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Original:

Celletti, S., Fedeli, R., Ghorbani, M., Loppi, S. (2023). Impact of starch-based bioplastic on growth and biochemical parameters of basil plants. SCIENCE OF THE TOTAL ENVIRONMENT, 856(2) [10.1016/j.scitotenv.2022.159163].

Published:

DOI: http://doi.org/10.1016/j.scitotenv.2022.159163

Impact of starch-based bioplastic on growth and biochemical parameters of basil plants

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Abstract

The recent use of bioplastics in agriculture is considered an ecological choice, aimed at limiting the environmental impact of plastics, in line with the Sustainable Development Goals of the United Nations. However, the impact of bioplastic residues on the environment is unclear as knowledge is lacking. This is the first study investigating the effect of a starch-based bioplastic on the growth and biochemical parameters of basil. Bioplastic was experimentally prepared and added to the soil at 2.5% (w/w), corresponding to twice the concentration of plastic mulch film residues currently found in cultivated soils, in view of the increasing agricultural use of bioplastics. Basil plants were grown without (controls) and with bioplastic addition for 35 days, under controlled experimental conditions. Compared to the control, plants exposed to bioplastic showed stunted growth (in terms of shoot fresh weight, height, and number of leaves). Significant reductions in the content of chlorophyll, protein, ascorbic acid, and glucose were also observed. Finally, the treatment caused oxidative stress, as evidenced by the increased content of malondialdehyde in the shoots. The addition of bioplastic increased the electrical conductivity and reduced the cation exchange capacity of the cultivation soil. These results suggest that bioplastic in soil may promote the onset of stressful conditions for plant growth in a similar manner to plastic. They will be complemented by further investigations to unravel the mechanisms underlying these responses, involving different doses and types of bioplastics and other crop species.

Keywords: antioxidant; bio-based plastic; biodegradable plastic; corn starch; lipid peroxidation.

1. Introduction

Plastic pollution is one of the most serious and pressing environmental concerns, as plastic is an indispensable resource from which affordable and useful products are obtained to satisfy the needs of human society (Andrady and Neal, 2009). However, it is also an emblem of waste, pollution and ecotoxicity, being an artificial polymeric material derived from fossil fuels such as petroleum (a source that will run out) and not readily biodegradable (Amobonye et al., 2021; Thompson et al., 2009). Furthermore, plastic has been recognized as hazardous to natural as well as agricultural ecosystems and human health (Hartmann et al., 2019; Ullah et al., 2021), as it is pervasive, ubiquitous and accumulates (Lebreton et al., 2018), by miniaturising into tiny particles, known as microplastics (MPs) and nanoplastics (NPs) (Thompson et al., 2004).

Due to the growing awareness of the importance of environmental sustainability and ecological transition, in 2015, all UN Member States negotiated the “2030 Agenda for Sustainable Development”, to promote human well-being and protect the environment, with a focus on reducing the carbon footprint and dependence on fossil fuels, in a broader effort to mitigate climate change. In this context, extensive research has been devoted to exploring industrial techniques to produce “green” materials, which are not harmful to the environment but have the same favourable characteristics as plastic (Moshood et al., 2022). Among these, bioplastic has attracted considerable attention as it is a type of polymeric material that is either biodegradable or bio-based (made at least partly from biological matter such as renewable feedstocks, *e.g.*, agricultural biomass) or has both characteristics. Most bioplastics currently produced belong to the group of 100% biodegradable and renewable feedstocks (as for instance the starch blends), having two main advantages compared with conventional plastics: (i) they decompose much faster (in 4-5 years on average, depending on the chemical composition) and are therefore also easier to recycle, requiring lower energy costs; (ii) being derived from biomass waste, they do not present the problem of feedstock depletion from a circular economy perspective (Lamberti et al., 2020; Rosenboom et al., 2022).

Bioplastics are attractive in packaging for the food sector (not only for environmental protection, but also for food safety) and in numerous medical and biomedical applications (Parisi et al., 2015). Worthy of attention is the use of bioplastic in agriculture (Coppola et al., 2021). A potentially important source of bioplastic to cultivated soils are the mulch films, which are now largely manufactured from starch-based bioplastics and their use plays undoubtedly a valuable role in reducing residual plastic pollution in agricultural soils and thus significantly mitigating the impact that plastic residues have on crop quality and yield (Colzi et al., 2022).

It has been suggested that, like conventional fossil-based plastics, also bioplastic may be of environmental and health concern owing to the release and decomposition of substances (*i.e.*, additives and toxic chemicals) into small molecules, such as monomers and oligomers (Spaccini et al., 2016; Zimmermann et al., 2020). Furthermore, bioplastics could disintegrate even faster than traditional plastics and could adsorb many pollutants with various physico-chemical effects, thus representing an additional threat (Wang et al., 2022). Although bioplastics can change soil properties, affect crop growth and yield (Jiang et al., 2017; Zhang et al., 2016), and potentially enter the food chain (Huerta Lwanga et al., 2017), to the best of our knowledge, only a very few experimental studies (Huerta-Lwanga et al., 2021; Liwarska-Bizukojc, 2021; Meng et al., 2021; Mroczkowska et al., 2021; Qi et al., 2018; Rillig et al., 2019; Sforzini et al., 2016; Wang et al., 2022) have so far evaluated the effects of bioplastics on agroecosystems, also reporting quite controversial results, sometimes showing stimulating effects (Abe et al., 2022; Huerta-Lwanga et al., 2021; Liwarska-Bizukojc, 2021; Mroczkowska et al., 2021) and, in other cases, inhibiting and toxic effects (Abe et al., 2022; Huerta-Lwanga et al., 2021; Liwarska-Bizukojc, 2021; Meng et al., 2021; Qi et al., 2018; Wang et al., 2022). Therefore, based on the limited evidence of effects, especially of starch-based bioplastic, this is a completely new scenario that needs urgently to be investigated to shed light especially on the effects of bioplastics in the plant-soil system before the use of these materials becomes excessive and can cause ecotoxicity.

In this regard, the aim of this study was to investigate whether the addition of a corn starch-based bioplastic to the soil affected the growth and biochemical parameters of basil (*Ocimum basilicum* L.), which was chosen as model crop species, being a very important medicinal plant and culinary spice, widely cultivated in many countries under natural and greenhouse conditions and marketed fresh, dried, or frozen. In view of the increasingly massive use of bioplastics in agriculture, soil was therefore supplemented with approximately double the concentration of bioplastic (2.5%, w/w) compared to formulations with the highest concentration described in the literature (on average about 1.4%, w/w) to simulate plastic mulch film residues in cultivated soils (Meng et al., 2021; Ng et al., 2018; Qi et al., 2018; Sforzini et al., 2016). Analyses of changes in soil characteristics caused by bioplastic were accompanied by analyses performed on a series of useful physiological and biochemical indicators related to plant growth and health status.

