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Exploring sustainable alternatives: Wood distillate alleviates the impact of bioplastic in basil plants

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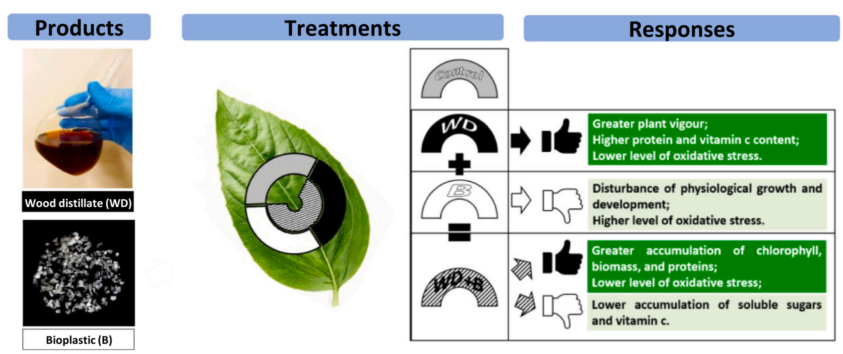
HIGHLIGHTS

- First study to examine the basil responses to the WD and bioplastic combination.
- WD enhanced the nutrition value (increased protein and vitamin C levels) of basil edible parts.
- Starch-based bioplastic addition hindered the physiological development of plants.
- WD showed potential in mitigating the oxidative stress caused by bioplastic.

GRAPHICAL ABSTRACT



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ABSTRACT

The growing interest in bioplastics and bio-based crop management products in agriculture is driven by the Sustainable Development Goals of the 2030 Agenda. However, recent research has raised concerns about the sustainability of bioplastics due to their potential negative impact on crop growth and yield, with implications for the environment and human health. In this study, wood distillate (WD) was evaluated as a natural enhancer of plant growth and defence system to mitigate the negative impact of a starch-based bioplastic on basil (*Ocimum basilicum* L.) plants. The study analyzed physiological and biochemical changes in basil plants subjected for 35 days to single or combined treatments of WD and bioplastic by measuring biomarkers of healthy growth, such as soluble proteins, sugars, vitamin C, and malondialdehyde (MDA). The results showed that WD promoted basil development, whereas the presence of bioplastic hindered it. Interestingly, WD did not affect sugars but increased vitamin C by 12 %, which is considered a positive effect as changes in sugar levels could indicate plant stress. In contrast, bioplastic resulted in reduced sugars (−41 %) and increased (+17 %) MDA level, while vitamin C content remained unchanged. However, when WD was added to plants grown with bioplastic, it elevated the levels of all examined parameters, except for sugars and vitamin C, which experienced reductions (−66 % and 33 %, respectively). Intriguingly, despite this reduction, the observed direct correlation between sugar and vitamin C contents was maintained, indicating that the decrease in sugar content may have reached a critical threshold. This study suggests that the use of WD has the potential to alleviate the adverse effects of

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bioplastic on basil growth and development and highlights the importance of adopting sustainable practices in agriculture, as well as the need for a critical assessment of the environmental impact of new technologies and products.

1. Introduction

Plastic pollution is among the main challenges facing contemporary human society, invading all compartments of our Earth: atmosphere, biosphere, lithosphere, and hydrosphere (Rhodes, 2019). This current scenario is mainly due to two reasons. The first is that the global demand for the use of plastic has increased dramatically over the years since 1950 (Statista, 2020; Zhao et al., 2022) and it is expected that, with the exponential increase in population growth, its production will double in the next 20 years, due to the essential properties that distinguish this material (light weight, strength, relatively low cost, and long life) (Atiwesh et al., 2021). The second is that of the 300 million tons of plastic produced each year (Rocha-Santos and Malafaia, 2022), only part of it is recycled (in Europe, recycling rates are around 30 %) and most of it is instead accumulated in landfills, generating huge piles of waste, or ends up in incinerators, which could be a useful route to implement for energy recovery if combustion plants are properly managed (Barnes et al., 2009). Moreover, it is worth emphasizing that plastic is toxic to natural and agricultural systems and human health, being an artificial polymer derived from non-renewable energy sources, such as petroleum, and it is also persistent and not easily biodegradable, remaining in the environment for hundreds or even thousands of years (Müller et al., 2012).

For these reasons, recent government policies (UN Sustainable Development Goals), focused on preserving ecosystem life and decreasing plastic pollution, are pushing research towards the production of "green" materials, *i.e.,* sustainable, renewable, and environmentally friendly in processing and consumption. Among these, bioplastics are highly valued polymeric materials as they share the favorable characteristics of conventional plastics, such as affordability, lightweight nature, and adaptability. However, unlike conventional plastics, that come from fossil sources, bioplastics may either originate from renewable materials (bio-based) or materials that undergo natural degradation (biodegradable), or both (Zimmermann et al., 2020; Vijayalaksmi et al., 2022). In view of the ecological transition, global production of bioplastics is estimated to increase from around 2.4 million tonnes in 2021 to 7.5 million tonnes in 2026 (European Bioplastics, 2022). Starch-based bioplastics are both bio-based and biodegradable and among the most widely used, accounting for approximately 50 % of the market share of all bioplastics produced (Marichelvam et al., 2019). This type of bioplastic finds applications in a variety of sectors, including food packaging, medical and biomedical (Parisi et al., 2015), and agriculture (*e.g.*, to produce mulch films, fastening technologies, plant pots and fertilizer spreading rods) (Colzi et al., 2022; European Bioplastic, 2021).

