



## Can microplastics threaten plant productivity and fruit quality? Insights from Micro-Tom and Micro-PET/PVC



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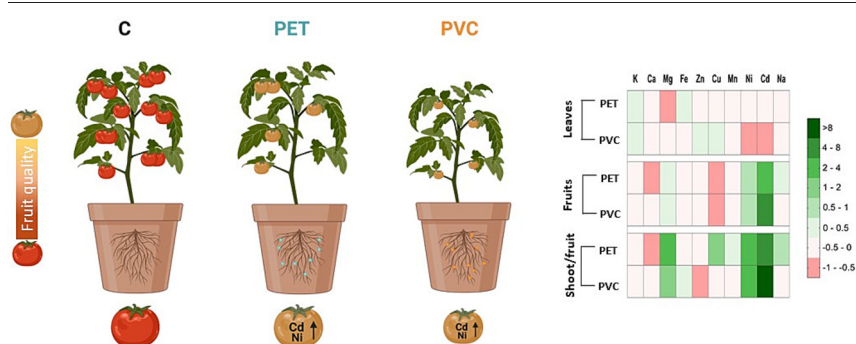
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### HIGHLIGHTS

- *Solanum lycopersicum* L. was treated with PET/PVC microplastics to test their toxicity.
- PET/PVC had negligible effects on plant growth, but decreased the number of fruits.
- Ni and Cd increased in fruits from PET/PVC-treated plants.
- Lycopene, soluble solids and phenols decreased in fruits from PET/PVC-treated plants.

### GRAPHICAL ABSTRACT



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### ABSTRACT

*Solanum lycopersicum* L., a crop grown worldwide with a high nutritional value for the human diet, was used to test the impact of microplastics on plant growth, productivity, and fruit quality. Two of the most represented microplastics in soils, polyethylene terephthalate (PET) and polyvinyl chloride (PVC), were tested. Plants were grown in pots with an environmentally realistic concentration of microplastics and, during the whole crop life cycle, photosynthetic parameters, number of flowers and fruits were monitored. At the end of the cultivation, plant biometry and ionome were evaluated, along with fruit production and quality. Both pollutants had negligible effects on shoot traits, with only PVC causing a significant reduction in shoot fresh weight. Despite an apparent low or no toxicity during the vegetative stage, both microplastics decreased the number of fruits and, in the case of PVC, also their fresh weights. The plastic polymer-induced decline in fruit production was coupled with wide variations in fruit ionome, with marked increases in Ni and Cd. By contrast there was a decline in the nutritionally valuable lycopene, total soluble solids, and total phenols. Altogether, our results reveal that microplastics can not only limit crop productivity but also negatively impact fruit quality and enhance the concentration of food safety hazards, thus raising concerns for their potential health risks for humans.

### 1. Introduction

Plants are the core basis for life on Earth and, for humans, they represent the major source of calories, proteins, and fat. They also are the staple food

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for livestock (Prescott-Allen and Prescott-Allen, 1990). While the global population is expected to reach 10 billion by 2050, our planet can sustain 15 billion on plant-based diets or only 5 billion on a mixed diet, thus posing a major challenge for agricultural systems in ensuring food supply (Ingram, 2011). This critical task is further aggravated by ever-rising losses in agricultural yield due to climate change. Abiotic stresses are the primary cause of crop growth reductions, accounting for up to 80 % of global yield losses (Oshunsanya et al., 2019). In comparison biotic stresses only reduce agricultural productivity by 20–40 % (Bommarco et al., 2013). Regarding abiotic stresses, in addition to well-studied factors, such as drought, high temperature, salt, organic and inorganic pollutants, etc., the life of plants, as well as of any other living organism, is now also threatened by a new generation of contaminant, plastics (Gibson et al., 2021).

Due to their convenience, plastic items are used worldwide, resulting in widespread dispersal and accumulation of plastic debris in all terrestrial and aquatic environments (Bläsing and Amelung, 2018; Dris et al., 2016; Eerkes-Medrano et al., 2015). The degradation of plastic polymers produces smaller particles classified as microplastics, when smaller than 5 mm, or as nanoplastics, when smaller than 1  $\mu\text{m}$  (Gigault et al., 2018; Rochman, 2018). The increasing occurrence of plastic in terrestrial and aquatic environments, progressively reaching ever more remote areas, has made plastic pollution one of the most pressing environmental issues that our society has to deal with (Hu et al., 2022; Prata et al., 2021).

The negative effects of microplastic pollution have been extensively studied on humans and animals (Patil et al., 2022; Zolotova et al., 2022). A growing body of evidence has begun to unravel how plants are affected by microplastics present in soil and water (de Silva et al., 2021; Ge et al., 2021; Karalija et al., 2022), and even in air (Falsini et al., 2022). The most common ways microplastics can, directly or indirectly, alter plant growth include: reductions in shoot and root biomass, germination rates and photosynthetic activity; genotoxic and oxidative damage; and alterations of plant ionome and metabolic profile (see e.g. (Colzi et al., 2022; Jiang et al., 2019; Lian et al., 2020; Pignattelli et al., 2020)). Nevertheless, a wide range of effects (from positive to negative) has been reported depending on the plant species and the type of polymer used (Rillig et al., 2019).

Among the various environmental sinks of microplastic pollution, agroecosystems have been identified as one of the most affected (Khalid et al., 2020; Ng et al., 2018). This is the result of continuous inputs from many management practices (Büks and Kaupenjohann, 2020; Nizzetto et al., 2016; Rillig et al., 2017), from plastic mulching to the use of fertilizers from contaminated sewage sludge and contaminated water for irrigation (Zhu et al., 2019). Alarming evidence points to a negative impact of microplastics on all aspects of agricultural systems. Indeed, plastic particles have been reported to alter soil physical and chemical properties, disturb soil microbial communities and act as vectors of pathogens and contaminants, thus affecting food-crop productions (Hartmann et al., 2022; Okeke et al., 2022; Pérez-Reverón et al., 2022). Hence, there is a pressing need to investigate if and how microplastics affect agroecosystems, particularly considering that agricultural lands occupy almost half of Earth's land surface, and that healthy soils are the basis for global crop production and food safety (FAO, 2021).

Tomato (*Solanum lycopersicum* L.) is included in the top 30 crops that account for the majority of the world's plant-based food supply (Menda et al., 2013; Prescott-Allen and Prescott-Allen, 1990; Rothan et al., 2019). Economically, it is the second most important crop after potato, with a global planting area of  $5.03 \times 10^6$  ha, an annual production of  $1.81 \times 10^8$  t and an export value of 14.1 billion dollars in 2019 (FAO, 2018). The fruit is an exceptional source of nutrients and antioxidant compounds, essential for human health, including mineral elements, vitamins C and E, carotenoids, organic acids, phenolic compounds, and flavonoids (Chaudhary et al., 2018). The consumption of tomato is also associated with lower risks of developing some types of cancers (Franceschi et al., 1994; Giovannucci et al., 1995).

Despite the agronomic importance of this crop, the impact of microplastic pollution on tomato remains poorly understood (see e.g. Shi

et al., 2022, 2023). The limited literature available on physiological responses in tomato reveals the multifaceted negative effects of microplastics during the vegetative phase, ranging from reduced germination, growth, and photosynthetic activity to the induction of oxidative stress and changes in the elemental and metabolic profiles (Shi et al., 2022, 2023). However, very little is known about the impact of microplastic pollution on fruit yield and nutritional value, with only one study reporting a reduction in fruit numbers in soil-grown plants treated with microplastic-containing sewage sludge (Hernández-Arenas et al., 2021). Furthermore, to date, most of the studies investigating how tomato responds to microplastic were conducted using hydroponics cultivation methods. Hence, further investigations in soil systems are needed, not only to check/validate the deleterious effects of these pollutants on plant growth, but especially to assess their outcomes in terms of fruit production, quality, and nutritional value in this fundamental crop.

Therefore, the aim of this study was to evaluate the effects of environmentally realistic concentrations of microplastics on the growth, photosynthetic performance, and elemental profile in soil-grown tomato plants, focusing on fruit yield and quality. To address this, we monitored the impact of polyvinyl chloride (PVC), and polyethylene terephthalate (PET) on *S. lycopersicon* var. Micro-Tom, whose short life cycle and abundant yield make it an excellent model system for the proposed study. PVC and PET were selected as they are two of the most diffused polymers in the environment and in agricultural soils (Dioses-Salinas et al., 2020; Yu et al., 2022).

