Evaluating the Impacts of Microplastics on the Soil Biochemical Environment and Cherry Tomato (*Solanum lycopersicum*) Productivity

By

Kenny Famakinwa

A Thesis Submitted in Partial Fulfillment of the

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This thesis has been examined and approved by the following members of the student's committee:

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Advisor
Christopher T. Ruhland, Ph.D.

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Committee Member
Mriganka De, Ph.D.

______________________________ Committee Member J
Mark W. Bowen, Ph.D.

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Microplastics are a global contaminant with serious consequences for ecosystems and human health, yet their effects on terrestrial soil and plants are poorly understood. We conducted the systematic research to reveal the impact of polyethylene microplastics (PE MPs), polypropylene (PP) MPs and Mixture of PE + PP, size (0.1 – 1 vs 2 – 3.5 mm), concentration (1% vs 5%) on soil biochemical and physiochemical of tomato (*Solanum lycopersicum* L) health. The results showed that the addition of MPs resulted in elevated soil pH, particularly at 5% concentration and size 2 – 3.5mm. There was a remarkable 30.6 – 47% decreased in soil respiration and inorganic nitrogen levels, after expose to PE, PP, and PE + PP, while plant height, chlorophyll content, and light adapted yield remained unaffected. Microplastics negatively impacted leaf production and fruit production, with 2- 3.5mm MPs sizes leading to 9-20% decreased in leaf number, fruit count and biomass. Additionally, MPs concentration played a role in shoot biomass, with 1% concentrations resulting in 9% reduced biomass. These findings are expected to provide valuable insights into the complex interactions between PE, PP, PP + PE on soil and plant health.

Chapter I

INTRODUCTION: MICROPLASTIC TYPES, SOURCES, AND EFFECTS ON PEOPLE, PLANTS, AND SOILS

Background

Plastics are type of synthetic polymer that are similar to natural resins found in trees and other plants that is derived from natural gas, coal, and crude oil through a process known as polymerization (Kluenen et al., 2020; Colzi et al., 2022). This process allows for the manipulation of molecular structures, resulting in a wide range of plastics with diverse properties. Common types include polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS), and polyethylene terephthalate (PET), each serving unique purposes due to their distinct characteristics (Choi et al., 2021; Fuller et al., 2016; Guo et al., 2020; Kluenen et al., 2020). Since wide scale production started in the 1930s, there has been a dramatic increase of Microplastics (MP) created between 1950 and 2015 with at least 8.3 billion tons being made (Azeem et al., 2021; Gao et al, 2019; Kluenen et al., 2020). Polymers play a crucial role in various industries, providing efficient solutions for packaging, construction, healthcare, electronics, and more. They revolutionize manufacturing processes and enable the creation of diverse products such as food packaging, medical devices, and electronics components. Despite their benefits, plastics pose significant environmental challenges. One of the most pressing issues is plastic pollution, particularly in oceans and waterways. Single-use plastics, such as bottles, bags, and straws, contribute to this pollution, endangering marine life and ecosystems (Subhankar Chatterjee and Shivika Sharma, 2019). Additionally, one crucial aspect of this impact is the breakdown of plastics into smaller particles over time. Transitioning from large macroplastics to microplastics, which can persist in the environment for hundreds of years, and understanding this process is essential for comprehending the full scope of plastic pollution (Azeem et al., 2021; Gao et al, 2019; Kluenen et al., 2020).

Breakdown Processes

Plastic sources can be divided into two main groups based on their origins, such as whether the particles are originally produced with those dimensions (primary) or derived from breakdown of larger debris through physical, biological, and chemical degradation processes (secondary; Tursi et al., 2022). The main sources of primary MPs are plastic containers, personal care products, tires, road markings, marine coatings, and synthetic textiles. Azeem et al., 2021; Gao et al, 2019; Kluenen et al., 2020; Tursi et al., 2022). Secondary sources are mainly photodegrading by sunlight caused by exposure to UV-B radiation, biodegradation by microorganism, or hydrolysis by sea water (Azeem et al., 2021; Tursi et al., 2022). Plastics undergo physical and chemical weathering, leading to fragmentation into smaller pieces (Azeem et al., 2021; Tursi et al., 2022).