Research has long been devoted to finding mulch films for use in agriculture that perform similarly to conventional plastics, but degrade within a few months without the need to remove them from the field at the end of the crop cycle (Kijchavengkul et al., 2008). However, little has been investigated about the possible threats posed by the massive and increasing use of bioplastics, as they miniaturize and degrade, on cultivated species and the possible accumulation of additives contained in bioplastics in food products and, consequently, their possible effects on human health (Brizga et al., 2020). Only recently, attention has been directed towards investigating the possible impact of the nascent use of bioplastics in agroecosystems; in this respect, the scientific results obtained are rather divergent (Abe et al., 2022; Huerta-Lwanga et al., 2021; Liwarska-Bizukojc, 2021) and some of these studies have only observed toxic effects on crop growth (Brown et al., 2023; Celletti et al., 2023; Meng et al., 2021; Qi et al., 2018; Wang et al., 2022; Zhou et al., 2023).

The current ecological transition is prompting the use in agriculture of biostimulants and corroborants, enhancing plant growth and boosting crop resistance against pathogens, with the fundamental requirement of being of natural origin, obtained from agricultural waste, and, therefore, have a low environmental impact in a perspective of circular economy and resource efficiency. Among the vastness of natural products used in agricultural field, wood distillate (WD – also known as wood vinegar or pyroligneous acid) deserves special attention as an innovative corroborant, thanks to the significant positive effects of its application on crop performance and yield (Fedeli et al., 2023c; Fedeli et al., 2022; Ofoe et al., 2022; Mungkunkamchao et al., 2013); additionally, it possesses biodegradability characteristics and do not alter the vitality of non-target soil organisms (Hagner et al., 2010) and sensitive bioindicators (*e.g.*, lichens and mosses) (Fačkovcová et al., 2020), and, hence, its use is considered absolutely safe for the environment. According to the Italian legislation (Italian Ministerial Decree n. 6793/, 2018), WD has recently been included in the list of agri-products, which can even be used in organic farming. More in detail, WD is a brownish liquid, containing >300 bioactive molecules (Wei et al., 2010a, 2010b), obtained from plant biomass waste by cooling and re-condensing the spent gases released during the pyrolysis for energy purposes (Yargicoglu et al., 2015; Grewal et al., 2018). Currently, this product is effectively used in field conditions by soil fertigation (where plants absorb molecules only by roots) and foliar spraying (where plant absorb molecules mainly from the leaves, but also from the roots) (Fedeli et al., 2022; Mungkunkamchao et al., 2013). The beneficial effects on plants are mainly attributable to the presence of antioxidants and growth-stimulating phytochemicals with hormone-like action (*e.g.*, polyphenols, flavonoids, tannins, organic acids, alkanes, alcohols, and esters) able to improve nutrient assimilation (Mu et al., 2006; Wei et al., 2010a, 2010b; Zulkarami et al., 2011). Furthermore, these molecules also play an important role in preserving cultivated plant health by protecting them from pathogen attack and suppressing weeds (Mourant et al., 2005; Ratanapisit et al., 2009). Wood distillate is also particularly valued in agriculture for its beneficial effects on soil quality and fertility (Shibayama et al., 1998; Tsuzuki et al., 2000), such as, for instance, increasing the availability and supply of important soil nutrients (Jeong et al., 2015; Pan et al., 2017; Polthanee et al., 2015) and enhancing the soil biochemical activities (Cardelli et al., 2020), with positive crop yields.

In this context, based on the results obtained in our recent study (Celletti et al., 2023), which evidenced that a 2.5 % (w/w) accumulation of starch-based bioplastic in soil would result in deleterious effects on basil (*Ocimum basilicum* L.) plant growth and considering the numerous positive effects that WD treatment provides to plant functioning, this is the first study aimed at analyzing the variations in physiological and biochemical parameters of basil plants subject to grow with WD alone or in combination with bioplastic and with only bioplastic, in order to verify whether WD could mitigate bioplastic-induced stress in basil.

2. Materials and methods

2.1. Bioplastic

The small bioplastic pieces were obtained from the biodegradable and bio-based bioplastic film prepared following the procedure described in Celletti et al. (2023). Briefly, the desired amount of bioplastic was obtained from a base dose consisting of 1.5 g of powdered corn starch, 10 mL of distilled water, 1.5 g of glycerol, and 1 mL of glacial acetic acid. The resulting milky mixture was continuously stirred with a glass rod on a heated plate at 85 °C until it thickened and became clearer. Firstly, the hot soft paste was spread on a glass plate and oven-hardened at 100 °C for 1 h. Afterwards, it was left to dry completely for a week at room temperature. Finally, the bioplastic film was manually removed from the plate and cut with scissors into small pieces of uniform length (approx. 5 mm) to be added to the soil.

2.2. Plant growth conditions

Basil (*Ocimum basilicum* L., cv. Riviera Ligure) seeds were imbibed in distilled water for 1 h, sown between layers of wet paper in a vertical tray, and allowed to germinate for 6 days at 22 °C in the dark. Subsequently, uniform seedlings were transplanted into glass pots (3 seedlings/pot), covered with aluminium foil, containing 80 g of a commercial growing medium (VigorPlant Italia srl). This latter was a mixture of acid peat, non-composted organic amendment, pumice, perlite, organic fertilizer and presented the following characteristics: 43 % of moisture content; 92 % of porosity; 5.30 ± 0.03 of $pH_{(H2O:1:20, w/v)}$; 1.12 ± 0.01 mS cm^{-1} of electrical conductivity [$EC_{(H2O:1:20, w/v)}$]; 56.89 ± 2.67 meq 100 g_{DW}^{-1} of cation exchange capacity (CEC).

The experimental design consisted of four treatments with 8 replicates each: control (C), without fertigation and bioplastic; fertigation (WD), with 0.5 % (v/v) wood distillate and without bioplastic; bioplastic (B), with 2.5 % (w/w) of bioplastic and without fertigation; bioplastic and fertigation (B + WD), with 2.5 % (w/w) of bioplastic and 0.5 % (v/v) wood distillate.