2. Materials and methods

2.1. Bioplastic preparation

The biodegradable corn starch-based bioplastic was made in our laboratory by the casting technique according to similar methodologies (de Azevedo et al., 2020; Shafqat et al., 2021), with some modifications. Briefly, 3.75 g of corn starch powder (practical grade, Saint Louis, MO, USA) were mixed vigorously in 25 mL of distilled water until a homogenous white dispersion was formed. Subsequently, 3.75 g of glycerol (≥ 99.5%, Honeywell, Muskegon, MI, USA) and 2.5 mL of glacial acetic acid (100%, Merk KGaA, Darmstadt, Germany) were added to the mixture; in particular, the former increases the flexibility of the bioplastic as it acts as a plasticiser, while the latter dissolves the starch more easily as it adds ions to the mixture. The resulting milky suspension was heated to 85 °C until it thickened and became transparent and clearer. At this step, gentle agitation was carried out continuously with a glass rod to avoid the formation of bubbles and lumps. This soft gelled paste was poured while still hot and immediately spread onto a glass plate (diameter = 15 cm) to obtain a bioplastic film (Fig. 1). Before manually peeling off, the film was first incubated to harden in an oven at 100 °C for 1 h and then left to dry completely at room temperature (25 °C) for one week before use.

2.2. Potting soil preparation

The soil utilized in this study was a commercial growing medium containing different components (*i.e.*, acid peat, compost-free soil organic conditioner, pumice, perlite, organic fertilizer), purchased from VigorPlant Italia srl, and with the following physical-chemical characteristics: 43% of moisture content; 92% of porosity; 5.30 ± 0.03 of $\mathrm{pH}_{(\mathrm{H_2O}:1:20,\ \mathrm{w/v})}$; 1.12 ± 0.01 mS cm^{-1} of electrical conductivity [$\mathrm{EC}_{(\mathrm{H_2O}:1:20,\ \mathrm{w/v})}$]; 56.89 ± 2.67 meq 100 $\mathrm{g}_{\mathrm{DW}}{}^{-1}$of cation exchange capacity (CEC).

Glass pots (diameter = 5 cm, height = 7 cm) were covered with an aluminum foil and filled with 80 g of soil without (C = control) or supplemented with 2.5% (w/w) bioplastic (B = bioplastic), previously cut into pieces of uniform size (approximately 5 mm) using sharp blades and scissors.

In view of the increasingly massive use of bioplastics in agriculture, B soil was supplemented with about twice the concentration of bioplastic described in the literature (on average about 1.4%, w/w), to simulate plastic mulch film residues in cultivated soils (Meng et al., 2021; Ng et al., 2018; Qi et al., 2018; Sforzini et al., 2016).

Pots were initially irrigated to 60% water holding capacity. During the plant growth period, this condition was maintained by weighing each pot daily and by adding water when necessary.

2.3. Plant growth

Basil plants (cv. Riviera Ligure) were obtained by seeds previously soaked in distilled water for 1 h and then germinated in darkness at 22 °C between layers of distilled water-soaked paper. Subsequently, homogeneous 4-day-old seedlings were transplanted into the C and B-treated pots (3 seedlings/pot) and after 11 days from transplanting only one seedling per pot was left. Plants were grown for 35 days (which is the typical growth stage for farm supplies in the Italian market) (Sgherri et al., 2010) under controlled experimental conditions [temperature (25/20 °C, day/night), relative humidity (70%), photoperiod (16/8 h, day/night), and light intensity (250 µmol m^{-2} s^{-1} PAR)] in a climatic chamber. To account for possible microclimatic conditions within the growth chamber, the pots were randomly rotated every day.

2.4. Plant analyses

2.4.1. Chlorophyll and biometric parameters

At the end of plant growth period (corresponding to harvest: 35 days from seedling transplanting), the chlorophyll content of basil leaves was measured using a portable and non-destructive chlorophyll content meter (CCM – 300, Opti-Sciences Inc, Hudson, NH, USA). Specifically, 6 values per leaf were measured at pairs of points diametrically opposite the midrib in the following order: two along the proximal, two along the central, and two along the distal part from the base to the apex of the leaf, thus 12 measurements per plant were taken on the youngest and fully expanded leaves. The chlorophyll content was expressed on a surface basis (mg m^{-2}).

Before harvest, several biometric parameters were recorded. The plant height was measured with a ruler (considering the distance between the plant apex and the soil surface), the number of leaves was counted, and the aboveground biomass was first weighted (expressed in terms of shoot fresh weight) and then immediately frozen at 20 °C for subsequent analyses.

2.4.2. Proteins and sugars

The contents of total soluble proteins and soluble sugars (fructose, glucose, and sucrose) were determined in the extracts of basil shoots obtained following the methods described in Fedeli et al. (2022), with slight modifications. Specifically, a total of 0.250 g of frozen material was homogenised in 1.5 mL of distilled water. The extract solution was centrifuged (PK110 centrifuge, Alc International S.r.l., Cologno Monzese, MI, Italy) at 3000 rpm for 5 min at room temperature.

The supernatant was recovered and centrifuged again at 12000 rpm (Z 233 MK-2, Hermle, LaborTechnik GmbH, Wehingen, Germany) for 7 min at room temperature.

For protein determination, an aliquot of the extract (20 µL) was diluted to 1 mL with distilled water and then 0.4 mL of the diluted sample were combined with 1.6 mL of the Bradford dye reagent solution (Thermo Fisher Scientific Inc., Waltham, MA, USA) (Bradford, 1976). After 20 min, the absorbance of the samples was measured at 595 nm using a UV-Vis spectrophotometer (8453, Agilent, Santa Clara, CA, USA). Results were expressed as mg g^{-1} using bovine serum albumin (BSA) (Sigma-Aldrich, USA) as standard.

For sugar determination, the remaining extract was filtrated using 45 µm syringe filters (diameter = 25 mm, Lab Logistic Group GmbH, Meckenheim, Germany) and 150 µL of the filtrate was transferred in new tubes placed in a vacuum evaporator (Jouan RC 10-10 Vacuum Concentrator Centrifugal Evaporator, Analytical Instruments Brokers LLC, Golden Valley, MN, USA) at 40 °C until completely dried. Subsequently, the samples were resuspended in 30 µL of distilled water and directly analysed by HPLC (600E System, Waters, Milford, MA, USA). Sugar separation was allowed using distilled water as mobile phase at a flow rate of 0.5 mL min^{-1} and an ion-exchange column (10 µm, 300 × 6.5 mm, Sugar-Pak I, Waters, Milford, MA, USA), kept constantly at 90 °C by means of an external temperature controller (Column Heater Module, Waters, Milford, MA, USA). The sugars were detected by a refractive index detector (2410 RI, Waters, Milford, MA, USA). Quantification was obtained by preparing individual stock solutions, using sugar reagent-grade analytical standards (D-Fructose, a-D-Glucose, Sucrose, Merk KGaA, Darmstadt, Germany).