Size matters

Plastic pollution comes in different size, due to fragmentation and degradation of debris. Macroplastics refer to fragments $\geq$ 20mm such as bottles, bags, and packaging materials. Mesoplastics are intermediate-sized plastic particles, ranging from 5 – 10 mm in diameter. Microplastics are tiny plastic particles $\leq$5mm outer diameter (OD). Nanoplastics are the smallest form of plastic particles, measuring $\leq$ 0.1 micrometer (µm) OD (Gao et al., 2019; Guo et al., 2020; Zhao et al., 2023). The ecological consequences of these plastics depend on the size, with large organisms being more vulnerable to ingesting entire plastic bags, while smaller organisms may ingest smaller plastic particles, which cause reduction in feeding capability and ultimately weight loss and decrease in growth. (Gao et al., 2019; Guo et al., 2020; Subhankar Chatterjee and Shivika Sharma, 2019).

Consequences of microplastics on aquatic organisms

While there has been considerable research on the effects of MPs in aquatic ecosystems, less attention has been paid to the potential effects of MPs in the terrestrial environment (Sabor et al., 2022; Baraki et al., 2021; Guo et al., 2020; Kluenen et al., 2020). Studies on effects of MPs in aquatic ecosystems shows that the tiny plastic fragments are persistent in aquatic ecosystems and are mistaken as food and digested by a range of aquatic biota such as phytoplankton, zooplankton, coral, sea urchins, lobster, fish, crayfish, and they eventually get transferred to higher trophic levels (Subhankar Chatterjee and Shivika Sharma, 2019). A study carried out in Australia's Great Barrier Reef detected the presence of MPs in the mesenterial tissue within the gut cavity of scleractinian corals, which has negative effects on coral's health (Subhankar Chatterjee and Shivika Sharma, 2019). Several experimental studies also revealed that zooplankton can ingest polystyrene beads of dimension of 1.7 – 30.6 μm (Subhankar Chatterjee and Shivika Sharma, 2019). A study on Centropages typicu (copepod), showed microplastic ingestion of size 7.3 μm, which resulted in low feeding ability and negatively affected their health (Subhankar Chatterjee and Shivika Sharma, 2019). Another study on freshwater shrimp when exposed to MPs such as polymethylmetacrcylate (PMMA) and polydroxybutyrate (PHB) shows decreased growth of this organism (Subhankar Chatterjee and Shivika Sharma, 2019). The polyethylene (PE) MPs uptaken in benthic organism freshwater amphipod caused decrease in the growth and reproduction processes (Subhankar Chatterjee and Shivika Sharma, 2019).

Microplastic effects on humans

Microplastics have also been found in the human body, such as human blood, which could be a result of ingesting fruits or organism with MPs (Ya et al., 2021). A study carried out by Conti et al. (2020) found microplastics in apples and carrots. Despite this finding, the exact implications of microplastics on human health remain inadequately understood. Studies show humans may experience immune system disruption, cytotoxicity, inflammation, neurotoxicity, and transfer to other tissues after being exposed to them (Md. Simul Bhuyan, 2022; Yan et al., 2021). A study carried out by Yan et al. (2021) shows inflammatory bowel disease was significantly higher in patients with MPs than people without MPs.

Microplastic concentration in terrestrial environment

Several studies show that >80% of the MPs wastes that end up in aquatic ecosystems comes from the land (Sabor et al., 2022; Ya et al., 2022, Azeem et al., 2021). It is estimated that 4-23 times more MPs are released and retained in terrestrial environments compared to the ocean (Ren et al., 2021; John et al., 2022; Kluenen et al., 2020). Therefore, because of the high retention of terrestrial MPs pollution, it is necessary to shift our focus to consequences of these pollutants on the terrestrial environment. Several studies on terrestrial environments revealed how MPs affects altered soil physical and chemical properties, reduce plant growth, and inhibit animal feeding abilities (Subhankar Chatterjee and Shivika Sharma, 2019; Sabor et al., 2022; Ya et al., 2022, Azeem et al., 2021).