The WD was provided by BioDea® (Arezzo, Italy) and obtained from sweet chestnut (*Castanea sativa* Mill., 1768) sap distilled in a steam current during the gasification. Its main physico-chemical characteristics are listed in Table 1.

Pots were initially irrigated to 60 % water holding capacity. During the growth period, this soil condition was kept by weighing each pot daily and by adding water when necessary. For WD treatment, 0.5 % (v/v) WD was added to the soil once a week, instead of the standard irrigation, for 4 weeks. The dosage of WD supplied in fertigation was chosen according to the producer's recommendations (BioDea®, Arezzo, Italy), while that of the bioplastic (2.5 %, w/w) was established in the light of the expected high increase in the use of bioplastics in agriculture in the near future driven by the ecological transition (Celletti et al., 2023).

From transplanting, plants were grown in a climate chamber with 16/8 h photoperiod, 25/20 °C (day/night) temperature, 250 µmol m^{-2} s^{-1} PAR light intensity, and 70 % relative humidity. At the 11st day from transplanting, only one plant per pot was left. During the experimental growth period, pots were randomly rotated and moved every day to minimize any possible influence of microclimatic conditions inside the climate chamber. After 35 days of growth, the plants were harvested and assessed as described below.

2.3. Aboveground biomass indices and total chlorophyll content

At harvest, the height of each plant was measured considering the distance between the soil surface and apex of the plant, the number of leaves was counted, and the shoot fresh biomass was weighted and then immediately stored in the freezer at −20 °C until analyses.

The total content of chlorophyll per unit area was determined in attached leaves of basil plants using a portable chlorophyll meter, model CCM – 300 (Chlorophyll Content Meter) (Opti-Science Inc., Hudson, NH, USA). Twelve measurements were taken on the two youngest fully expanded leaves (six measurements/leaf), avoiding the central rib, and averaged for each plant (Fedeli et al., 2023a).

2.4. Total content of soluble proteins and sugars

The determination of the total contents of both soluble proteins and soluble sugars (given by the sum of fructose, glucose, and sucrose) was performed in the extracts of basil shoots according to Celletti et al. (2023). In brief, frozen tissue was homogenized (ULTRA-TURRAX® – T 10 basic, Werke GmbH & Co. KG, Staufen, Germany) in distilled water in a ratio of 1:6 (*w/v*) and then centrifuged (PK110 centrifuge, Alc International S.r.l., Cologno Monzese, MI, Italy) at 3000 rpm for 5 min. The supernatants were collected and centrifugated (Z 233 MK-2, Hermle, LaborTechnik GmbH, Wehingen, Germany) at 12000 rpm for 7 min to obtain the extracts.

Protein content was determined in the extracts according to Bradford (1976) method, using bovine serum albumin (BSA) as standard, by reading the absorbance of samples at 595 nm (UV–Vis spectrophotometer – 8453, Agilent, Santa Clara, CA, USA).

Sugar content was determined in the extracts, which were filtered (45 µm pore size, 25 mm diameter, CA syringe filters, Lab Logistic Group

Table 1
Main physico-chemical characteristics of wood distillate (BioDea® - Arezzo, Italy), obtained by steam-distilling sweet chestnut (*Castanea sativa* Mill., 1768) sap during the gasification process.

Parameter	Value	Method
TOC (% DW)	58.03	CHNS Elemental Analysis
TN (% DW)	1.06	CHNS Elemental Analysis
H (% DW)	7.27	CHNS Elemental Analysis
S (% DW)	0.07	CHNS Elemental Analysis
pH	4	UNI EN ISO 10523:2012
Density (g mL^{-1})	1.05	
Flash point (°C)	>60	ASTM D6450-16a
Total organic compounds (g L^{-1})	33.8	
Acidity (mg L^{-1})	1289	APAT CNR IRSA 2010 B Man 292,003
Organic acids (mg L^{-1})	32.3	
Acetic acid (mg L^{-1})	21.5	
Polyphenols (g L^{-1})	24.5	
Phenols (g L^{-1})	3	
PCBs (mg L^{-1})	<0.2	CNR IRSA 24b Q 64 Vol 31,988
Hydrocarbons C < 12 (mg L^{-1})	<0.1	EPA 5021A 2014 + EPA 8015D 2003
Hydrocarbons C10-C40 (mg L^{-1})	<0.1	UNI EN ISO 9377-2:2002
16 US-EPA PAHs (mg L^{-1})		EPA 3550C 2007 + EPA 8310 1986
Acenaphthene	<0.05	
Acenaphthylene	<0.05	
Anthracene	<0.05	
Benzo[*a*]anthracene	<0.05	
Benzo[*a*]pyrene	<0.05	
Benzo[*b*]fluoranthene	<0.05	
Benzo[*g,h,i*]perylene	<0.05	
Benzo[*k*]fluoranthene	<0.05	
Chrysene	<0.05	
Dibenz[*a,h*]anthracene	<0.05	
Fluoranthene	<0.05	
Fluorene	<0.05	
Indeno[1,2,3-*cd*]pyrene	<0.05	
Naphthalene	<0.05	
Phenanthrene	<0.05	
Pyrene	<0.05	
Macronutrients (mg L^{-1})		Alkaline melting + ICP-MS analysis
Ca	325.50	
K	23.49	
Mg	6.79	
P	7.28	
Micronutrients (mg L^{-1})		Alkaline melting + ICP-MS analysis
Cu	0.18	
Fe	21.16	
Mn	0.58	
Mo	0.0007	
Zn	3.22	
Other nutrients		Alkaline melting + ICP-MS analysis
Al	1.96	
Ba	0.06	
Cr	0.03	
Na	103.59	

TOC: total organic carbon. **TN**: total nitrogen. **PCBs**: polychlorinated biphenyls. **16 US-EPA PAHs**: list of 16 priority polycyclic aromatic hydrocarbons as classified by the United State Environmental Protection Agency. **Al**: aluminium; **Ba**: barium; **C**: carbon; **Ca**: calcium; **Cr**: chromium; **Cu**: copper; **Fe**: iron; **K**: potassium; **Mg**: magnesium; **Mn**: manganese; **Mo**: molybdenum; **N**: nitrogen; **Na**: sodium; **Zn**: zinc.