## 2. Materials and methods

### 2.1. Plant growth and experimental conditions

Experimental setup and procedures for the microplastic treatments were carried out according to already standardized methodologies (Colzi et al., 2022; Pignattelli et al., 2020). Plants were grown on the same soil adopted by Pignattelli et al. (2020) and Colzi et al. (2022) with the following characteristics: silty-clayey soil, pH (in  $\text{H}_2\text{O}$ ) 7.5, electrical conductivity  $0.5 \text{ dS m}^{-1}$ , density  $0.3 \text{ g cm}^{-3}$ , total porosity 84 % v/v, field capacity 45 %, organic matter 1 %, microplastic concentration  $< 0.5 \text{ mg kg}^{-1}$ .

Following a digestion of the samples using 10 mL of 69 %  $\text{HNO}_3$  in a microwave digestion system (Mars 6, CEM), soil elemental concentrations were estimated by atomic absorption spectroscopy (PinAAcle 500, Perkin Elmer) as in Bettarini et al. (2019). The average values for the measured elements were:  $4.3 \pm 0.3$ ,  $18.6 \pm 1.4$ ,  $2.2 \pm 0.7 \text{ mg g}^{-1}$  d.w. for K, Ca and Mg, respectively, and  $208.1 \pm 13.1$ ,  $69.02 \pm 1.6$ ,  $37.9 \pm 0.5$ ,  $16.9 \pm 1.7$ ,  $113.9 \pm 4.0$ ,  $323.4 \pm 7.3$ ,  $6.4 \pm 0.2 \mu\text{g g}^{-1}$  d.w. and below detection limit or Fe, Mn, Cu, Ni, Zn, Na, Cd, and Pb, respectively. The microplastics used were the same as those used in the above-mentioned papers, with spherical shape, diameters between 40 and 50  $\mu\text{m}$  (included in the range of dimensions that can be found in agricultural soils, as reported in (Pérez-Reverón et al., 2022)), and density PVC =  $1.4 \text{ g cm}^{-3}$  and PET =  $1.68 \text{ g cm}^{-3}$  (Sigma-Aldrich, quality level 100, CAS-number 9002–86–2 and 9003–07–0 respectively). For the treatment, a concentration of 0.5 % microplastic/soil (w/w) was chosen based on concentrations commonly used in controlled environments to simulate microplastic contamination in soils (see e.g., Chen et al., 2022a, 2022b; Dong et al., 2021; Qi et al., 2020; Xu et al., 2020). This is an environmentally relevant concentration especially in areas where plastic mulching is widely used (Guo et al., 2023). However, the level of microplastic pollution in the soil is highly variable worldwide (Zhang et al., 2022), with concentrations as high as 7 % in industrial areas (Fuller and Gautam, 2016).

Externally dark-coated glass pots (10 cm diameter x 10 cm depth) were individually prepared by adding 650 mL of soil that was previously oven-dried at  $60^\circ\text{C}$  and sieved with a stainless-steel sieve that allowed only particles smaller than 3 mm to pass. The addition of microplastics was performed inside glass containers and the plastic particles were carefully mixed with the substrate to homogenize their distribution. No significant differences in the above-mentioned soil characteristics were detected after PVC/PET addition.

Seeds of *Solanum lycopersicum* var. Micro-Tom (purchased from Gargini Sementi, Lucca, Italy) were then stratified for one day in the dark at room temperature to synchronise germination. One seed per pot and 12 seeds per treatment (36 plants in total) were sown. Plants were grown in a climatic chamber with the following conditions: 24/16 °C day/night, light intensity 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , 16-h (day) photoperiod and relative humidity 60–65 %. For irrigation, distilled water obtained directly from a glass water distiller was used (two times a week with 100 mL). The water irrigation volume was determined based on the soil field capacity, following Pignattelli et al. (2020), and then checked to ensure that the soil in the pots was adequately wet prior to the experiment. Plants were harvested and sampled 15 weeks after germination, when all fruits had reached maturity. Plants were cut at the crown and the whole shoots were weighted to determine fresh and dry weights. The percentage of shoot water content calculated as  $((F_W - D_W)/F_W) * 100$ .

Due to the very fine architecture of secondary roots, we did not attempt to analyse these organs. We found the root tissue to be so tightly entangled to the soil particles that extraction of material was impossible without a substantial damage or loss to the roots, thus invalidating the reliability of any data collected.

## 2.2. Fluorescence parameters

The measurement of fluorescence parameters began two weeks after germination, when the first fully expanded leaves were present, and stopped 11 weeks after germination, when leaves started to show the first signs of discoloration indicating the onset of senescence.

Chlorophyll a fluorescence was measured on 15-min dark-adapted leaves using a portable fluorimeter (Plant Efficiency Analyzer - Handy PEA, Hansatech Instruments Ltd). Leaves were flashed for 1 s with a saturated red-light pulse (1800  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , 650 nm) from a LED into the fluorimeter sensor. Leaf health was evaluated using the maximum quantum yield of photosystem II ( $F_v/f_m$ , where  $F_v$  = maximal  $F_m$  fluorescence – minimal  $F_0$  fluorescence; a parameter that is a reliable indicator of photochemical activity of the photosynthetic apparatus, Bussotti et al., 2012), and the performance index ( $P_{\text{index}} = [1 - (F_0/f_m)]/[M_0/V_J] [(F_m - F_0)/F_0] [(1 - V_J)/V_J]$ , where  $M_0$  = initial slope of the fluorescence transient after 300  $\mu\text{s}$  normalized on  $F_v$  and  $V_J$  = variable fluorescence at 2 ms, Bussotti et al., 2012), that expresses the potential ability for energy conservation. The optimal value of  $F_v/f_m$  in non-stressful conditions for C3 plants is around 0.83 and its decrease is a sign of reduced efficiency of PSII reaction centre and/or photoinhibition (Björkman and Demmig, 1987); concerning  $P_{\text{index}}$ , its values are variable because various parameters are taken into consideration, but its decrease indicates an alteration of the photosynthetic process in general, from absorbed photons in PSII to the reduction of electron acceptors (Bussotti et al., 2012; Strasser et al., 2000). Chlorophyll (Chl) content was measured using a Chl content meter (Multi-Pigment-Meter MPM-100, Opti- Sciences) and expressed as total Chl per  $\text{m}^2$  of leaf material ( $\text{mg m}^{-2}$ ) (Gitelson et al., 1999).

We monitored leaf area development as an additional parameter to assess the impact of microplastics in the early stages of the vegetative growth (2 and 4 weeks after germination). The leaf area was determined using the ImageJ v. 1.51 software (Schneider et al., 2012). As the tomato plants matured, the overlapping leaves did not allow for further non-destructive monitoring of leaf area. At the harvest stage, leaf surface was also not evaluated as the margins of most leaves were dry and slightly curled due to senescence.

## 2.3. Flower and fruit counting

Floral buds started to appear four weeks after germination and on the fifth week the first flowers blossomed. The total number of flowers produced by plants over time was determined through a cumulative count of all the new blossomed flowers. The counting ended around 7 weeks after all floral buds had turned into flowers. Fruits began to appear six weeks after germination and their cumulative number was recorded over time.

From the 11th week, fruits were continuously harvested when they reached their full maturation status, which was assessed by measuring fruit colour with the portable colour digitizer application ColorGrab (Loomatix, Haifa, Israel). Measurements started when fruit colour changed from “deco” (RGB: 197, 207, 140) to “jonquil” (RGB: 225, 243, 137). When the colour turned close to “red” (RGB: 255, 0, 0) the fruit was considered ripe and was harvested, snap-frozen in liquid nitrogen and stored at  $-80$  °C for later analysis.

The net fruit yield was calculated as the product of the average number of fruits and their average fresh weight.