Microplastic sources in soil

Microplastics can enter soil through compost and sewage sludge, plastic mulching, deposition, irrigation, and other ways (Azeem et al., 2021; Kluenen et al., 2020). Agricultural activities have been identified as one of the main anthropogenic activities that generates MPs pollution in soil due to biosolid application. Recent studies estimate the annual input of MPs to farmlands through biosolids ranges from 43,000 to 430,000 tonnes/year in Europe and North America (Nizzetto et al., 2016), and from 2,800 to 19,000 tonnes/year in Australia (Ng et al., 2018). In southern China the concentration of plastic particles found in agricultural soil ranges from 7,100 to 42,960 particles/kilogram (kg^{-1}; mean 18,760 particles kg^{-1}) annually, (Zhang and Liu, 2018).

Most common plastics found in agricultural soils

The most common MPs in terrestrial environments are polyethene (PE), polypropylene (PP), polystyrene (PS), and polyvinyl chloride (PVC; Choi et al., 2021; Fuller et al., 2016), with PE and PP emerging as the predominant types among the seven types of plastic (i.e., PP, PE, PES (polyester), acrylic, PA (polyamide), PVC, and PS) found in 30 farmland studies, constituting 65.96% and 18.29%, respectively, among the surveyed farmlands (Hu et al., 2022).

Effects of microplastics on soil

Microplastics accumulation in soil has been shown to cause deleterious effects to soil health and function (Colzi et al.,2022; Guo et al., 2020), such as altering soil pH which in turn affects the microbial community, bioavailability of metals and other micro- and macro-nutrients along with biochemical processes in the rhizosphere (Azeem et al., 2021; Colzi et al.,2022; Guo et al., 2020; Liu et al., 2022). Nitrogen (N) is an essential plant nutrient and soil microorganisms influence the availability of N in the soil through nitrogen fixation (Nannnipieri and Eldor, 2009; Shi et al., 2022). Ya et al. (2022) demonstrated that the addition of PE microplastic pollution to soil altered the soil microbial community composition. Another study shows that PE microplastics significantly decreased soil nitrate (NO_3^-), mineral N (MN), total dissolved N (TDN), the net cumulative N nitrification (Nn) at elevated temperatures (Shi et al., 2022). Furthermore, Ma et al. (2023) found that the addition of PE into the soil decreased the soil pH by 3.06%.

Effects of microplastics on plants

MPs appear to have detrimental impacts on plant growth, chlorophyll concentrations, and photosynthesis rates (Boot et al., 2019; Colzi et al., 2022; de Souza Machado et al., 2019; Shi et al., 2022b; Wu et al., 2022). Xin et al. (2014) found that maize heights were 3.3%, 11.9%, and 16.8% lower at the seedling stage in treatments with plastic residuals of 180, 360, and 720 kg per hectare, respectively, compared to those without residual treatment. A study on garden cress (*Lepidum sativum*), demonstrated a 56%, 46%, and 21% reduction in germination rates after 8 hours at 103 to 107 particles per millimeter (mL^{-1}) of NPs and MPs at three difference sizes (50, 500, and 4800 nm). Another study investigated root growth of onion (*Alluim cepa*) and found a 41.5% reduction in root length compared to control treatments after 72 hours of polystyrene (PS) size (50mm) exposure, (Giorgetti et al, 2020). Reductions in growth were attributed to the accumulation of MP particles obstructing the growth of roots or interfering with nutrient uptake, leading to stunted growth (Bosker et al., 2019; Giorgetti et al, 2020). Gao et al. (2019), also found that MPs greatly reduced photosynthetic rate and chlorophyll content of lettuce (*Lactuca sativa)* possibly through reductions in electron flow in photosynthesis as a result of less water uptake. Furthermore, penetration of MPs into the cell wall of plants results in less absorption by chlorophyll (Chatterjee and Sharma, 2019). Recently, a study carried out on tomato plants (*Lycipersicon esulentum* L.) demonstrated an inhibitory effect ranging from 10.1%

to 23.6% on seed germination, with PE identified as the most toxic among the three different types of microplastics PS, PP, and PE used (Shi et al., 2022b).