GmbH, Meckenheim, Germany), completely vacuum-dried at 40 °C and re-suspended in 30 μL of distilled water. This volume was inserted into HPLC (600E System, Waters, Milford, MA, USA). Sugars were separated using an ion-exchange column (10 μm, 300 × 6.5 mm, Sugar-Pak I, Waters, Milford, MA, USA), kept constantly at 90 °C *via* an external temperature controller (Column Heater Module, Waters, Milford, MA, USA), and distilled water as mobile phase at a flow rate of 0.5 mL min^{-1}. For the detection of the sugars, HPLC was equipped with a refractive index detector (2410 RI, Waters, Milford, MA, USA). Standards for each analyte were prepared as individual stock solutions, using sugar reagent-grade analytical compounds (D-Fructose, a-D-Glucose, Sucrose, Merk KGaA, Darmstadt, Germany).

2.5. Ascorbic acid content

The content of L-ascorbic acid (vitamin C) was determined in the shoots of basil plants, following the method reported in Celletti et al. (2023). In brief, 0.2 g of frozen tissue were homogenized with 0.8 mL of 10 % (w/v) TCA, using an ULTRA-TURRAX® (T 10 basic, Werke GmbH & Co. KG, Staufen, Germany). The homogenates were filtered on gauze and, prior to centrifugation (3000 rpm for 5 min), were kept for 5 min in an ice bath. An aliquot (0.4 mL) of limpid supernatant was diluted with distilled water in a ratio of 1:4 (v/v). Finally, 0.2 mL of 0.2 M Folin – Ciocalteu reagent (Carlo Erba, Cornaredo, MI, Italy) were added to the diluted samples. They were shaken vigorously and kept for 10 min at room temperature. Subsequently, the absorbance was measured at 760 nm using a UV–Vis spectrophotometer (8453, Agilent, Santa Clara, CA, USA). The content of ascorbic acid was estimated using the calibration curve prepared by taking 0.05–0.2 mL of ascorbic acid (BioXtra, ≥99.0 %, crystalline) stock solution (100 μg mL^{-1}).

2.6. Lipid peroxidation level

The level of lipid peroxidation was estimated according to Fedeli et al. (2023b) and expressed as malondialdehyde (MDA) content, as MDA is considered a biomarker of oxidative damage, being a highly reactive metabolite produced by the breakdown of peroxidated PUFAs (Poly-Unsatured Fatty Acids). Briefly, 0.5 g of frozen shoot of basil plants was homogenized in 5 mL of pre-chilled reagent previously prepared by dissolving 0.25 % (w/v) 2-thiobarbituric acid (TBA) in 10 % (w/v) TCA. After incubation at 95 °C for 30 min, the reaction was stopped by cooling the samples on ice. Afterwards, the samples were centrifuged at 5000 rpm for 20 min and the absorbance was measured in the supernatants at 532 nm and 600 nm (UV–Vis spectrophotometer – 8453, Agilent, Santa Clara, CA, USA). The correction for non-specific turbidity was obtained by subtracting the absorbance value measured at 600 nm. Lipid peroxidation level was calculated using a molar extinction coefficient (155 mM^{-1} cm^{-1}) of the formed MDA – TBA complex.

2.7. Statistical analysis

Data normality was verified with the Shapiro-Wilk test. Data are presented as means of 8 biological replicates ± standard error run in triplicate. All results were subjected to one-way ANOVA and LSD as post-hoc test ($p < 0.05$), using the software CoStat Version 6.45 (CoHort, Barkeley, CA, USA).

3. Results

3.1. Growth parameters and greenness

At the end of the experimental growth period, basil plants from the four treatments showed clearly different development stages visually (Fig. 1). The trend of the treatments was the same in all three analyzed biometric parameters (*i.e.*, plant height, number of leaves, and shoot fresh weight) (Fig. 2A, B and C, respectively), with the WD-fertigated plants showing the highest values, followed by C plants > B + WD-treated plants > B-treated plants. Specifically, WD plant height, number of leaves, and aboveground biomass were 17 % (Fig. 2A), 14 % (Fig. 2B), and 10 % (Fig. 2C) higher than the related controls (C plants). The B plants showed reductions in plant height by 70 % (Fig. 2A), leaf number by almost half (−54 %, Fig. 2B) and shoot fresh weight by 90 % (Fig. 2C) in comparison with the corresponding C plants. On the other hand, these parameters were significantly less affected by the combined treatment (B + WD), which caused lower reductions than the B treatment alone in plant height (−49 %, Fig. 2A), leaf number (−41 %, Fig. 2B), and shoot fresh weight (−76 %, Fig. 2C), compared to the relative controls. Indeed, comparing B and B + WD treatments, it can be found that plant height, leaf number, and shoot fresh weight increased by 68 % (Fig. 2A), 29 %

Fig. 1. Visual differences in the development stage of basil plants grown in soil for 35 days under the four different treatments: C = control; WD = fertigation with 0.5 % (v/v) wood distillate; B = 2.5 % (w/w) bioplastic mixed with soil; B + WD = 2.5 % (w/w) bioplastic mixed with soil coupled with fertigation with 0.5 % (v/v) wood distillate.