## 2.4. Elemental profile of shoots and fruits

Plant parts (shoots and mature fruits) were carefully washed with demineralized water and dried at 70 °C for two days. The concentration of elements was measured by wet-digestion of about 100 mg of oven-dried material in 10 mL of 69 %  $\text{HNO}_3$  in a microwave digestion system (Mars 6, CEM) with maximum temperature of 200 °C for 10 min (as in Bettarini et al., 2019). Atomic absorption spectroscopy (PinAAcle 500, Perkin Elmer) was used to determine the amount of K, Ca, Mg, Fe, Zn, Cu, Mn, Ni, Na, Cd and Pb and concentrations were expressed on a dry weight basis. Certified reference materials (grade BCR, Fluka Analytical, Sigma-Aldrich) were used to verify the reliability and accuracy of the method (values <10 % and < 5 % RDS respectively).

## 2.5. Fruit analysis

One subgroup of tomatoes was defrosted and each whole fruit immediately ground by hand into a homogeneous pulp. The juice was then extracted by centrifugation at 15000 rpm for 10 min. The collected supernatant was used to determine total soluble solids (TSS), titratable acidity (TA) and pH as in Baek et al. (2021) with some small modifications. Briefly, TSS, expressed as °Brix, were measured using a hand refractometer (N1 Atago) initially calibrated with distilled water (0°Brix TSS). For TA, 0.5 mL of juice was diluted with 4.5 mL MilliQ water and titrated against 0.1 N NaOH to a pH of 8.2 using a pH meter. Acidity was computed and expressed as percent citric acid (Nielsen, 2017).

The fruits in the second subgroup were freeze-dried and ground into a fine powder and used to determine lycopene,  $\beta$ -carotene, soluble sugars and total phenol concentrations.

Lycopene and  $\beta$ -carotene were determined as in Branisa et al. (2014). Briefly, the ground freeze-dried tomatoes (0.1 mg) were extracted twice in 1 mL acetone–hexane mixture (2:3) and the samples sonicated for 15 min (10 s pulse, 10 s pause) in an ice-water bath to prevent overheating. The supernatant was collected after centrifuging the samples at 5000 rpm for 10 min at 20 °C. The absorbance of each supernatant was measured at 453, 505, 645 and 663 nm with a multiplate reader (Tecan, Männedorf, Switzerland). Lycopene and  $\beta$ -carotene content were calculated from the following equations:

$$\text{Lycopene (mg/100 ml)} = -0.0485A_{663} + 0.204A_{645} + 0.372A_{505} - 0.0806A_{453}$$

$$\beta - \text{carotene (mg/100 ml)} = 0.216A_{663} - 1.22A_{645} - 0.304A_{505} + 0.452A_{453}$$

where A = absorbance reading

Soluble sugar concentrations were measured using the anthrone colorimetric method (Yemm and Willis, 1954). Ground freeze-dried tomatoes were extracted in boiling 80 % ethanol, twice, and the supernatant collected and used to measure total sugars. Total sugars (as hexose equivalents) were determined by measuring the absorbance of the samples at 620 nm in a multiplate reader (Tecan, Männedorf, Switzerland) and using a standard curve for glucose.

Total phenol concentrations were determined as in Baek et al. (2021) with slight modifications. Briefly, 1 mL of 75 % methanol was added to

the ground freeze-dried samples (0.1 g) and vortexed. The tubes were then sonicated as above described for the pigments and the samples centrifuged at 5000 rpm for 10 min at 20 °C. Total phenol content was measured in the supernatant by mixing 0.1 mL of the extract, with 0.2 mL of Folin-Ciocalteu reagent (10 % v/v). Then, 0.8 mL of 700 mM Na<sub>2</sub>CO<sub>3</sub> was added to the mixture and total phenolics were determined after 1 h of incubation. A microplate reader a microplate reader (Tecan, Männedorf, Switzerland) was used to measure the absorbance of the reaction mixture at 750 nm. Total phenolics concentration was expressed as mg of gallic acid equivalents (GAE) 100 g<sup>-1</sup> dry weight of the sample.

## 2.6. Statistics

The experiment comprised of three treatments (control plants and plants treated with the two kinds of MPs) with 12 biological replicates each. When measurements were performed over time (fluorescence parameters and flower/fruit appearance), the same plant was evaluated at different times.

One-way ANOVA was used to check the significance of differences ( $p < 0.05$ ) among means at the same experimental times, using GraphPad Prism 7 (GraphPad Software, San Diego, CA). A HSD-Tukey test was run for post-hoc comparisons. Data normality was checked with the Shapiro-Wilk test, whereas Bartlett's test was used for checking homogeneity of variances.

Photosynthetic parameters and flower/fruit numbers were analysed using linear mixed-effects models (LMMs) in a repeated measurement ANOVA design, using plant identity as a random effect factor to account for the temporal correlation of observations. Time was used as an ordinal variable. The impact of each treatment was investigated in a full factorial design using leaf  $F_v/fm$ ,  $P_{index}$ , Chl data and flower/fruit number as response variables and the treatments with plastic particles as explanatory variables. The significance of the fixed effects and of associated interaction factors was verified using an ANOVA type III table, with Satterthwaite's method. The analyses were conducted using the software R v.4.1.3 (R Core Team, 2020). LMM computations were conducted using the lmer function of the lme4 package version 1.1–12 for fitting the models.

The relationships among treatments and shoot or fruit traits were evaluated using a Principal Component Analysis (PCA) implemented in the package FactoMinerR (Lê et al., 2008) under the R software. Two PCA were performed considering the type of analysed organs. PCA was performed for 16 main shoot traits (fresh and dry weight, water content,  $F_v/fm$ ,  $P_{index}$ , chlorophyll content and concentration of 10 metal ions); these traits reflect the condition of the plants at the end of the experiment, with the exception of  $F_v/fm$ ,  $P_{index}$  and chlorophyll content, whose measurements necessarily stopped 11 weeks after germination. All of them are useful to differentiate the effects of PET/PVC on tomato plant growth, development and photosynthesis. For fruits, 21 main traits were considered (fresh and dry weight, number of flowers and fruits, water content, 6 biochemical parameters, concentration of 10 metal ions). Overall, this analysis was used to assess how plant productivity and fruit quality were altered by the presence of microplastics in soil.

## 3. Results

### 3.1. Effects of microplastics on plant growth and photosynthetic parameters

Two weeks after germination, the presence of microplastics in the root-zone induced a 17 % decrease ( $F = 3.13$ ,  $P = 0.045$ ) in the leaf area of PET-treated tomato plants compared with control plants (Fig. 1). After four weeks, although PET-treated plants still showed a 8 % decrease of leaf area mean value in respect to controls, no significant differences were found among the treatments ( $F = 2.34$ ,  $P = 0.11$ ).

Differences in shoot fresh and dry weights at the end of the cultivation period are reported in Fig. 2a and b. A significant effect of treatment was scored only in the lower fresh weight of PVC-treated plants compared to controls (20 % decrease,  $F = 6.42$ ,  $P = 0.0044$ ), which was associated to

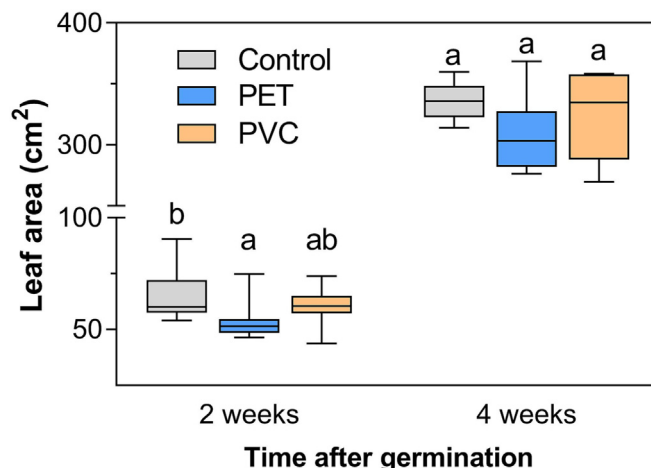


Fig. 1. Box-plot of the leaf area of *Solanum lycopersicum* plants grown in presence of PET or PVC 0.5 % for 2 and 4 weeks. Whiskers from minimum to maximum value of the data sets are reported. Lower case letters indicate significant differences (one-way ANOVA, at least  $p < 0.05$ ) among mean values of control and treated samples within each sampling time (2 or 4 weeks after germination).

a decline (0.26 %) in tissue water content in PVC-exposed plants ( $71.6 \pm 5.0b$ ,  $67.6 \pm 12.9b$ ,  $52.7 \pm 8.2a$  for control, PET-treated and PVC-treated samples respectively,  $F = 13.79$ ,  $P < 0.0001$ ).