CHAPTER II

Evaluating the Impact of Microplastics on the Soil Biochemical Environment and Cherry Tomato (*Solanum lycopersicum*) Productivity

Introduction

The presence of microplastics (MPs) in the environment is a serious concern due to their high persistence, bioaccumulation, and extensive use in agricultural production (Azeem et al., 2021; Li et al., 2021). Microplastics are a diverse group of polymers which are extremely small debris, mostly produced from petrochemicals extracted from oil and gas products (Colzi et al., 2022; Kluenen et al., 2020). Since wide scale production started in the 1930s, there has been a dramatic increase of MPs created between 1950 and 2015 with at least 8.3 billion tons being made (Azeem et al., 2021; Gao et al, 2019; Kluenen et al., 2020). Despite the large production, over half of the MPs that have been produced get thrown away as waste (Gao et al, 2019). Given the high durability of plastics and being non-biodegradable, most of this waste is still present in the environment, and some are even found in remote places such as Antarctica (Leistenschneider et al., 2021).

Plastic pollution comes in different sizes, due to fragmentation and degradation of debris. Particles with sizes <5 mm are typically classified as MPs while sizes <0.1 um are referred to as nanoplastics (NPs; Mariano et al., 2021). Ecological consequence of these plastics depends upon the size, with smaller particles capable of being absorbed and transported through plant roots (Booker et al., 2019; Gao et al., 2019), or consumed by soil microbes (Gao et al., 2019; Guo et al., 2020; Chatterjee and Sharma, 2019). Plastics comes in various types, each with distinct properties (Fuller et al., 2016; Guo et al., 2020). The most common MPs in terrestrial environmental are polyethene (PE), polypropylene (PP), polystyrene (PS), and Polyvinyl chloride (PVC; Choi et al., 2021; Fuller et al., 2016), with PE and PP emerging as the predominant types among the seven types of plastic (PP, PE, PES (polyester), acrylic, PA (polyamide), PVC, and PS) found in 30 farmland studies, constituting 65.96% and 18.29%, respectively, among the surveyed farmlands (Hu et al., 2022).

While there has been considerable research on the effects of MPs in aquatic ecosystems, less attention has been paid to the potential effects of MPs in the terrestrial environment (Baraki et al., 2021; Guo et al., 2020; Kluenen et al., 2020; Sabor et al., 2022). Several studies show that >80% of the MP wastes that end up in aquatic ecosystems comes from the land (Azeem et al., 2021; Sabor et al., 2022; Ya et al., 2022), yet it is estimated that 4-23 time more MPs are released and retained in terrestrial environments compared to the ocean (Horton et al., 2017). Therefore, because MPs are highly retained on the landscape, it is necessary to shift the focus to consequences of these pollutants on the terrestrial environment. Microplastic can enter into soil through compost and sewage sludge, plastic mulching, deposition, irrigation, and other ways (Azeem et al., 2021; Kluenen et al., 2020). Recent studies estimate, the annual input of MPs to farmlands through biosolid ranges from 43,000 to 430,000 tonnes/year in European and North American (Nizzetto et al., 2016), from 2,800 and 19,000 tonnes/year in Australia (Ng et al., 2018). In southern China the concentration of plastic particles found in agricultural soil ranges from 7100 to 42,960 particles/kilogram (kg^{-1}; mean 18,760 particles kg^{-1}) annually, (Zhang and Liu, 2018).

Microplastics accumulation in soil have been shown to cause deleterious effects to soil health and function (Colzi et al.,2022; Guo et al., 2020), such as altering the pH of soils which in turn affects microbial community, bioavailability metals and other micro- and macro-nutrients along with other biochemical processes in the rhizosphere (Azeem et al., 2021; Colzi et al.,2022; Guo et al., 2020; Liu et al., 2022). Nitrogen (N) is an essential plant nutrient and soil microorganisms influence the availability of N in the soil through nitrogen fixation (Nannnipieri and Eldor, 2009; Shi et al., 2022). Ya et al. (2022) demonstrated that the addition of PE microplastic pollution to soil altered the soil microbial community composition. Another study shows that PE microplastics significantly decreased soil Nitrate (NO_3^-), mineral N (MN), total dissolved N (TDN), the net cumulative N nitrification (Nn) at elevated temperatures (Shi et al., 2022). Furthermore, Ma et al. (2023) found that the addition of PE into the soil decrease the soil pH by 3.06%.