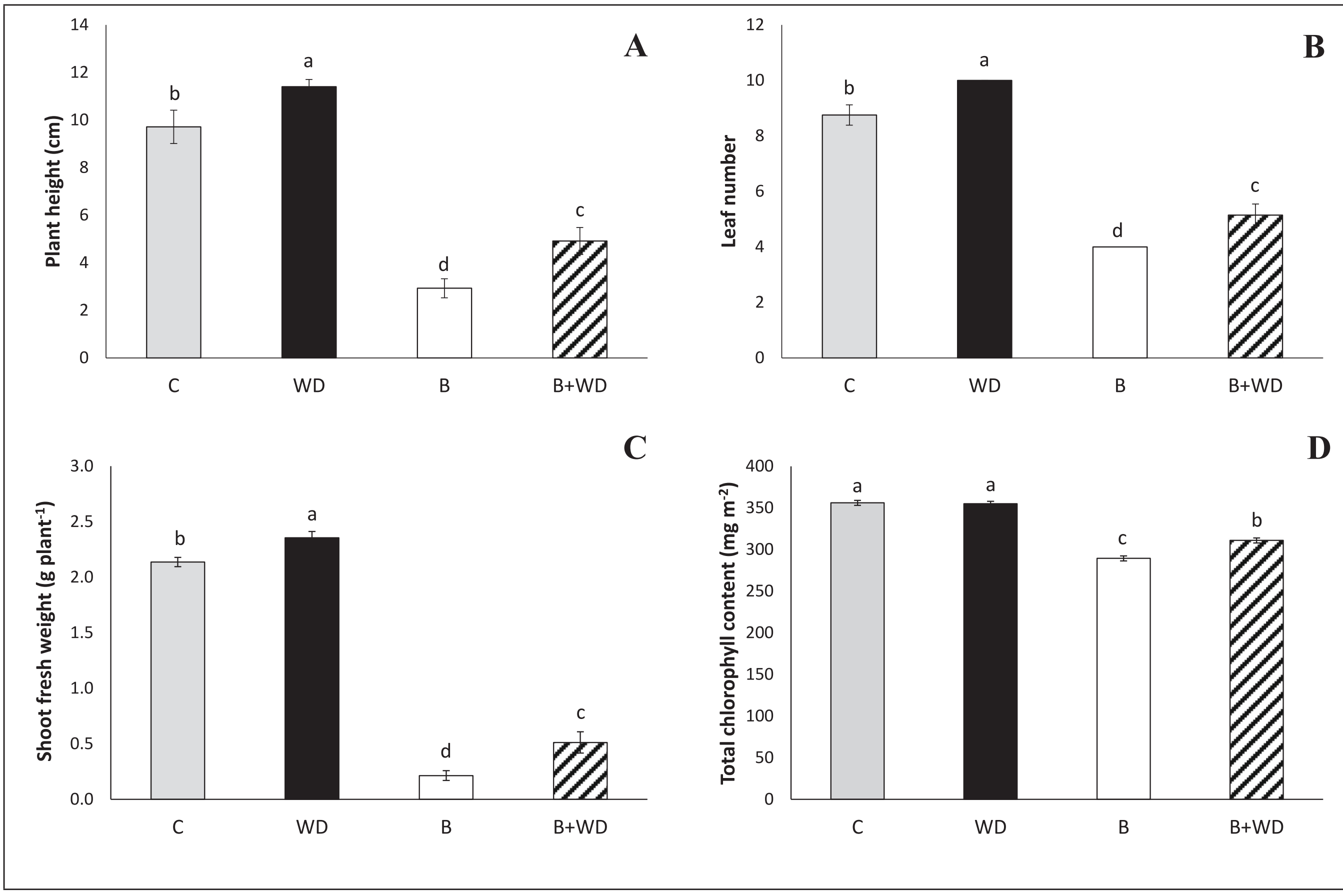

Fig. 2. Changes in the height (A), leaf number (B), shoot fresh weight (C), and in the total content of leaf chlorophyll (D) of basil plants grown in soil for 35 days under the four different treatments: C = control; WD = fertigation with 0.5 % (v/v) wood distillate; B = 2.5 % (w/w) bioplastic mixed with soil; B + WD = 2.5 % (w/w) bioplastic mixed with soil coupled with fertigation with 0.5 % (v/v) wood distillate. Data are presented as mean ± SE. Different letters indicate the statistical significance between the four different treatments according to the one-way ANOVA followed by LSD test with $p < 0.05$.

(Fig. 2B), and 117 % (Fig. 2C), respectively.

The changes in total chlorophyll content in the leaves of basil plants are showed in Fig. 2D; this physiological parameter also followed a similar trend to that of the biometric parameters. The only exception was the plants fertigated with WD, which did not differ significantly from the C plants, reaching values of around 350 mg chlorophyll m^{-2}; whereas the chlorophyll content decreased by 19 % when the plants were grown only with bioplastic (B *vs.* C). Although in the plants exposed to the combined treatment the reduction in chlorophyll content was still significantly evident (−13 %, B + WD *vs.* C), these plants however exhibited a 7 % increase compared to the treatment with bioplastic alone (B + WD *vs.* B) (Fig. 2D).

3.2. Macromolecule content

Treatment with WD promoted an increase in the total soluble protein content in the shoots of basil plants by >50 % compared to that determined in the shoots of C plants (Fig. 3A). In contrast, an inverse result was observed when the basil plants were grown with only bioplastic, regardless of the presence of WD. However, it is interesting to note that treatment with the sole bioplastic reduced the protein content much more strongly (−83 %) than when bioplastic was combined with fertigation (−52 %) compared to C. The addition of WD to the B-treated plants noticeably increased (+179 %) the protein content at shoot level (B + WD *vs.* B) (Fig. 3A).