At two and four weeks after germination the chlorophyll fluorescence data (Fig. 3) highlighted significantly lower  $F_v/fm$  values for treated plants in comparison to controls, for both plastics and only PVC respectively ( $F = 16.61$  and  $13.15$ ,  $P < 0.001$ ).  $P_{index}$  in treated plants never differ from the controls, except for the PET treatment that caused a decrease of about 15 % at the end of the experiment ( $F = 6.065$ ,  $P = 0.0057$ ). Independent of treatment, in all plants,  $F_v/fm$  and  $P_{index}$  significantly increased after four weeks of growth, reaching stable levels that declined at the end of the experiment, with  $P_{index}$  declining faster than  $F_v/fm$  ( $19.85 < F < 35.83$  and  $20.87 < F < 51.25$  for  $F_v/fm$  and  $P_{index}$  respectively, always  $P < 0.0001$ ).

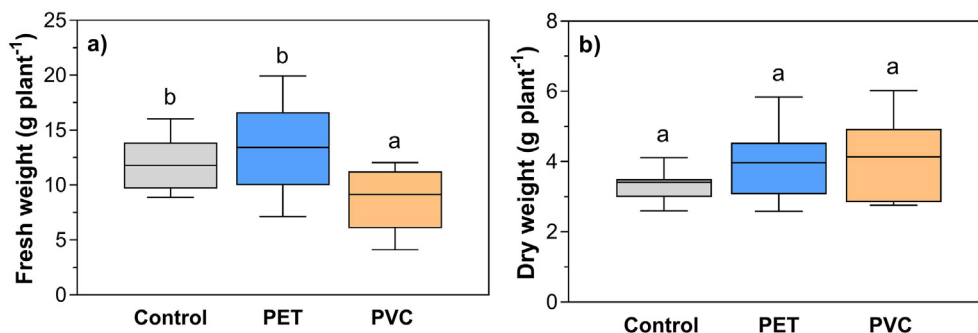
Regular monitoring of leaf chlorophyll contents revealed that PET-treated plants had always significantly lower values compared to control plants, with the decline ranging from 11 % (at four weeks) to 21 % (at ten weeks). By contrast, significant declines in leaf chlorophyll contents in PVC-treated plants only occurred four, six and eight weeks after germination ( $6.683 < F < 22.99$ ,  $P < 0.01$ , Fig. 3), with a decrease of 14, 14, and 7 % compared to controls, respectively. Within each treatment, as for fluorescent parameters, there were age-dependent variations in leaf chlorophyll contents, with significant increases at the beginning of the experiment and decreases toward the end ( $24.88 < F < 29.85$ ,  $P < 0.0001$ ).

The variation over time of the two photosynthetic indexes and of the leaf chlorophyll content significantly depended on the polymers used for the treatment (treatment/time significant with at least  $P < 0.05$  for both: Tables S1, S2, and S3).

### 3.2. Effects of microplastics on flower number and fruit yield

The total number of flowers produced across treatments (Fig. 4) was the same over time, except at five weeks after germination in PET-treated plants, where the number of flowers decreased by 28 % compared to controls ( $F = 4.162$ ,  $P < 0.05$ ). Starting from seven weeks after germination, there was a decline in the number of fruits per plant in microplastic-treated plants (20–28 % decrease, Fig. 5), except for PVC treated samples at nine and ten weeks ( $7.062 < F < 18.38$ , at least  $P < 0.001$ ). While the type of polymer did not influence the changes over time in the cumulative number of flowers (Table S4), it affected the number of fruits (Table S5).

Fruits fresh weight in PVC-treated plants declined by 25 % compared to control plants (Fig. 6a,  $F = 16.73$ ,  $P < 0.0001$ ) while this did not occur in



**Fig. 2.** Box-plot of the a) shoot fresh weight, and b) shoot dry weight of *Solanum lycopersicum* plants grown in presence of PET or PVC 0.5 % for 15 weeks. Whiskers from minimum to maximum value of the data sets are reported. Lower case letters indicate significant differences among the mean values (one-way ANOVA, at least  $p < 0.05$ ).

PET-treated plants. On the other hand, the calculated net fruit yield displayed significantly different values among all treatments, with the highest score for the control plants and the lowest one for PVC treated plants ( $\text{g}^{-1}$  per plant,  $81.7 \pm 8.21$ ,  $65.7 \pm 12.7$  and  $51.1 \pm 4.3$  for control, PET-treated and PVC-treated plants respectively,  $F = 34.01$ ,  $P < 0.0001$ ).

While the presence of plastic particles in soil did not affect fruit dry mass (Fig. 6b,  $F = 1.891$ ,  $P = 0.1669$ ), fruit water content significantly declined (5 % decrease) in PVC-treated plants in comparison to control plants (% ,  $91.3 \pm 1.8b$ ,  $90.9 \pm 1.8b$ ,  $87.0 \pm 1.7a$  for control, PET-treated and PVC-treated samples respectively,  $F = 19.95$ ,  $P < 0.0001$ ).

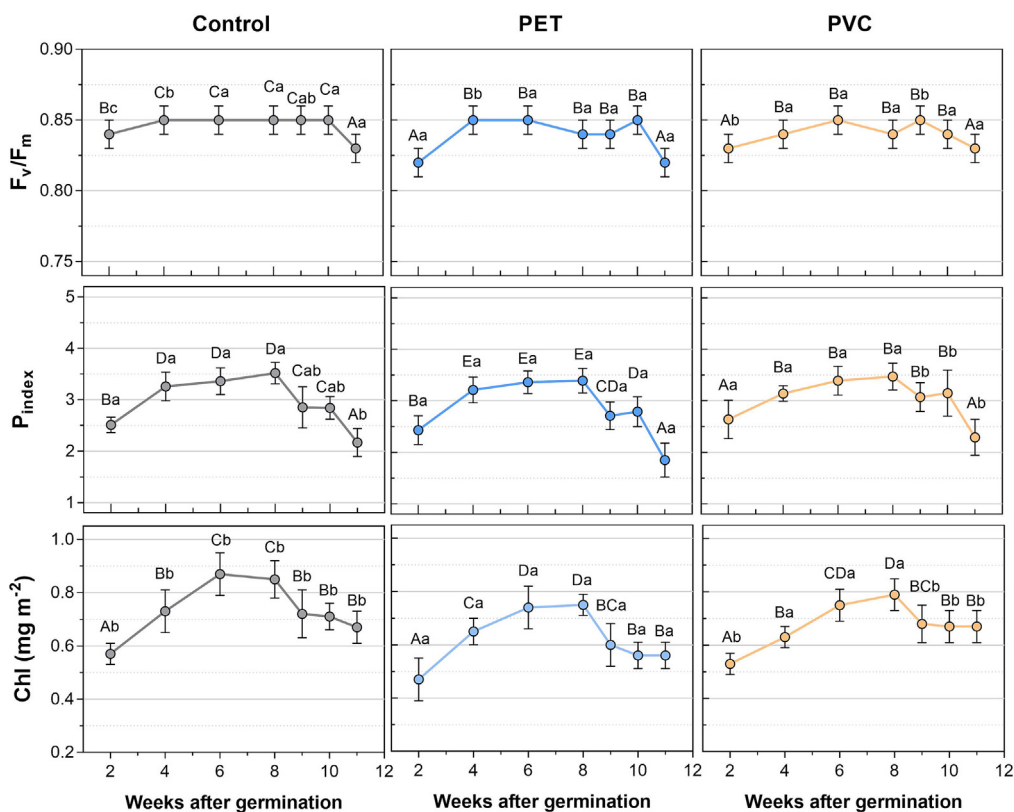
### 3.3. Effects of microplastics on leaf and fruit ionome

In microplastic-treated plants there was a decline in the concentration of Mg, Mn, Ni, Cd and Na in leaves (Table S6,  $F = 29.64$ ,  $P < 0.0001$ ,  $F = 7.513$ ,  $P = 0.002$  and  $F = 35.59$ ,  $P < 0.0001$ ,  $F = 46.91$ ,

$P < 0.0001$ ,  $F = 11.35$ ,  $P = 0.0002$  for Mg, Mn, Ni, Cd and Na respectively).