2.4.3. Ascorbic acid

The ascorbic acid (vitamin C) content was estimated colorimetrically following the method by Jagota and Dani (1982), with some modifications. In brief, 0.8 mL of 10% (w/v) trichloroacetic acid (TCA) (99.5%, Panreac, Castellar del Vallès, Barcellona, Spain) extraction solution were added to 0.2 g of frozen shoot material. The samples were homogenised with an ULTRA-TURRAX® (T 10 basic, Werke GmbH & Co. KG, Staufen, Germany) prior to filtration on gauze. Subsequently, the filtrates were kept in an ice bath for 5 min and then centrifuged (PK110 centrifuge, Alc International S.r.l., Cologno Monzese, MI, Italy) at 3000 rpm for further 5 min at room temperature. An aliquot of 0.4 mL of supernatant was transferred into tubes containing 1.6 mL of distilled water. Then, 0.2 mL of 0.2 M Folin – Ciocalteu reagent (Carlo Erba, Cornaredo, MI, Italy) were added to the diluted supernatants and shaken vigorously. The mixtures were incubated for 10 min at room temperature and thereafter the absorbances were measured at 760 nm (8453, UV – Vis Spectrophotometer, Agilent, Santa Clara, CA, USA). Calibration was done with 0.05 – 0.2 mL of a 100 μg mL^{-1} L-ascorbic acid (BioXtra, ≥99.0%, crystalline) stock solution. The ascorbic acid content of the samples was expressed as μg g^{-1}.

2.4.4. Malondialdehyde

Oxidative stress of membrane lipids was estimated by analysing the content of malondialdehyde (MDA) as a metabolite reactive to 2-thiobarbituric acid (TBA) according to Quagliata et al. (2021), with slight modifications. Briefly, 0.5 g of frozen shoots were homogenised with an ULTRA-TURRAX® (T 10 basic, Werke GmbH & Co. KG, Staufen, Germany) in 5.0 mL of extraction solution, obtained by dissolving 0.25% (w/v) TBA (≥ 98.0%, Merk KGaA, Darmstadt, Germany) in 10% (w/v) TCA (99.5%, Panreac, Castellar del Vallès, Barcellona, Spain). The homogenates were incubated at 95 °C for 30 min in a hot-water bath (GBath 1800 Digital Thermostatic Bath, F.lli Galli G. & P., Milano, MI, Italy) and then immediately cooled on ice to stop the reaction. Once completely cold, the samples were centrifuged (PK110 centrifuge, Alc International S.r.l., Cologno Monzese, MI, Italy) at 5000 rpm for 20 min at room temperature. After centrifugation, absorbance was measured on the recovered supernatants with a UV-Vis spectrophotometer (8453, Agilent, Santa Clara, CA, USA). To correct the absorbance at 532 nm from the interference of non-specific turbidity, absorbance at 600 nm was subtracted from the reading. The MDA content was expressed as μg g^{-1} using the molar extinction coefficient of the formed MDA – TBA complex of 155 mM^{-1} cm^{-1}.

2.5. Planted soil analyses

After harvesting the aboveground plant biomass, the entire potting soil was taken and considered as rhizosphere soil as the pots were completely rooted. Soil samples were oven-dried at 105 °C for a week to get constant weight and then crushed to pass through a 2-mm sieve before analysis.

2.5.1 pH and electrical conductivity

The soil pH and EC were measured according to (Celletti et al., 2021b)in the limpid supernatants (soil:dH_2O ratio of 1:20, g_{DW}:mL), after 5 min of centrifugation at 4000 rpm (PK110 centrifuge, Alc International S.r.l., Cologno Monzese, MI, Italy) and paper filtration from a 2 h-initial shaking (711, VDRL STIRREL, ASAL srl, Cernusco sul Naviglio, MI, Italy). The pH was determined using a pH-meter (edge® HI2002, HANNA Instruments Inc., Woonsocket, RI, USA) and EC using a conductimeter (BASIC 30, EC – meter, Crison Strumenti SpA, Carpi, MO, Italia).

2.5.2 Cation exchange capacity

The CEC was determined as described by Bascomb (1964) with some modifications. In brief, 2 g of soil were weighed in centrifuge tubes and 25 mL of 10% (w/v) $BaCl_2 \times 2\ H_2O$ solution buffered with 8.1 pH triethanolamine solution were added and shaken for 3 min, left to rest for 5 min and then shaken again for 3 min. The samples were centrifuged (PK110 centrifuge, Alc International S.r.l., Cologno Monzese, MI, Italy) at 3000 rpm for 5 min at room temperature and the supernatants were discarded. The sedimented soils were resuspended by adding 25 mL of distilled water, shaken, centrifuged, and the supernatants discarded. Further 25 mL of 0.1 N $MgSO_4 \times 7\ H_2O$ solution were added to the washed soils, again shaken and centrifuged. An aliquot (10 mL) of each clear supernatant was immediately transferred to a conical flask, containing 100 mL of distilled water and

10 mL of 30% ammonium hydroxide solution. These solutions were titrated under slow stirring with 0.05 N ethylenediaminetetraacetic acid disodium salt (EDTA – Na_2) solution, using 2 drops of 0.1% (w/v) Eriochrome Black T indicator. In parallel, a blank sample was titrated by pipetting 10 mL of 0.1 N $MgSO_4 \times 7 H_2O$ solution into the flask instead of the supernatant. The endpoint of sample titration was indicated by the colour change from clear blue to reddish purple. The results were expressed as meq 100 g^{-1}, according to the formula:

$$CECofthesoil = \frac{(m-n) * 0.05 * 2.5 * 100}{p}$$

where,

m = volume EDTA – Na_2 (mL) used to titrate the blank sample;

n = volume EDTA – Na_2 (mL) used to titrate the soil sample;

0.05 = normality of EDTA – Na_2 solution;

2.5 = conversion factor to relate the 10 mL titrated to the 25 mL $MgSO_4$ solution added;

p = weight of the soil sample (g).

2.6. Statistical analysis

Data normality was verified with the Shapiro-Wilk test. The results are presented as mean ± standard error (SE) from five biological replicates ($n = 5$). The experiment was replicated 3 times. Significant differences ($p < 0.05$) between C and B means were evaluated with the Student's t-test. Calculations were run using the free software R version 4.0.3 (R Core Team 2022). For correlation analysis, the Pearson correlation coefficient was used.