Fig. 3B shows the shoot total soluble sugar content, given by the sum of fructose, glucose, and sucrose content. No significant differences were observed for this parameter between the WD and C treatments, reaching values of 3.254 and 3.016 mg $g^{-1}_{shoot\ FW}$, respectively. In contrast, the presence of bioplastic reduced the total soluble sugar content, regardless of the presence or absence of WD. In particular, the treatment with bioplastic alone reduced it by 41 %, whereas, in this case, it was the combination of bioplastic with WD fertigation which exhibited the greatest reduction (−66 %), in comparison with the sugar content analyzed in the C shoots (Fig. 3B). Although no significant changes were evident between B plants supplemented with WD (B + WD treatment) compared to the plants treated with the bioplastic alone (B treatment), a strong percentage decrease (−42 %) can be detected (Fig. 3B).

3.3. Biomarker content

A significant increase (+12 %) in ascorbic acid content was observed in WD shoots compared to C (Fig. 4A). On the other hand, the B-treated plants did not differ statistically from the C plants in this parameter. Only when the plants were simultaneously subjected to the B and WD treatment, the ascorbic acid accumulation was considerably reduced (−33 %), compared to that determined in the C shoots. Comparing the combined treatment (B + WD) with B, the reduction was 27 % (Fig. 4A).

The highest level of lipid peroxidation of the cell membrane (estimated as MDA content) was observed in the shoots of basil plants grown in the presence of bioplastic (B treatment), which increased significantly by 17 % compared to the C-treated plants (Fig. 4B). Fertigation with WD reduced MDA content by 9 % for WD treatment and 17 % for B + WD treatment compared to C. In addition, however no significant differences in MDA content were evident between the WD and B + WD treatments, showing values around 0.6 μg $g^{-1}_{shoot\ FW}$. The combination of WD with

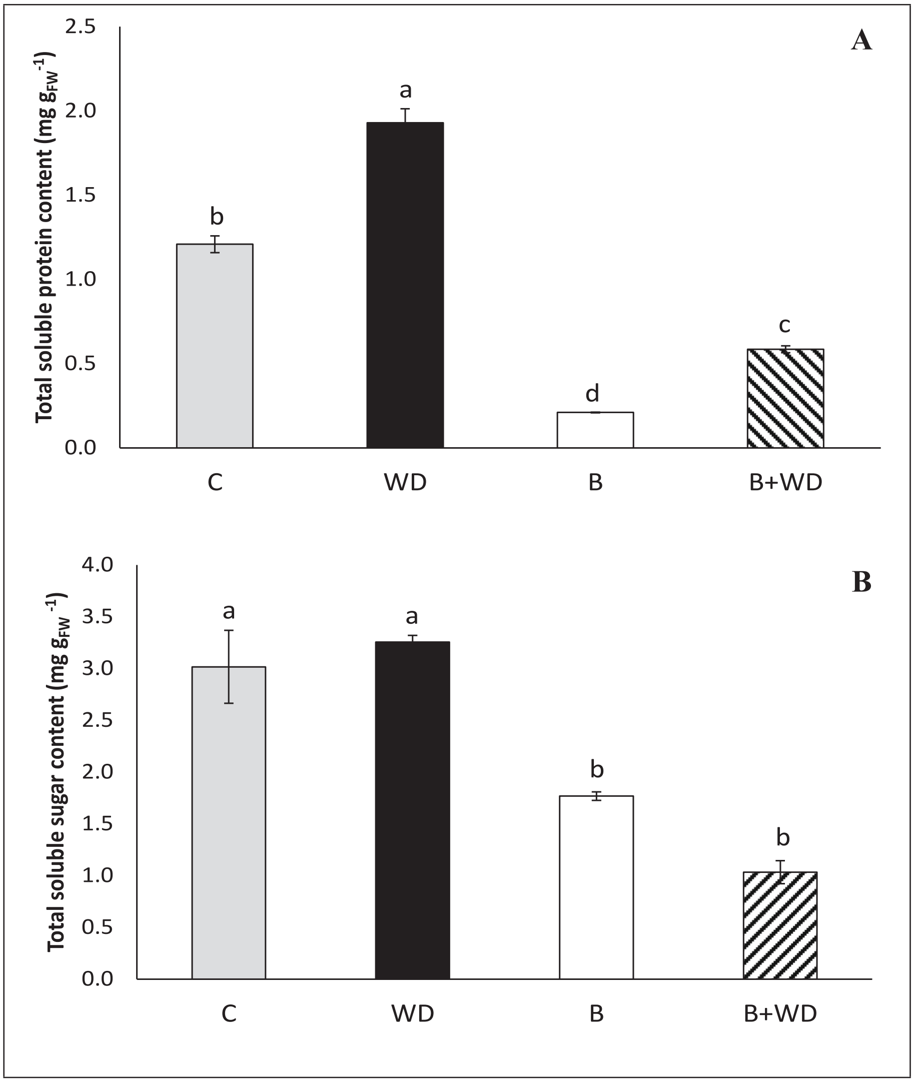

Fig. 3. Changes in the total content of soluble proteins (A) and soluble sugars (given by the sum of fructose, glucose, and sucrose content) (B), determined in the shoots of basil plants grown in soil for 35 days under the four different treatments: C = control; WD = fertigation with 0.5 % (v/v) wood distillate; B = 2.5 % (w/w) bioplastic mixed with soil; B + WD = 2.5 % (w/w) bioplastic mixed with soil coupled with fertigation with 0.5 % (v/v) wood distillate. Data are presented as mean ± SE. Different letters indicate the statistical significance between the four different treatments according to the one-way ANOVA followed by LSD test with $p < 0.05$.

bioplastic (B + WD) resulted in a reduction of 29 % with respect to B-treated plants (Fig. 4B).