Concerning the fruit ionome (Table S7), microplastic-treated plants had significantly greater fruit Mg, Ni and Cd concentrations ( $F = 12.82$ ,  $P < 0.0001$ ,  $F = 14.32$ ,  $P < 0.0001$  and  $F = 119.9$ ,  $P < 0.0001$ , for Mg, Ni and Cd respectively). Fruits from PET-treated plants showed significantly lower Ca and Mn concentrations ( $F = 46.93$ ,  $P < 0.0001$ ,  $F = 11.53$ ,  $P = 0.0002$  for Ca and Mn respectively), while K, Mn and Zn concentrations decreased only in those from PVC-treated plants ( $F = 4.42$ ,  $P < 0.05$ ,  $F = 11.53$ ,  $P = 0.0002$  and  $F = 10.7$ ,  $P = 0.0003$  for K, Mn and Zn respectively). Compared to fruits from control plants, Na concentrations increased when plants were exposed to PET and decreased in those exposed to PVC ( $F = 27.48$ ,  $P < 0.0001$ ). All analysed tissues had Pb concentration below the detection limit.

Shoot-to-fruit ratio was calculated to characterise the microplastic-induced changes in element translocation between these two organs



**Fig. 3.** Values of  $F_v/f_m$ , performance index ( $P_{index}$ ), and chlorophyll content in *Solanum lycopersicum* plants grown in presence of PET or PVC 0.5 %. Values are mean of 12 replicates  $\pm$  standard deviation. Letters indicate the significant differences among the treatments; capital case for comparison of the same treatment over the different times, lower case for comparisons among the different treatments within the same exposure time (at least  $p < 0.05$ ).

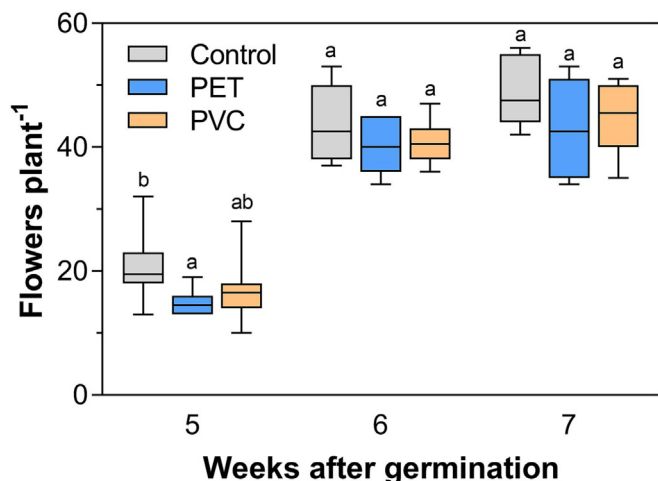


Fig. 4. Box-plot of the number of flowers per plant of *Solanum lycopersicum* grown in presence of PET or PVC 0.5 %. Whiskers from minimum to maximum value of the data sets are reported. Lower case letters indicate significant differences among the mean values (at least  $p < 0.05$ ).

(Table S8). Significant increases in shoot-to-fruit ratio were scored in plants grown in the presence of both polymers for Mg, Ni and Cd ( $F = 30.41$ ,  $P < 0.0001$ ,  $F = 27.53$ ,  $P < 0.0001$ ,  $F = 368.4$ ,  $P < 0.0001$ , respectively) and for Cu and Na for PET only ( $F = 23.76$ ,  $P < 0.0001$  and  $F = 25.38$ ,  $P < 0.0001$ ). Calcium shoot-to-fruit ratio was lower in microplastic-treated samples ( $F = 71.42$ ,  $P < 0.0001$ ). The same occurred for K and Zn ratio in PVC-treated samples ( $F = 7.798$ ,  $P = 0.017$  and  $F = 7.963$ ,  $P = 0.0015$ , respectively).

Fig. 7 represents the amplitude heatmaps of the treatment-induced changes in element concentration and shoot-to-fruit ratio, highlighting their microplastic-, element- and organ-dependencies (red colour indicates a decreased element concentration compared to the corresponding control and green colour an increased element concentration compared to the corresponding control). In general, plastic-induced variations were evident in fruits but less so in leaves, with mainly decreases in leaves and both decreases and increases in fruits. Overall, PVC was the polymer that induced the greater variations in element concentrations. Similarly, also for the shoot-to-fruit ratio, anomalies/departures from control were scored, with PVC treatment resulting in the greater changes.

#### 3.4. Effects of the microplastics on fruit properties

Fig. 8 reports the parameters measured on fruits from control and PET and PVC treated plants. Regarding the concentration of the studied

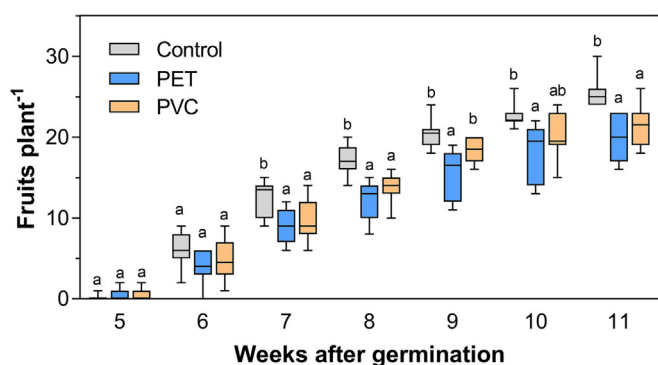


Fig. 5. Box-plot of the number of fruits per plant of *Solanum lycopersicum* grown in presence of PET or PVC 0.5 %. Whiskers from minimum to maximum value of the data sets are reported. Lower case letters indicate significant differences among the mean values (at least  $p < 0.05$ ).

nutritionally valuable carotenoids, only PVC induced a 20 % decline in lycopene levels in fruits compared with control plants ( $F = 3.783$ ,  $P = 0.0332$  and  $F = 0.6572$ ,  $P = 0.5250$  for lycopene and  $\beta$ -carotene respectively). The presence of PET in the soil provoked a 10 % decrease of in fruit TSS ( $F = 9.203$ ,  $P = 0.0007$ ), while the total soluble sugars and TA was similar in all samples ( $F = 0.1423$ ,  $P = 0.8679$  and  $F = 3.067$ ,  $P = 0.0600$ , for total soluble sugars and TA respectively).

Finally, total phenols were 15 % lower in fruits from PVC-exposed plants in comparison to those from plants grown in non-contaminated soil ( $F = 3.82$ ,  $P = 0.0322$ ).

#### 3.5. PCA results

Treatment groups and shoot or fruit traits were merged in a single biplot graph to help visualize the contribution of traits in separating plants grown in absence or in presence of PET/PVC. PCA biplot for shoots (Fig. 9a) explained 47.2 % of total variability with the two principal components (28 % and 19.2 % for PC1 and PC2 respectively), showing a clear separation of the three treatments. Control plants were separated from PET/PVC-treated ones especially along the PC1 dimension, where the most discriminating traits were shoot Ni and Cd concentrations (Fig. 9a, Table S9a). PVC-treated plants were distant from PET-treated plants and partially from control plants along the PC2 dimension, with shoot fresh weight and water content as main vectors in determining the separation (Fig. 9a, Table S9a). Concerning PCA biplot for fruits (Fig. 9b), 41.2 % of total variability was explained with PC1 and PC2 (24.6 % and 17.2 % for PC1 and PC2 respectively). Also in this case, the three treatments were remarkably separated, even more than what happened in the PCA biplot for shoot traits. Fruits of control plants were particularly distant from the ones of PVC-treated plants along the PC1 dimension and the two most discriminating traits were Cd concentration and water content (Fig. 9b, Table S9b). Instead, fruits of PET-treated plants were separated from those of the other treatments along the PC2 dimension, following the vector of Mg and Ca concentration (Fig. 9b, Table S9b).

## 4. Discussion

In this work, the impact of soil polluted with PVC and PET microplastics on shoot growth, photosynthesis, and fruit development and quality in *Solanum lycopersicum* was investigated. For the biometric parameters, our results show that the degree of microparticle toxicity depended on the phenological stage considered, rather than the polymer used.