3. Results

After 35 days of growth in the presence of the corn starch-based bioplastic, basil plants showed a significant decline (-8%) in the content of leaf chlorophyll (Fig. 2A). Bioplastic also negatively affected the biometric parameters of plant growth and development: plant height, number of leaves, and aboveground fresh weight were all strongly reduced by -68%, -42%, and -82% (Fig. 2B, C, and D), respectively. Figure 2E displays visually these considerable differences in the growth of basil plants cultivated without (C = control, on the left) and with bioplastic (B = bioplastic, on the right) added in soil.

Only the content of glucose was significantly decreased (-22%) by the addition of bioplastic, while changes were insignificant for fructose and sucrose at shoot level (Fig. 3A). The total content of soluble sugars (given by the sum of fructose, glucose, and sucrose) showed an overall decreasing trend after plastic supplementation.

The addition of bioplastic to the soil caused a significant reduction (-44%) in the content of the soluble proteins in the aboveground part of basil plants (Fig. 3B).

Also, the content of ascorbic acid (vitamin C) in basil shoots was significantly decreased (-9%) after bioplastic addition (Fig. 4A).

When basil plants were grown in soil added with bioplastic, the content of MDA in shoots was significantly increased by 17% (Fig. 4B).

The correlation analysis performed between the content of ascorbic acid and MDA in the shoots showed a negative linear correlation between these two biomarkers (Pearson's r = -0.561), but this was not statistically significant ($p = 0.092$) (Fig. 4C).

At soil level, the presence of the corn starch-based bioplastic did not modify the pH (remaining stable at ~7.6), even though it was more than 2 units higher than that found at the beginning of the experiment (Fig. 5A); on the contrary, EC increased by 16%, while CEC decreased (-7%), albeit slightly, but significantly (Fig. 5B, and C, respectively).

4. Discussion

In agriculture, the challenge of replacing conventional fossil-based plastics, commonly used to produce mulch films, with bioplastics (especially bio-based and biodegradable ones, such as starch-based ones) is of utmost importance for the climate, in line with the Sustainable Development Goals of the United Nations 2030 Agenda. In contrast to plastics, it is not yet clear what impact the massive use of bioplastics will generate on the environment, and particularly on agricultural soils, both in the short- and long-term, as knowledge on these aspects is so far lacking and controversial. It is known that bioplastics decompose by releasing chemicals (Spaccini et al., 2016) and, therefore, like plastics, they may imbalance soil characteristics and, consequently, affect the growth and yield of crops (Jiang et al., 2017; Zhang et al., 2016), eventually accumulating in the parts of edible crops and entering the food chain (Huerta Lwanga et al., 2017) with possible negative implications for human health (Li et al., 2020; Muncke et al., 2020).Within this scenario, aware and conscientious research is therefore extremely urgent and essential to decipher the impact of bioplastics on the environment according to a sustainability perspective (Lamberti et al., 2020; Rosenboom et al., 2022).

The present study investigated the impact of a corn starch-based bioplastic, added to the soil, on the growth and development of basil, which is a highly interesting food crop plant. Our study focused on the analysis of the shoot alone, as this is the commercial part of interest for basil plants. The bioplastic tested was obtained experimentally in our laboratory by mixing various components (such as corn starch, glycerol, and acetic acid) in the appropriate proportions. Accordingly, knowledge of the single components of bioplastics is an advantage which will allow to investigate in the foreseeable future what component will prevail and how the different components individually will affect plant growth and soil characteristics. The concentration of bioplastic was established at 2.5% (w/w), based on the current use of bioplastics in agriculture (mostly in the form of mulch films), which amounts to more than 1% on average (Ng et al., 2018; Qi et al., 2018; Sforzini et al., 2016), but which could likely increase to extremely high levels in the near future in view of a predictive increase in the use of bioplastics due to the ecological transition in this sector (Meng et al., 2021).

The possible interaction of bioplastic with the plant was investigated by monitoring changes in growth and biochemical features associated with vegetative development and plant health.

The reductions in chlorophyll content and fresh biomass of bioplastic-treated basil plants observed in this study are consistent with the phytotoxicity effects exerted by the addition of another bio-based and biodegradable bioplastic, the polylactic acid (PLA), at 10% and 2.5% (dry soil weight), on the leaves of common bean and maize plants, respectively (Meng et al., 2021; Wang et al., 2020). On the other hand, in the same study by Wang et al. (2020), it has been evaluated also the effect of polyethylene (PE), which is a bio-based but non-biodegradable bioplastic, and it seemed not to cause any phytotoxic effect on maize plants. Conversely, Pignattelli et al. (2021) demonstrated that garden cress (*Lepidium sativum* L.) exposed to the lower size (61 – 499 μm) of polyethylene terephthalate (PET), having the same characteristics of PE, decreased the photosynthetic efficiency. Therefore, the studies mentioned above suggest that different types of bioplastic polymers affected differently plant growth and that differences are most likely a function of the bioplastic's level of degradability and size. In our experiments, at harvest, the added bioplastic left no physical traces after careful visual inspection, indicating that it had totally dissolved in the soil (Mroczkowska et al., 2021) and therefore, being readily bioavailable, was probably taken up by the root system.

In addition, our data showing a negative regulation of plant growth parameters was in line with the outcomes of recent studies (Qi et al., 2018; Sun et al., 2020; van Weert et al., 2019), reporting the effects of MPs and NPs on different plant species. Hence, we can suggest that, in this case, the bioplastic exerted effects similar to plastic.

Combining the observed negative effects on basil growth parameters by bioplastic and the fact that, in plants, sugars, produced by photosynthesis, are used to support all aspects of plant growth and development (Ciereszko, 2018; Sami et al., 2016), we evaluated how the content of soluble sugars changed in the shoot when basil plants were grown with bioplastic. Specifically, we determined the content of the disaccharide sucrose and that of both its two distinct monosaccharide constituents (*i.e.*, fructose and glucose). Interestingly, only the content of glucose dropped significantly. This lower glucose level could explain the lower biomass accumulation observed in the shoots of the bioplastic-treated plants, as glucose acts as a signal molecule and phytohormone affecting the expression of many different genes involved in key processes such as leaf growth (Moore et al., 2003). Indeed, the bioplastic tested in our study seemed to behave like a type of plastic (*i.e.*, polystyrene – PS) used in the experiments by S. Li et al. (2021), where PS particles, once absorbed by the roots of barley plants, caused an inhibition of energy supplementation and biomass accumulation. Moreover, the overall decreasing trend resulting from summing up all sugars also agreed with the observations in cucumber fruits by Z. Li et al. (2021), where treatment with PSNP of different sizes significantly reduced the soluble sugar content.