4. Discussion

Our results clearly corroborate the well-known biostimulatory action of WD on vegetable growth and development. Indeed, numerous pieces of scientific evidence widely discussed the beneficial effects of WD on the growth of various crop plants, such as, for example, rice (Berahim et al., 2014), tomato (Mungkunkamchao et al., 2013), lettuce, rapeseed, and cucumber (Mu et al., 2006). Furthermore, our results support some very recent scientific research concerning the impact of bioplastics on crop growth (Abe et al., 2022; Celletti et al., 2023; Huerta-Lwanga et al., 2021; Meng et al., 2021; Qi et al., 2018). The beneficial effects of WD are largely attributable to its richness in organic molecules (especially organic acids and polyphenols) promoting various plant development processes, including the induction of the plant hormone network and photosynthesis (Mu et al., 2006). On the contrary, the specific compounds within bioplastic responsible for the observed negative effects on plant growth are still unknown, as well as how they interfere with plant functions. Although the mechanisms of action of WD remain unclear, the existing literature consistently supports the notion that the increased plant growth observed with WD treatments could be due to the synergetic action of different bioactive molecules in the WD that enhance plant growth in a broader sense (Zhu et al., 2021; Wang et al., 2019; Mu et al., 2006), but also to a higher nutrient supply, WD being rich in nutrients, in particular, calcium, among the macronutrients and, iron (Fe), among the micronutrients. This effect also appeared in our experiment when WD was combined with bioplastic. In this case, the WD was able to significantly attenuate the growth-inhibiting effect of the bioplastic and thus promote the production of leaf biomass, even if it did not allow the control levels to be reached at least with the dosage tested.

In our case, the addition of WD did not result in any change in chlorophyll content, although many studies have revealed that WD applications increased chlorophyll content in several crop plants such as, for instance, lettuce (Vannini et al., 2021), mustard (Benzon and Lee, 2017), and rice (Theerakulpisut et al., 2017; Berahim et al., 2014). Conversely, the presence of the bioplastic resulted in a strong decline in chlorophyll content, aligning with a reduction in growth and protein content of these plants, as chlorophyll is a nitrogen (N)-containing molecule and N is an important part of the compounds that regulate plant growth and structure and is the protein characterizing element (Wen et al., 2019). It is important to note that the beneficial effect of WD in enhancing chlorophyll content was evident when it was supplied to the soil of plants grown with the bioplastic. When plants are exposed to stressful conditions, such as the presence of bioplastic, the activities of enzymes responsible for chlorophyll biosynthesis could be impaired, presumably because bioplastic may have negatively interfered with the root system, leading to reduced nutrient uptake and triggering

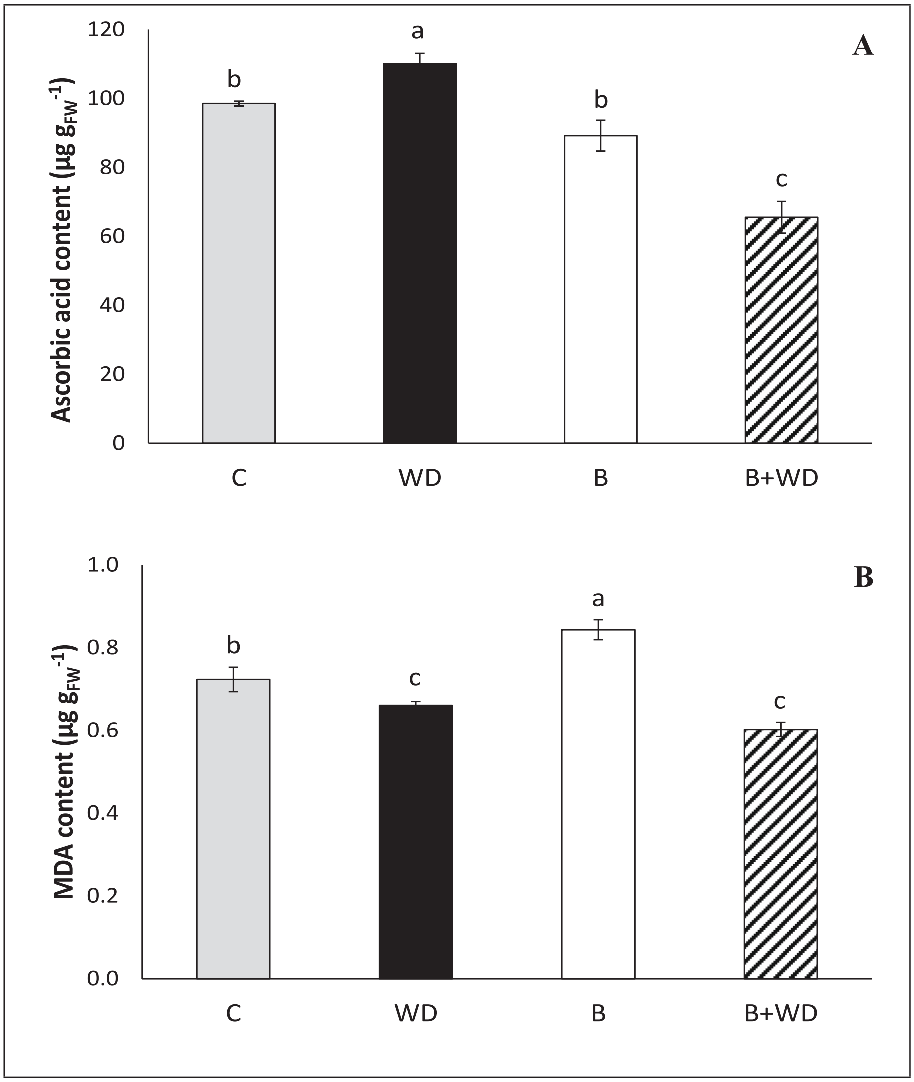

Fig. 4. Changes in the content of ascorbic acid (vitamin C) (A) and malondialdehyde (MDA) (B), determined in the shoots of basil plants grown in soil for 35 days under the four different treatments: C = control; WD = fertigation with 0.5 % (v/v) wood distillate; B = 2.5 % (w/w) bioplastic mixed with soil; B + WD = 2.5 % (w/w) bioplastic mixed with soil coupled with fertigation with 0.5 % (v/v) wood distillate. Data are presented as mean ± SE. Different letters indicate the statistical significance between the four different treatments according to the one-way ANOVA followed by LSD test with $p < 0.05$.