Specifically, when comparing fresh biomass productions at the end of the experiment, it was found that PET did not limit shoot growth. This finding is consistent with earlier studies (Chen et al., 2022a, 2022b; Colzi et al., 2022) and is likely to be associated with the reduced impact of PET on soil structure compared to other plastic polymers (Souza Machado et al., 2018). By contrast, PVC impaired plant development, with a decline in fresh weight confirming its toxicity. This has recently been associated with the strong ability of this polymer to induce oxidative stress or alter plant ionome (Colzi et al., 2022; Pignattelli et al., 2020). Indeed, PVC can produce radical species (Rånby et al., 1978) and the highly dangerous HCl by-product (Yuan et al., 2014). Given the lack of PVC-induced shoot dry mass changes, our results indicate that it exerted its toxicity mainly by altering plant water relations, as already reported for polymer particles (Khalid et al., 2020). This probably occurred through multifaceted mechanisms. Among them, in addition to a direct root malfunctioning due to the above-mentioned toxicity of PVC, the presence of plastic particles on the root surface could have increased its water repellency and decreased its hydraulic conductance, given the lower wettability of this polymer compared to PET (as inferred from contact angle values, Hild Frank (2023) and postulated for shoots of the rootless plant *Tillandsia usneoides* (Falsini et al., 2022)).

The continuous assessment of various parameters throughout the entire crop cycle also indicated that PVC, but not PET, was toxic for this crop. This monitoring was done to determine whether all parameters were similarly

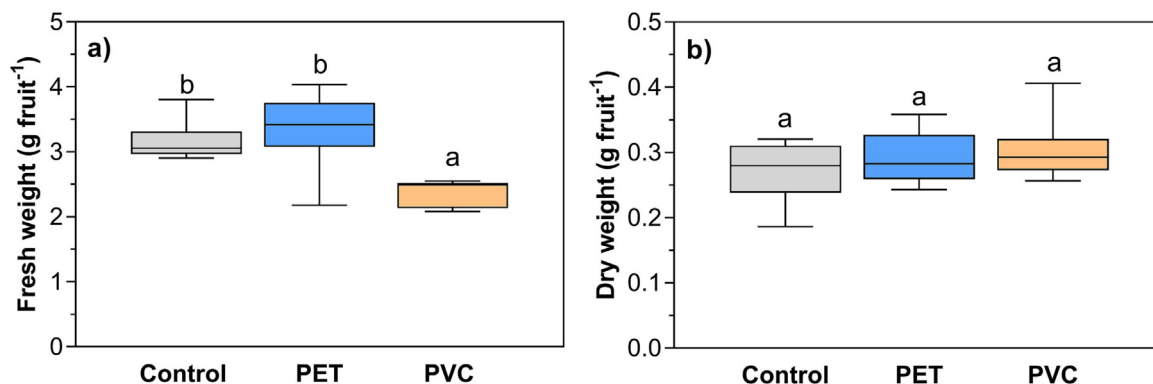


Fig. 6. Box-plot of the fruit fresh and dry weight (a and b) of *Solanum lycopersicum* plants grown in presence of PET or PVC 0.5 %. Whiskers from minimum to maximum value of the data sets are reported. Lower case letters indicate significant differences among the mean values (at least  $p < 0.05$ ).

affected by the pollutants and to find possible mechanisms underlying plastic particle toxicity. The evaluation of leaf area alone, possible only during the first stages of plant growth, resulted in the opposite ranking of plastic toxicity, with PET, but not PVC, impairing leaf development. However, this negative effect was short-termed and already not visible four weeks after germination. This again highlights the fact that plant's phenological stage influences responses to microplastics and the limitations of drawing conclusions based solely on short-term studies that only consider part of the crop life cycle.

The photosynthetic parameters were evaluated throughout the entire crop life cycle, showing an increase at the beginning and a decrease at the end. The presence of PET and PVC microplastics in the soil are known to dampen  $F_v/fm$ ,  $P_{index}$  and other photosynthetic parameters (e.g. photosynthetic pigments, maximum electron transport rate) in plants, even though with mechanisms still uncertain and surely multifactorial (see e.g. Colzi et al., 2022; Pehlivan and Gedik, 2021; Zhang et al., 2022). While a similar response was also observed in this study, the presence of microplastic only affected some of the measured parameters and to a limited extent. This minimal effect of the plastic particles on the leaf photosynthetic activity aligns with the non-toxic effect of PET and is unlikely to be the main factor underlying PVC toxicity. Nevertheless, when focusing only on the early stages of development (two weeks after germination) most of the photosynthetic parameters indicated PET as the more toxic material, in line with the early reduction in leaf area observed in PET-treated plants. Overall, when

considering our entire dataset, this clearly shows how snapshot measurements over a short period of time might be misleading in the evaluation of microplastic toxicity.

Regarding food security and safety, a key issue is understanding how fruit production and quality will change with increased microplastic pollution in agroecosystems. Despite the limited effects on plant growth, our results indicate that microplastics in the soil can reduce fruit numbers in *S. lycopersicum* cv. Micro-Tom. Since the experiment lasted until the end of the crop cycle, we were able to assess the real reduction in the final yield and not only transient delay in crop production generated by microplastics, as in the case of tomato plants exposed to plastic-bearing sewage sludge (Hernández-Arenas et al., 2021). Therefore, the threat posed by plastic particles to agroecosystems is not only real but also unpredictable, as it is independent from evident microplastic-induced reductions in shoot biomass. Among the causes of the lower number of fruits in plants grown in polluted soils (which declined by 25 % and 15 % with PET and PVC respectively), it is likely that the presence of microplastics resulted in early flower abortion as plastic particles did not negatively affect the number of flowers produced. The missed fruit development could be attributed, at least in part, to microplastic-induced alterations in the hormones mediating this process, as plastic particles have been shown to induce changes in hormone profile in hydroponically grown tomato seedlings (Bouaicha et al., 2022). In addition, the above-mentioned alterations in shoot water relations in PVC-treated plants could also contribute to this missed fruit

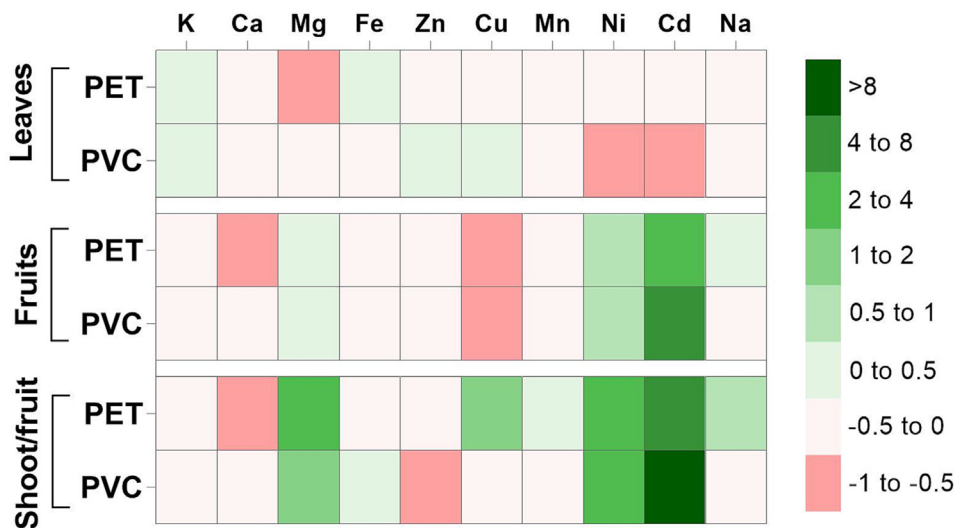


Fig. 7. Heatmap showing the variation of leaf and fruit ionome, and of shoot-to-fruit ratio of *Solanum lycopersicum* plants after 15 weeks of growth in presence of PET or PVC 0.5 %. Colour scale indicates decreased (red), unchanged (white) or increased (green) element concentration in respect to the corresponding control plants. The fold-number of variation is reported, together with asterisks indicating the significant difference between the element concentrations in treated and control plants (at least  $p < 0.05$ ).