After bioplastic exposure, basil plants also reacted by reducing the content of proteins, which are another class of primary organic compounds. This result is reasonable since proteins are biological macromolecules constituting essential building blocks of all living organisms, including plants, and, thus, a slowdown in their synthesis is a clear sign that the basil plants were in unfavourable growth condition (Murray et al., 2017). To the best of our knowledge, no study in the literature has documented any effect of bioplastics on changes in the protein amount in plants. Only the study by Z. Li et al. (2021) demonstrated an increase of this parameter on cucumber fruits, but using a plastic material, the PSNP, of different sizes. Since many proteins belong to the enzyme category, it can be speculated that, under these conditions, the addition of bioplastic to the soil might have hindered some biochemical enzymatic reactions of vital importance for plant metabolism, such as photosynthesis, as evidenced by the significant reduction in the content of chlorophyll, an essential molecule for the proper functioning of the photosynthetic process.

For its protective role against the effects of drought, ozone, and ultraviolet sunlight (Gallie, 2013), L-ascorbic acid (commonly known as vitamin C) is the most widespread non-enzymatic antioxidant compound in plants (Arrigoni and De Tullio, 2002; Ishikawa et al., 2006). Very worthy of mention, Dowdle et al. (2007) demonstrated the linkage between ascorbic acid and the growth and life of plants. These authors revealed ascorbic acid is biosynthesised from hexose sugars (including fructose and glucose), given the discovery of the existence of a specific enzyme, GDP-L-galactose phosphorylase, capable of synthesising ascorbic acid in plants (Dowdle et al., 2007). Our findings suggested that lower glucose accumulation probably led to a significant decrease in ascorbic acid level in bioplastic-treated basil shoots, further corroborating the evidence of the essential correlation between glucose and ascorbic acid. Experiments that evaluated the effect of different PSNP sizes on cucumber fruits also confirmed this relationship between the two molecules (Z. Li et al., 2021). These authors clearly demonstrated that treatment with PSNP of 500 nm in size significantly reduced both ascorbic acid and soluble sugar content, while treatment with PSNP of 100 nm significantly increased both. In addition, these findings support our results regarding the effect of bioplastic, which, like plastic, decreased both sugar and ascorbic acid content.

Plants subjected to environmental stress have been shown to increase the production of antioxidant compounds, in order to counteract the increased production of reactive oxygen species (ROS) (Hasanuzzaman et al., 2020); these species are harmful to plant vitality as they react with cell membrane lipids and cause their peroxidation (Su et al., 2019). In our study, we analysed the content of MDA, which is the main product formed at the end of the chain of radical reactions caused by a stressful environmental condition and, therefore, it is commonly used as a biomarker for detecting the extent of oxidative damage to biological membranes (Shulaev and Oliver, 2006). Exposure of basil plants to corn starch-based bioplastic significantly increased the MDA content in the shoots. On the other hand, however, as mentioned above, basil plants showed a rather weak ascorbic acid scavenging ability to cope with the high level of oxidative stress. Indeed, at the onset of oxidative stress, it has been widely observed that antioxidant compounds fail to counteract ROS production, which occurs normally in plant metabolism. Certainly, a more solid hypothesis could be formulated by also analysing the activities of antioxidant enzymes as well as the level of non-enzymatic antioxidants, such as ascorbic acid in this case (Wani et al., 2013). Thus, this type of bioplastic might have established a trade-off mechanism in the aerial part of the plant whereby when oxidative stress compounds increase, defence compounds decrease, although the correlation analysis between the two evaluated biomarkers did not statistically validate it. However, this hypothesis is not dissimilar to the study by Pignattelli et al. (2021), describing the effects of PET MP treatment in cress plants, in which an opposite trend was evident between the antioxidant defence response (which decreased) and the accumulation of ROS (which increased) in the leaf tissue. In contrast, Gao et al. (2019) reported that treatment with PE MP increased both the level of oxidative stress and the content of ascorbic acid in lettuce plants. Based on the high levels of MDA accumulation observed in the shoots and the knowledge that these high levels may damage cell membranes and even lead to cell death (Sharma et al., 2012), it could be a plausible explanation that the starch-based bioplastic, considered in our study, can be involved in the impairment of bio-membrane proteins, as supported by the drop in total protein content found in our basil shoots.

In this study, we can assume that most of the effects observed at the shoot level of basil plants are probably the indirect consequence of the actions exerted by bioplastic addition to the soil on the proper function of plant roots. As an example, these actions may include the subtraction of oxygen due to the degradation processes of the bioplastic by microorganisms and the depletion of water in the soil due to the hydrophilicity and water absorption by bioplastic (Abe et al., 2021). The changing physiological and biochemical responses observed in the leaf apparatus of basil plants exposed to bioplastic could reflect an imbalance in some fundamental, yet crucial, soil variables. Much is known about the changes in soil properties due to the persistence and accumulation of plastics (Bouaicha et al., 2022; Chen et al., 2022; Khalid et al., 2020; Liu et al., 2017). Likewise plastics, it should be stressed out that the assessment of the impact of bioplastic in agricultural soils is of paramount importance from an ecological and human food safety perspective (Qi et al., 2018). For these reasons, we analysed some inherent soil chemical characteristics, mainly pH, EC, and CEC in soils where basil plants were grown. One of the most important soil variables is pH, as it mainly affects the availability of nutrients to plants (Delgado and Gómez, 2016; Fageria and Stone, 2006). Estimating the EC of a soil can provide a lot of helpful information about the overall soil health. As an example, high EC levels can mean that a soil, or even a growing medium in general, contains a high content of salts (mainly sodium – Na), which are potentially harmful to plant vitality

(Celletti et al., 2021a; Hazelton and Murphy, 2007). On the other hand, CEC is an effective index to assess soil fertility. Indeed, this parameter measures the capacity of the soil to retain exchangeable positively charged ions (cations) through electrical attraction. For example, when a soil is rich in organic matter, it has a very high CEC and, therefore, means that nutrients can move through the soil and become available to plants (Hazelton and Murphy, 2007). In the studies by Boots et al. (2019) and Yu et al. (2020), it was observed that bio-based and non-biodegradable bioplastic (specifically as high-density polyethylene – HDPE and PE, respectively), altered the cation and proton exchange of the soil, resulting in a drop in soil pH and CEC. On the other hand, other experiments that tested PE and PLA at different dosages illustrated that these treatments increased pH (Wang et al., 2020). Looking back to our results, no pH-dependent change was visible, while that of CEC resulted in a significant reduction in agreement with the above-mentioned results. With regard to EC, on the other hand, we noticed a considerable increase. If we combine the values of increasing EC and decreasing CEC, we can generalise by hypothesising that these trends may be related to an increase in salt concentration (such as monovalent cations: Na and potassium, for instance) rather than to an increased release of potentially exchangeable nutrients (such as divalent cations: calcium and magnesium, for instance) and, thus, absorbable by plants.