nutritional stress in plants (Celletti et al., 2023). Among the nutrients affected, Fe holds particular importance as it is involved in chlorophyll synthesis and functions as a component of various enzymes (including catalase, peroxidase, cytochrome oxidase, ferredoxin, and flavoproteins), which participate in the redox reactions of photosynthesis (Kroh and Pilon, 2020). The effect of WD on total soluble protein content diverged from the observed effect on chlorophyll content; however, it resembled the response observed in parameters related to plant growth. This discrepancy can be attributed to the dual role of many plant proteins, which not only participate in enzymatic reactions, including the chlorophyll biosynthesis process (Rasheed et al., 2020; Murray et al., 2017), but also serve a significant structural and functional role, acting as essential biological macromolecules for the survival of all living organisms, including plants (Rasheed et al., 2020; Murray et al., 2017). In fact, the protein content measured in the shoots of basil plants that had received WD was almost double that of control plants, in line with results reported in rapeseed (Zhu et al., 2021), tobacco (Mao et al., 2019), and rice (Simma et al., 2017), which showed improved plant growth. The fact that the bioplastic lowered the protein content and that many proteins have the characteristic of being biological catalysts suggests that bioplastic might have hindered some enzymatic reactions vital for plant metabolism, as already hypothesized by Celletti et al. (2023). Among all the various parameters associated with plant growth, it was observed that shoot fresh weight exhibited the closest resemblance to protein content in terms of the degree of reduction caused by the presence of bioplastic and the recovery by plants grown with bioplastic along with the addition of WD. This finding supports the previously expressed hypothesis that, under favorable growth conditions, there is a direct correspondence between increased protein accumulation, enhanced plant structure, and consequently, higher biomass as indicated by fresh weight.

Considering that sugars also serve as signal molecules and their biosynthesis is enhanced when environmental factors are not beneficial to the plants' survival (Rolland et al., 2006; Rosa et al., 2009; Lastdrager et al., 2014). In contrast, the plant response to the bioplastic treatment resulted in a significant reduction in the shoot soluble sugar content. This could probably be the effect of the removal of sugars, accumulated by nutritional stress, to avoid the inhibition of photosynthesis (Pego et al., 2000). The sugar-mediated signaling mechanism in plants is costly in terms of metabolic energy (Rolland et al., 2002). If the B plants had allocated a substantial portion of their energy towards increasing sugar synthesis, it would have been detrimental to their overall survival. This represents an example of energy-saving responses that are crucial for stress adaptation (Tomé et al., 2014). In the case of the combined treatment (B + WD), the negative effect of bioplastic outweighed the beneficial effect of WD. Consequently, there was a further reduction in shoot sugar content of these plants, which was even more pronounced than in the B plants. This same effect was observed for the content of ascorbic acid (vitamin C), a potent antioxidant in plants (Smirnoff, 2000), when considering the combined treatment. The decrease in vitamin C level observed in the B + WD treatment could be attributed to the fact that the sugar level in these plants fell below a critical threshold limit (approximately 1 mg $g^{-1}_{shoot\ FW}$), which subsequently led to a decline in vitamin C content, since vitamin C is biosynthesized from hexose monosaccharides such as fructose and glucose (Celletti et al., 2023; Dowdle et al., 2007). Conversely, the sole application of WD was able to increase the endogenous vitamin C level in the shoot of basil plants. It can be inferred that WD, which contains substantial amounts of other antioxidant compounds such as phenolics (Loo et al., 2008), facilitated their accumulation and availability within the plants under stable growth conditions. These compounds likely strengthened the plants' comprehensive defence mechanisms, safeguarding cell membranes against potential environmental stress. The hypothesis is supported by the analysis of oxidative stress level (MDA). Specifically, the WD plants exhibited significantly lower oxidative stress level compared to the control plants. Surprisingly, the B + WD plants, despite having lower vitamin C content, showed oxidative stress level equivalent to those of the WD plants. On the other hand, the disturbing action induced by the bioplastic was evident as it strongly increased the level of cell membrane oxidative stress in B plants. This phenomenon is consistent with previous findings that demonstrate elevated oxidative stress in plants growing in the presence of plastic (Zhang et al., 2022), posing a threat to the normal vital functions of the plants (Alscher et al., 1997).

5. Conclusions

Notably, when WD was combined with bioplastic, it enhanced productivity, chlorophyll and protein content, while reducing oxidative stress. These findings indicate that WD effectively mitigates bioplastic-induced damage and supports healthy human nutrition. In this context, to effectively fill these gaps, a comprehensive chemical characterization of the specific molecules within WD, including hormones and proteins, needs to be undertaken. This effort would be helpful in proposing a structured scheme to unveil the precise mechanism through which WD enhances plant growth.

The study encourages further research to better understand the negative effects of bioplastic on plants and develop sustainable strategies using natural products, like WD, that align with the principles of sustainability in agriculture and the circular economy. Undoubtedly, the involvement of diverse plant species, varying dosages of both bioplastic and WD, as well as different soil types and field conditions, will be indispensable for future investigations targeting the elucidation of the underlying mechanisms governing the interplay between WD, bioplastic, and soil in plants.

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CRediT authorship contribution statement

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

Declaration of competing interest

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Data availability

Data are made available by the authors to any qualified researcher who makes a request.

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