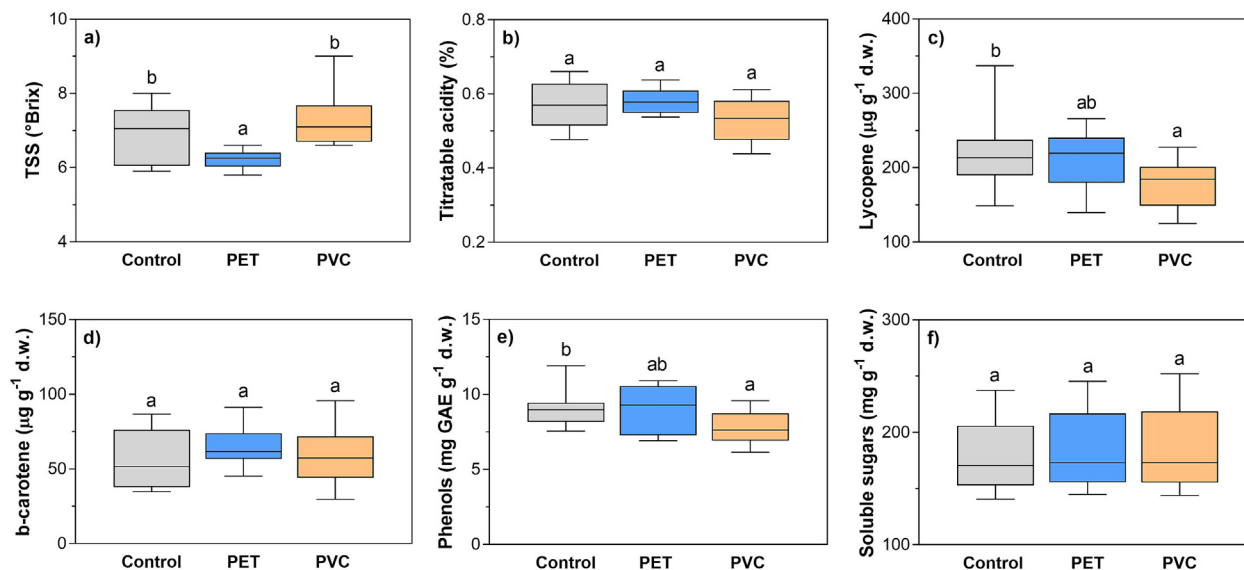


Fig. 8. Box-plot of the fruit quality parameters of *Solanum lycopersicum* plants grown in presence of PET or PVC 0.5 %: a) total soluble solids (TSS), b) titratable acidity (TA), c) lycopene concentration, d)  $\beta$ -carotene concentration, e) total phenol concentration, and f) soluble sugar concentration. Whiskers from minimum to maximum value of the data sets are reported. Lower case letters indicate significant differences among the mean values (at least  $p < 0.05$ ).

development, as drought stress increases flower drop (Lamin-Samu et al., 2021). As for mean fruit size, the impact of microplastic on plant weight mirrored those on fruit weight, with only PVC impacting the fresh mass, probably due to alteration in the plant water relationship as postulated above. The consequent lower fruit water content could be considered as a positive characteristic for fruit quality (Guichard et al., 2001). In any case, given the low number of fruits produced in all treated plants, our data strongly suggest that microplastics in the soil have a detrimental effect on net fruit yield, with a 20 % and 38 % decline for PET and PVC respectively. To the best of our knowledge, this is the first report about such negative effect of these new era contaminants on tomato yield, thus posing serious concern on the future of food production.

Microplastics can alter the plant ionome due to direct and indirect effects of plastic polymers on root functioning (Colzi et al., 2022). In the case of *S. lycopersicum* shoots, PET and PVC induced decreases for the same ions, namely Mg, Mn and Ni. These declines are hardly conceivable as the causes underlying microplastic toxicity as their concentrations did not fall outside the optimum range commonly reported for plant shoots (Marschner, 1995; Kabata-Pendias, 2000). Along with the nutrients, Cd, Pb and Na were also assessed due to their importance when evaluating vegetable nutritional value. While Pb was never detected in any of the samples, Cd and Na concentrations not only remained within the maximum established limits for shoots (Marschner, 1995; Kabata-Pendias, 2000), but also decreased in the samples grown in presence of plastics.

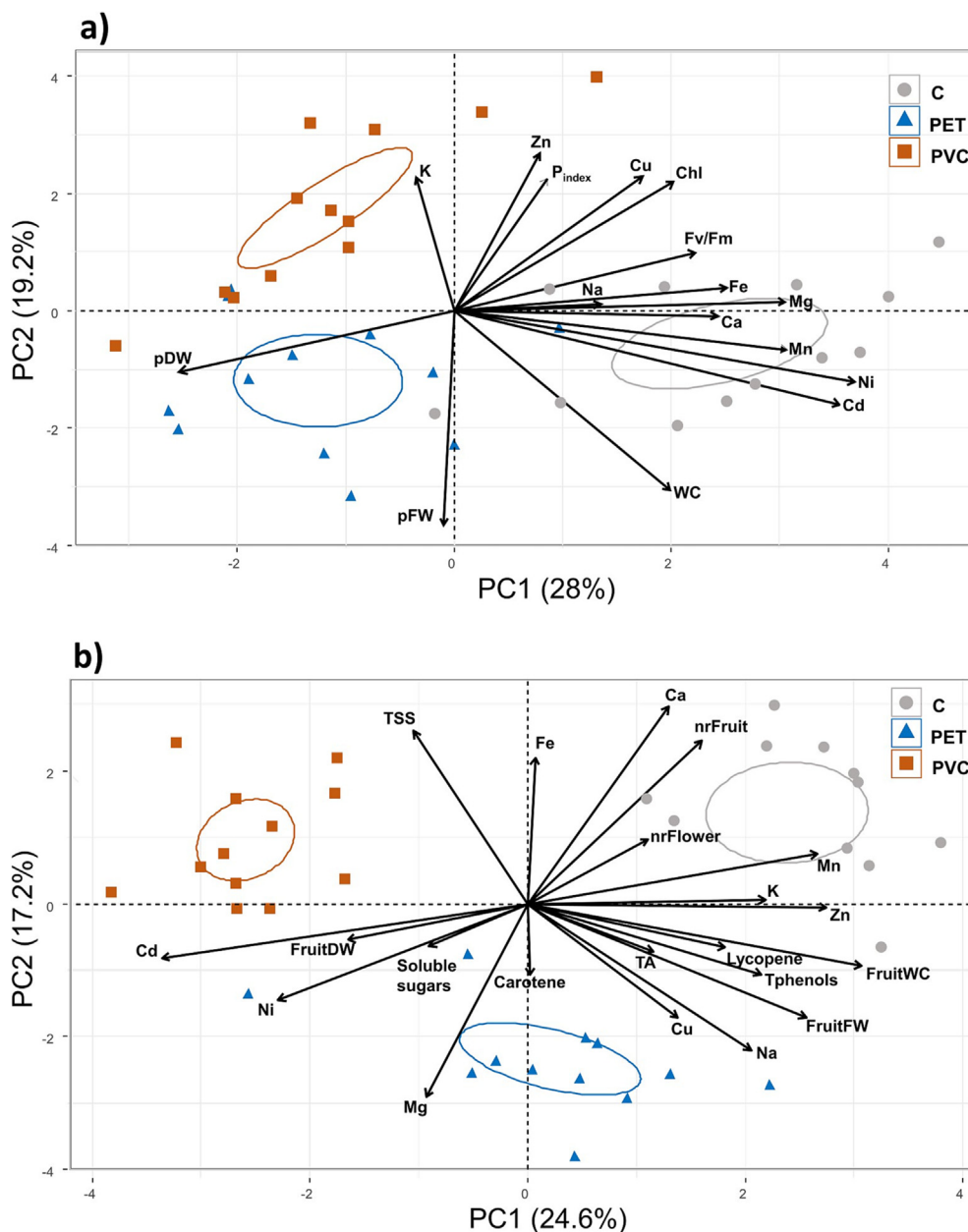
Fruit ionomics is a valuable proxy for productivity and quality in tomato (Komatsu et al., 2022; Watanabe et al., 2016). Unexpectedly, the slight elemental variations induced by the microplastics in shoots were linked to dramatic changes in fruit ionome, with material-dependent increases or decreases for several ions. In most cases, there was a decline in the concentration of nutrients in fruits from PET or PVC-treated plants compared with control plants. This suggest that soil microplastic pollution can have damaging effects for fruit nutritional value. A particular concern was raised by the declines in: (i) Ca, in opposition to the directives of food fortification (White and Broadley, 2009); and (ii) K, in PVC-treated plants only, since tomatoes are considered a valuable dietary source of this element (Perveen et al., 2015). Furthermore, while fruits from treated plants did not show any visual symptoms of disorders, lower Ca concentrations could represent an issue for cultivars with a longer life cycle and longer maturation time as they could potentially develop the typical syndrome of Ca deficiency, the so-called blossom-end rot (Taylor and Locascio, 2004). Conversely, the decline in K could explain, at least in part, the smaller fruit size, since this

element has been identified as crucial for tomato yield (Caretto et al., 2008; Daoud et al., 2020) and the increase in flower drop, as K deficiency is known to promote that process (Wood et al., 2010). Fortunately, the concentrations of Fe and Cu, critical elements for biofortification as they are among the most common micronutrient deficiencies in humans (Saghir Ahmad, 2015; Wairich et al., 2022; White and Broadley, 2009), were not affected by the microplastic treatments. The other fundamental element for biofortification, Zn, decreased only in the presence of PVC.