Thus, it was evident that the addition of a high amount of biodegradable material caused an alteration of the soil environment (specifically, an increase in EC and a decrease in CEC). Taken together, these changes could be related to the changes in growth and biochemical parameters observed in the shoots. Indeed, the impact of high salt concentrations in the soil on the reduction of plant vigour, leaf number and/or size, discoloured foliage and increased oxidative stress levels of the plants is well known (Ashraf, 2009; Parida and Das, 2005; Wani et al., 2013). Furthermore, excess salts (such as Na) can compete with nutrients, leading to an induction of a nutrient imbalance and reducing nutrient availability to the plants (Machado and Serralheiro, 2017; Wani et al., 2013). Among the nutrients, iron (Fe) plays a central role in photosynthesis and chlorophyll synthesis, as well as in respiration and nitrogen assimilation (Celletti et al., 2020; Connorton et al., 2017; Rout and Sahoo, 2015). Given the central role of this nutrient, the imbalance in Fe uptake due to the interference of the bioplastic could be one of the main factors that triggered the reduction in plant growth (Bartucca et al., 2017; Del Buono et al., 2015).

However, it is worth emphasizing that this is the first study investigating the impact of a corn starch-based bioplastic on the growth and biochemical parameters of basil plants. To the best of our knowledge, many key questions still need to be clarified, as there is currently little information concerning the impact of bioplastics on plants. Therefore, this phenomenon needs to be additionally explored at multiple levels to better understand the mechanisms underlying these physiological and biochemical responses. Further studies will be required to identify which component is mainly responsible for the effects on plants, to test the effect of this treatment on other crops and soil types, also using different types of bioplastics and different concentrations, to test whether the plant response is species- and/or dose-dependent, and to determine the threshold beyond which the plant is strongly induced to trigger intense reprogramming of metabolic processes with high energy costs.

5. Conclusions

Looking back at the initial hypothesis, the present study verified the possible interference on basil plants of the presence in the soil of residues of potentially contaminating emerging materials, such as corn starch-based bioplastic, which belongs to the group of biodegradable and bio-based bioplastics and is one of the most widely used on the market. The results clearly indicated that this type of bioplastic affected plant growth parameters and changed the properties of planted soil. Specifically, growth was stunted, the defense response weakened, and oxidative stress was induced in the aerial part of basil plants. Overall, these results provided some clues as to whether bioplastic added to the soil simulates the effects of plastic, favouring the onset of a stressful condition for plant survival and vitality.

Hence, within this framework of the results presented in this study, there is a need to examine the impact of bioplastics in agriculture, as there is currently little information on their effects on cultivated plants. From a more general point of view, it would be forward-looking to address the various challenges of evaluating bioplastic impacts to raise environmental and social awareness before the use of these materials may become excessive and cause ecotoxicity problems.

Acknowledgements

We would like to thank Massimo Guarnieri for his technical help with HPLC analysis.

Author contributions

Silvia Celletti: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Visualization, Writing – original draft. **Riccardo Fedeli:** Data curation, Investigation. **Majid Ghorbani:** Investigation. **Stefano Loppi:** Conceptualization, Resources, Supervision, Writing – review & editing. All authors have read and approved the final manuscript.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of Interest

The authors declare no conflict of interest.

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Figure captions

Fig. 1. Image showing bioplastic before drying obtained by the casting technique.

Fig. 2. Content of total chlorophyll (A) and biometric parameters [plant height (B), leaf number (C), and shoot fresh weight (D)] of basil plants grown for 35 days without (C = control, grey bar) and with (B = bioplastic, green bar) corn starch-based bioplastic added to the soil at the concentration of 2.5% (w/w). Image (E) comparing basil plants grown without (C = control, on the left) and with bioplastic (B = bioplastic, on the right) added in the soil. All data are reported as mean values ± SE. The statistical significance between C and B conditions was tested by Student's t-test (* = $p < 0.05$; *** = $p < 0.001$). t-value and p-value are indicated.

Fig. 3. Content of soluble sugars (*i.e.*, fructose, glucose, sucrose, and their sum) (A) and total soluble proteins (B) in shoots of basil plants grown for 35 days without (C = control, grey bar) and with (B = bioplastic, green bar) corn starch-based bioplastic added to the soil at the concentration of 2.5% (w/w). All data are reported as mean values ± SE. The statistical significance between C and B conditions was tested by Student's t-test (* = $p < 0.05$; ** = $p < 0.01$; ns = not significant). t-value and p-value are indicated.

Fig. 4. Content of ascorbic acid (vitamin C) (A) and malondialdehyde (MDA) (B) in shoots of basil plants grown for 35 days without (C = control, grey bar) and with (B = bioplastic, green bar) corn starch-based bioplastic added to the soil at the concentration of 2.5% (w/w). All data are reported as mean values ± SE. The statistical significance between C and B conditions was tested by Student's t-test (* = $p < 0.05$). t-value and p-value are indicated. Correlation analysis between the shoot content of ascorbic acid and MDA (C) of basil plants grown for 35 days without (C = control, grey dot) and with (B = bioplastic, green dot) corn starch-based bioplastic added to the soil at the concentration of 2.5% (w/w). Pearson correlation coefficient (r) and p-value are indicated.

Fig. 5. pH (A), electrical conductivity – EC (B), and cation exchange capacity – CEC (C) of the soil where basil plants were grown for 35 days without (C = control, grey bar) and with (B = bioplastic, green bar) corn starch-based bioplastic at the concentration of 2.5% (w/w). All data are reported as mean values ± SE. The statistical significance between C and B conditions was tested by Student's t-test (* = $p < 0.05$; *** = $p < 0.001$; ns = not significant). t-value and p-value are indicated.