In addition to having deleterious effects on soil communities, MPs appear to have detrimental impact on plant growth, chlorophyll concentrations, and photosynthesis rate (Boot et al., 2019; Colzi et al., 2022; de Souza Machado et al., 2019; Shi et al., 2022b; Wu et al., 2022). Xin et al. (2014) found that maize heights were 3.3%, 11.9%, and 16.8% lower in treatments with plastic residuals of 180, 360, and 720 kg per hectare (hm^{-2}), respectively, at the seedling stage compared to those without residual treatment. Reductions in maize height was attributed to the accumulation of MP particles obstructing the growth of root or interfering with nutrient uptake, leading to stunted growth (Bosker et al., 2019). Gao et al. (2019), also found that MPs greatly reduced photosynthetic rate and chlorophyll content of *Lactuca sativa* possibly through reductions in electron flow in photosynthesis as a result of less water uptake. Furthermore, penetration of MPs into the cell wall of plant results in less absorption by chlorophyll (Chatterjee and Sharma, 2019). Recently, a study carried out on tomato (*Lycipersicon esulentum* L.) demonstrated an inhibitory effect ranging from 10.1% to 23.6% on seed germination, with PE identified as the most toxic among the three different types of microplastics PS, PP, and PE used (Shi et al., 2022b).

Tomato (*Solanum lycopersicum*) is a widely grown horticultural crop in the United States, China, and Southern Europe, mostly under greenhouse conditions (Mori et al., 2008; Cheng et al., 2022). Prior research, has highlighted variations in toxicity among different MPs types, concentrations, and size (Shi et al., 2022b: Yu et al., 2020; Yu et al., 2022). Notably, PE was identified as the most toxic among MPs (PE, PP, PS; Shi et al., 2022b), the highest concentration (e.g., 15,000–17,000,000 particles/L), of MPs had the most significant impact (Yu et al., 2020; Yu et al., 2022), and the largest MPs size had the most negative impact on plant health among the sizes tested (50, 500, and 4800 nm; Booker et al., 2019). We hypothesized that the phytotoxicity effect of MPs on soil health and tomato physio-biochemical parameters will intensify with increasing concentration and size, and this trend will be observed within each MPs type with the mixture (PE + PP) anticipated to exhibit the highest level of toxicity. While previous studies have examined the effects of microplastics on tomatoes (Hernandez-Arenas et al., 2021; Shi et al., 2022b; Shi et al., 2023), none of these studies have examined how the mixing of plastics could affect plant physiological or altered soil biochemical processes. The aim of this study was (1) to investigate whether MP toxicity influenced soil biochemical and plant physiological processes in tomato, and whether if these effects were dependent on MP type, concentration and/or size; and (2) to assess whether the use of a mixture of MPs to simulate environmental conditions would result in a more pronounced phytotoxic effect. This study seeks to provide valuable insights into the complex interactions between microplastics, soil health, and plant physiology.

Methods

Experimental design

Soil sampling, processing, and background soil analyses

The experiment focused on studying the effects of various types, sizes, and concentration of MPs on tomatoes grown in pots in a greenhouse located at the Minnesota State University, Mankato, MN, USA (44° 0' 22.652" N, 94° 2' 18.428" W). The silt loam (sand: 8.3 ± 0.61%, silt: 77.6 ± 0.51% and clay: 14.0 ± 0.18%; Table 2) soil (classified as a fine, smectitic, mesic Aquertic Argiudolls; USDA web soil survey) used in this study was obtained from a local certified organic farm in Good Thunder, Blue Earth County, MN, USA (44° 0' 22.572" N, 94° 2' 18.132" W) in October 2021 after maize (*Zea mays* L.) was harvested. Soil was collected with a shovel from 0-15 cm depth using a simple random sampling technique. Collected soil was transported to the greenhouse for storage. Before use, air-dried soil was sieved (<4 mm mesh) to remove gravel and large roots/residues, and then ground using a mortar and pestle followed by passing through a 2 mm stainless-steel test sieve (US no. 10). Sieved subsamples (n = 5) were analyzed in June 2021 before the start of the experiment to examine initial soil physical (texture, gravimetric water content, bulk density, and maximum water holding capacity), chemical (total C, N, P, K, Ca, Mg, S, Mn, Fe, Mn, Zn, Cu, Na, cation exchange capacity, pH, and electrical conductivity), and biological (soil organic matter, inorganic N and soil respiration) properties (Table 2).