While microplastics led to a decline in the concentration of ions positively linked with fruit quality, the opposite was true for anti-nutritional factors, with increases in Mg, Na (only in case of PET-treated plants), Ni and Cd. Only the increase in Mg did not represent a lowering of the nutritional values of tomatoes, since it is included in the nutrients to encourage, while fruits with higher Na are less desirable (Drewnowski, 2010). More alarmingly, Ni and Cd can represent a health safety hazard in food webs and environmental matrices. While Ni is an essential nutrient for some microorganisms, plants, and animal species, its nutritional value for humans has yet to be recognised (White and Brown, 2010). On the other hand, Ni is reported to negatively affect human health through a variety of side effects, ranging from allergy to chronic diseases (Genchi et al., 2020). Despite this, a specific legislation on Ni in food is missing and currently there is only a recommendation of a tolerable daily intake of  $13 \mu\text{g Ni kg}^{-1}$  body weight set by the European Food Safety Authority (Schrenk et al., 2020). Our data suggests that the presence of microplastics in the soil has the potential to increase Ni uptake and translocation to shoot tissues. Given that tomatoes are already considered naturally rich in Ni (Roccotello et al., 2022), this could potentially raise Ni fruit concentrations to levels that exceed the acceptable daily dose. Cadmium is classified as carcinogen (ATSDR, 2007; IARC (International Agency for Research on Cancer), 2016) and, for vegetables like tomato, is allowed at a maximum level of  $0.050 \mu\text{g g}^{-1}$  fresh weight (Commission Regulation (EC) No 1881/2006 of 19 December 2006 Setting Maximum Levels for Certain Contaminants in Foodstuffs, 2006). Considering that the water content of fruits was around 90 %, the tomatoes from control plants showed a Cd concentration within the maximum acceptable limit. This however was not the case for fruits from microplastic-treated plants which raises concerns for human health that are far more alarming than the already serious declines in net fruit production.

Shoot-to-fruit ratio was calculated to uncover some of the mechanisms underlying the observed alterations in fruit ionome, as this parameter reflects element mobility in the phloem and the efficiency of its xylem transport into the fruit (Watanabe et al., 2016). This analysis indicates that





**Fig. 9.** Principal Component Analysis (PCA) biplot of selected traits of *Solanum lycopersicum* shoots (a) and fruits (b) after growth in absence or in presence of PET/PVC. The length of the vectors shows the contribution of shoot/fruit traits on first and second dimensions (PC1 and PC2); the circles represent the dispersion of data for each treatment with respect to both PC1 and PC2.

microplastic-induced alterations in leaf and fruit ionome led to contrasting accumulation/depletion patterns in these two organs. For some ions there were parallel reductions (Mn, and Na in case of PVC, with no changes in shoot-to-fruit ratio) and increases (Mg, Ni and Cd, and Na in the case of PET, with an extremely large increase in S:F) in the measured concentrations both in leaves and fruits. By contrast, for other ions, there were no changes (Cu, Fe and Zn-PET) or their concentrations only declines in fruits but not in leaves (K, Ca and Zn-PVC, with a decrease in S:F). This suggests that microplastics in the soil interfered with one or both above-mentioned processes in an element-dependant way, without excluding the possibility that these polymers interfered with the element root uptake and shoot translocation as well.

Concerning the other investigated fruit qualities, the presence of microplastics in the root-zone decreased some of the beneficial compounds present in tomatoes, although to a lesser extent compared to changes in fruit ionome. Specifically, lycopene and total phenol concentrations were

lower in fruits from PVC-treated plants, thus jeopardising the intake of these major contributors of the fruit antioxidant capacity (Martínez-Valverde et al., 2002). Interestingly, this decline was caused by the plastic material more effective in inducing oxidative stress in plants (Pignattelli et al., 2020). On the other hand, PET-exposed plants produced fruits with reduced TSS, which is one of the most important traits positively related to consumer preference (Malundo et al., 1995). Therefore, the microplastic-induced decrease in fruit quality is not only of concern for human health but also for producers, as tomato consumption and profitability are predicted to increase with greater consumer satisfaction with the fruit quality (Kader, 2008).

Insights from PCA into the complex behaviour of the investigated shoot and fruit traits showed that microplastic induced effects were organ- and polymer-specific. The separation of the three treatments in the PCA biplots was clear (Fig. 9a,b). Nevertheless, when considering fruit traits, PCA more effectively discriminated PVC-treated samples from control ones compared

to PET-treated samples. This suggests that PVC has a greater toxicity on tomato fruits. Interestingly, the treated samples spread over two opposite directions in respect to control samples, but always following the direction of the Mg, Cd and Ni vectors. This is an indication that both polymers dramatically changed element concentration in the fruits and shoots of plastic-treated plants.

Further analyses are needed to understand the physiological mechanisms underlying the microplastic-induced variations in fruit ionome and properties. A complete evaluation of the possible food safety hazards associated with the presence of microplastics in such organs is also required. Indeed, even smaller plastic particles than those used in the present experiment have already been discovered in some fruits and vegetables (Oliveri Conti et al., 2020), which raises additional concerns about the impact of microplastics on health and safety (Kadac-Czapska et al., 2022),

## 5. Conclusions

PET and PVC microplastics in the soil had only marginally effects on *Solanum lycopersicum* growth and physiology in the vegetative phase, with only PVC inducing a slight reduction in shoot fresh mass at the end of the crop life cycle. These limited effects on shoot growth and physiology were not unequivocal predictor of changes in fruit production, since both plastic polymers induced a decrease in the number of fruits, which was also coupled with a decrease in fruit fresh weight in the case of PVC. In addition, both polymers, including the apparently not toxic PET, lowered fruit quality, with a concerning increase in anti-nutritional factors while several nutritionally valuable ones declined, such as lycopene and total phenols in the case of PVC. Our results highlight for the first time that microplastics may be a threat for agroecosystems, not only for yield reduction and economical losses, but also for food safety. Such worrying results deserve to be further investigated by both broadening the range of treatments, type, and size of plastic particles and scaling up from small pilot studies to large, field-based studies.

## CRediT authorship contribution statement

**Marco Dainelli:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – review & editing. **Sara Pignattelli:** Formal analysis, Investigation, Writing – review & editing. **Nadia Bazihizina:** Formal analysis, Investigation, Writing – review & editing. **Sara Falsini:** Formal analysis, Investigation, Writing – review & editing. **Alessio Papini:** Data curation, Resources, Writing – review & editing. **Ivan Baccelli:** Resources, Writing – review & editing. **Stefano Mancuso:** Resources. **Andrea Coppi:** Methodology, Resources, Writing – review & editing. **Maria Beatrice Castellani:** Resources, Writing – review & editing. **Ilaria Colzi:** Conceptualization, Formal analysis, Investigation, Data curation, Writing – review & editing, Supervision, Funding acquisition. **Cristina Gonnelli:** Conceptualization, Methodology, Resources, Writing – original draft, Writing – review & editing, Supervision, Project administration.

## Data availability

Data will be made available on request.

## Declaration of competing interest

The authors declare they do not have financial interests/personal relationships which may be considered as potential competing interests.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2023.165119>.

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