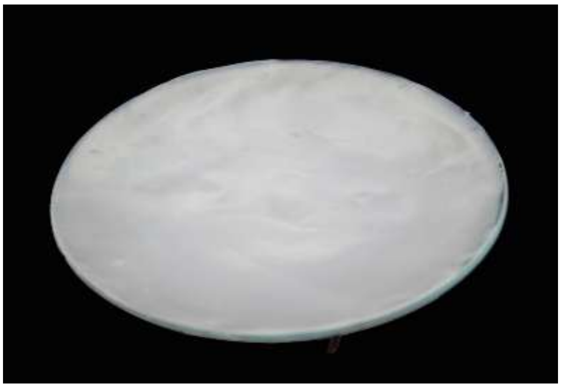

Fig. 1

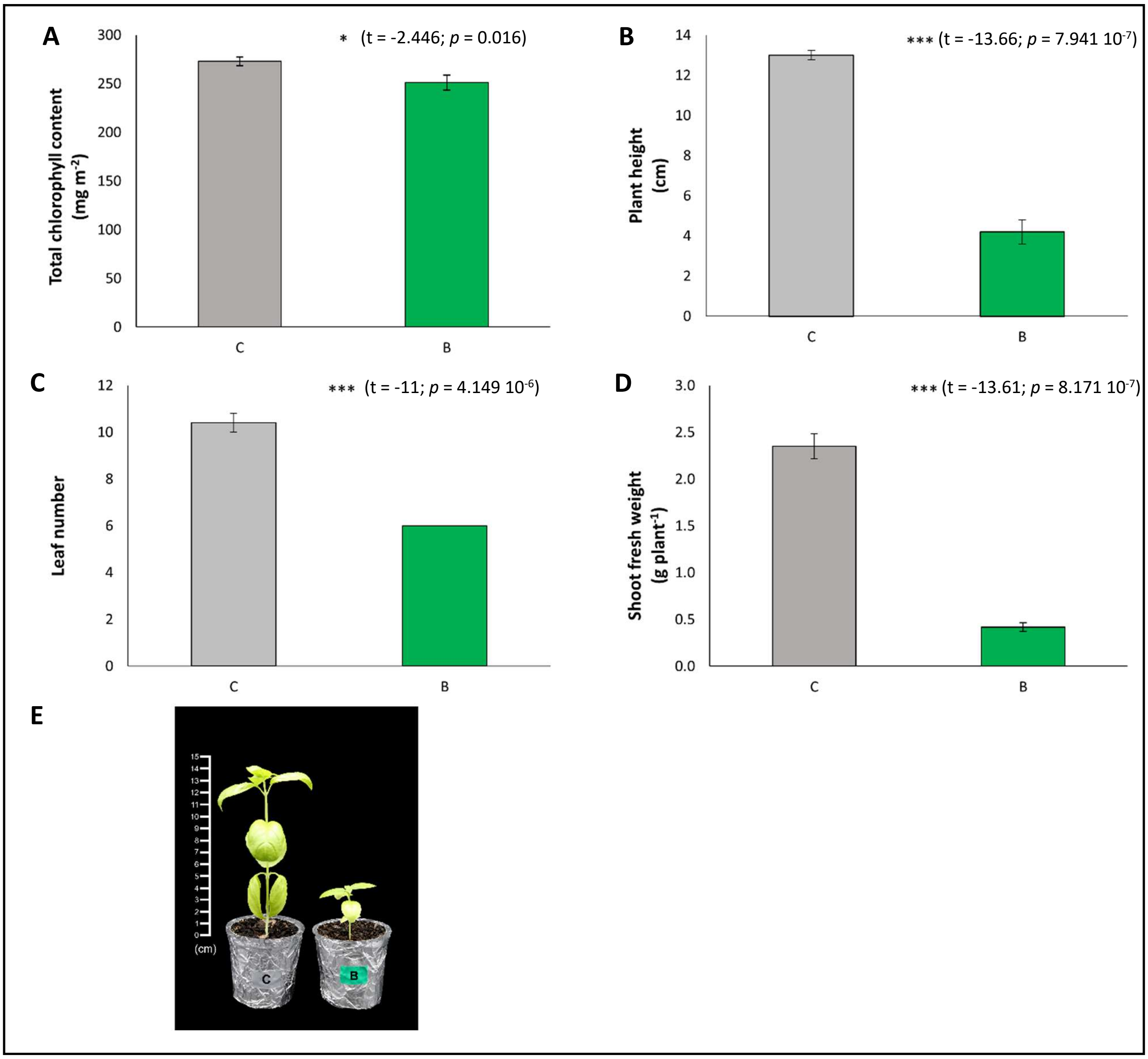
A
Total chlorophyll content (mg m-2)
300
250
200
150
100
50
0
* (t = -2.446; p = 0.016)
C
B
B
Plant height (cm)
14
12
10
8
6
4
2
0
*** (t = -13.66; p = 7.941 10-7)
C
B
C
Leaf number
12
10
8
6
4
2
0
*** (t = -11; p = 4.149 10-6)
C
B
D
Shoot fresh weight (g plant-1)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*** (t = -13.61; p = 8.171 10-7)
C
B
E
(cm)
C
B

Fig. 2

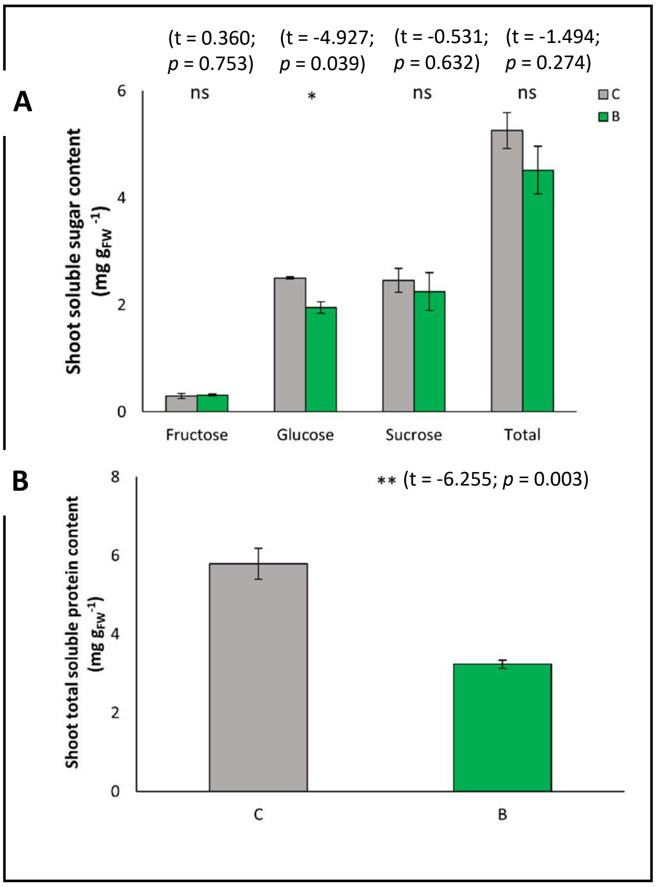
(t = 0.360; p = 0.753)
(t = -4.927; p = 0.039)
(t = -0.531; p = 0.632)
(t = -1.494; p = 0.274)
A
ns
*
ns
ns
C
B
Shoot soluble sugar content (mg gFW -1)
0
2
4
6
Fructose
Glucose
Sucrose
Total
B
** (t = -6.255; p = 0.003)
Shoot total soluble protein content (mg gFW -1)
0
2
4
6
8
C
B

