed to the increase in both soil cation exchange capacity (CEC) and N and P availability upon compost amendment to soil [8]. Indeed, greater content of K, Ca and Mg were found in compost-amended plants grown under salinity. Furthermore, a larger N uptake and amount of $NaHCO_3$-extractable P were observed in compost-fertilised soils [8]. The larger CEC was likely due to the large content of carboxyl groups in the humified fraction of compost [43, 44], whereas the larger availability of anionic nutrients should be attributed to the microbially mediated compost mineralisation. In fact, humic matter in compost represents a source of metabolic energy for microbial biochemical processes, after which N and P become more accessible for plant uptake [11]. It is noteworthy that compost application increases the pool of soil humus, that was recently reported to be involved in the mitigation of salinity stress in plants, since humic matter affects both the plant ionic balance and the biosynthesis of osmoprotectants [45, 46]. Furthermore, humic molecules were able to reduce the salt-induced degradation

of the HIGH-AFFINITY K^+ TRANSPORTER 1 (HKT1), thus allowing a continuous Na^+ transport into the xylem stream and then into the shoot and contributing Na^+ compartmentalisation and stress alleviation [46, 47]. Furthermore, humic molecules were indicated to affect transcriptional pathways of salinity-stressed plants, with an upregulation of several stress-related genes, such as those linked to the expression of redox and heat-shock proteins [45, 47]. Finally, humic substances were recently shown to help in salt stress mitigation by increasing the expression of the plasma membrane H^+-ATPase in tomato roots, thereby inducing a larger Ca^{2+} uptake. Their role as signalling compounds is well known, as well as improving the Na/K ratio [45, 48–50]. Overall, humified matter in compost was shown to mitigate salinity damages by multiple mechanisms, including a general plant reprogramming at transcriptional and metabolic levels.

Conclusions

Growth and yields of tomato plants are significantly reduced by salinity in irrigation waters, as compared to control, though we found that compost-amended soils helped reduce the consequences of the salt stress more than a mineral fertilisation. In fact, compost incorporation into soil increased the content of macro- and microelements in tomato plants, thus inducing a reprogramming of biochemical plant processes with synthesis of primary and secondary metabolite which ensured more efficient ROS scavenging, and cell respiration and accumulation of compatible solutes. In turn, an observed improved plant nutritional *status* may be attributed to both the larger availability of plant nutrients and enhanced biological activity in soil provided by the humified components of compost, which had already recognised to be directly involved in plant adaptation to salt stress. Overall, our results point out that amending with composted agricultural residues might represent a suitable alternative to mineral fertilisers to reduce the detrimental impact of irrigation with salinised water on tomato growth and development.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-022-00373-5.

Additional file 1: Table S1. Effect of salt dose (0, 40 and 80 mM Na Cl), type of fertilization (mineral or with green compost) ant their interaction as assessed by Two-way ANOVA ($p < 0.05$). Significant differences are highlighted in bold. **Table S2.** Effect of the fertilisation regime on metabolite levelsas assessed by Student's test ($p < 0.05$). Significant differences are highlighted in bold.

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Author contributions
DS conducted part of the experiments and wrote the manuscript; VC acquired funding, conceptualised the work, supervised the experiments, conducted part of the experiments and revised the work. MV and AA performed part of the experimental work; GV performed extraction, identification and quantification of plant hormones. AB and AM acquired funding and supervised the work. AP acquired funding, supervised the work and revised manuscript. All the authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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