Fig. 3

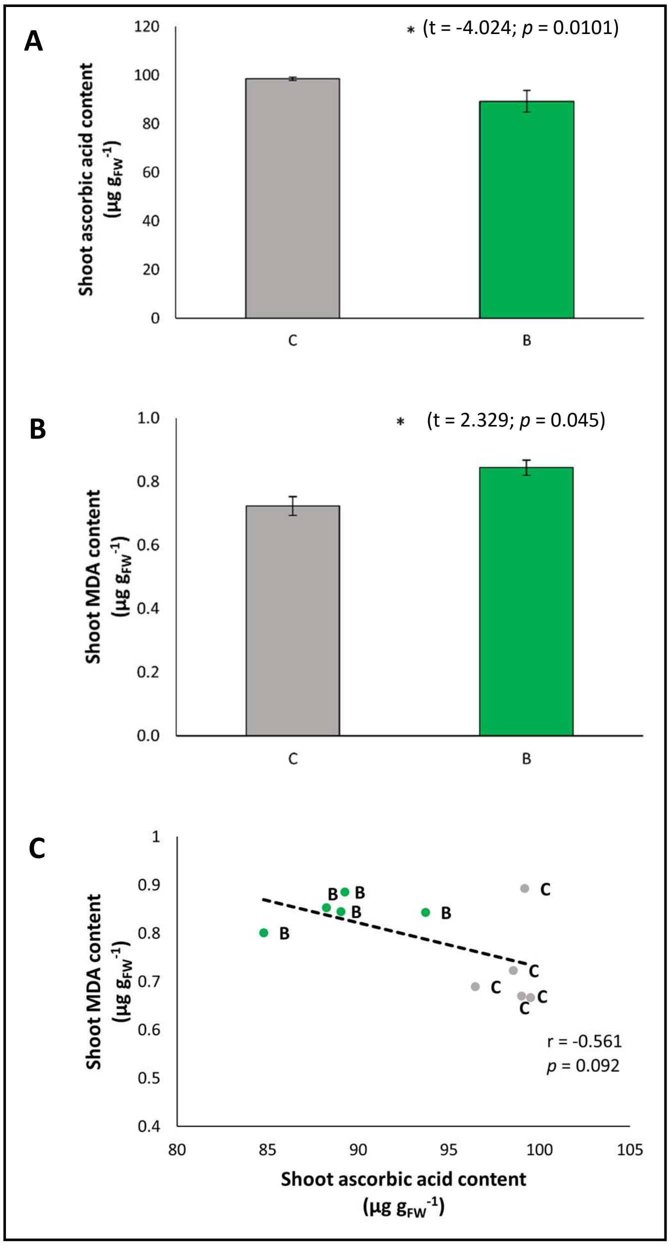
A
B
C
Shoot ascorbic acid content ($\mu g\ g_{FW}^{-1}$)
Shoot MDA content ($\mu g\ g_{FW}^{-1}$)
* (t = -4.024; p = 0.0101)
* (t = 2.329; p = 0.045)
r = -0.561
p = 0.092
C
B
0
20
40
60
80
100
120
0.0
0.2
0.4
0.6
0.8
1.0
0.4
0.5
0.6
0.7
0.8
0.9
1
80
85
90
95
100
105

Fig. 4

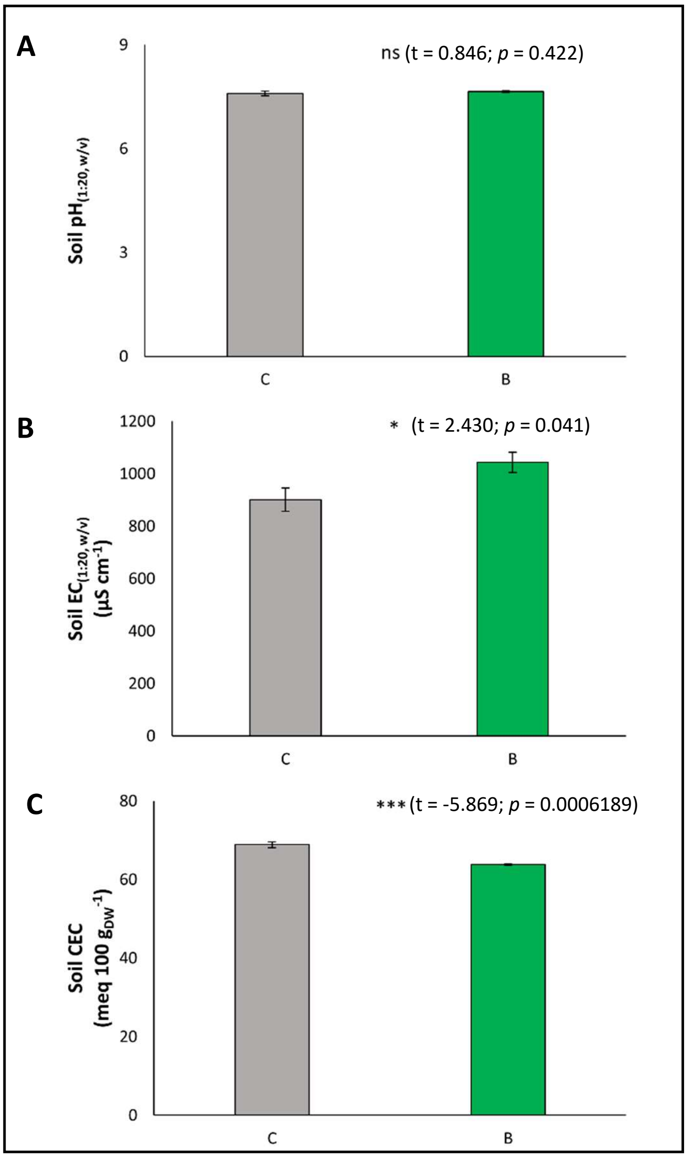
A
Soil pH$_{(1:20, w/v)}$
0
3
6
9
ns (t = 0.846; *p* = 0.422)
C
B
B
Soil EC$_{(1:20, w/v)}$ (µS cm^{-1})
0
200
400
600
800
1000
1200
* (t = 2.430; *p* = 0.041)
C
B
C
Soil CEC (meq 100 g$_{DW}$$^{-1}$)
0
20
40
60
80
*** (t = -5.869; *p* = 0.0006189)
C
B

Fig. 5