Briefly, particle size distribution was estimated using a Malvern Mastersizer 3000 laser diffractometer (Malvern Panalytical Ltd, Malvern, United Kingdom). The maximum water holding capacity (MWHC) was calculated as the difference in weight between a saturated soil that was allowed to drain for 6-h, and the weight after the soil was oven-dried for 48 h at 105ºC (De et al., 2020; Haney and Haney, 2010). Soil bulk density was determined at the time of soil collection using the core method (Blake & Hartge, 1986) to mimic the field soil bulk density in the greenhouse pot experiment. Gravimetric soil moisture content was determined using a technique described by Gardner (1986) to correct the soil mass to determine bulk density and inorganic N (IN; NH_4^+–N + NO_3^-–N). The loss-on-ignition method was used to measure the soil organic matter content (Cambardella et al., 2001).

A commercial laboratory (Ward Laboratories, Inc., Kearney, NE, USA) performed the chemical soil analyses, including total C and N (a dry combustion method as described by Nelson & Sommers, 1996); soil test P, Bray 1 (0.013 N HNO_3 and 0.015 N NH_4F extracting solution); soil test K, Ca, and Mg (1 N ammonium acetate extracting solution); soil test S (500 ppm calcium phosphate extractant); soil test Cu, Fe, Mn, and Zn (0.005 M DTPA extraction method); cation exchange capacity (1 M ammonium acetate extraction method of base cations followed by the summation of exchangeable base cations - Ca, Mg, Na, K, and Al); and electrical conductivity (1:1 soil-water method).

Microplastic treatments

Our study included a control (with no MPs) and 12 MP (three types × two sizes × two concentrations) treatments with five replicates for each treatment making a total of 65 pots (Table 1. Three types of MPs, polyethylene (PE; Product # ZL16BC003), polypropylene (PP; Product # ECUX860448) and a 50/50 proportion of PE + PP mixture, were used with two MP size ranges (0.1 – 1.0 mm and 2.0 – 3.5 mm), and two concentrations of MP suspensions (1% and 5%; w/w). We used a 1% (10,000 mg kg-1 dry soil; (w/w)) concentration gradient to mimic the current condition, while a 5% (50,000 mg kg-1 dry soil; (w/w)) concentration gradient was employed to represent the future scenario with higher levels of microplastics. This was done to simulate the anticipated increase in plastic mulch film residues in agricultural soil (Liu et al., 2022 and Zhang et al., 2022). The PE + PP mixture was used as a single treatment to mimic an environment that has both MPs in the soil. All the selected size ranges of MPs were provided by Brunk Plastic Services, Minneapolis, MN, USA.

Study plant and experimental set up

In December 2022, a pot experiment with completely randomized design was established in a greenhouse. One hundred seeds of large red cherry tomato (SimplyGro LLC, Manchester, NH, USA) were sown on 18 December 2022 in a seedling tray which contained the same soils used for the experiment. On 30 December 2022, at 2-3 leaf stage seedlings were transplanted into experimental pots (16.5 cm high, diameter of 12 cm at the bottom and 17 cm at the top) and thinned to one seedling per pot after 13 days of growth.

Each pot had two distinct layers: a mixture of quartz sand and gravel (~860 g) at the bottom (up to 2.54 cm depth) followed by 10 cm of 2-mm sieved soil (~2291 g) to achieve the bulk densities of 3.04 g cm^{-3} and 1.01 g cm^{-3} (similar to field bulk density), respectively. Before adding a mixture of quartz sand and gravel, a piece of mesh fabric was placed over the drainage holes in the bottoms of pots to prevent any material loss. The selected MP treatments were mixed with the soil at a concentration of 1% and 5% by manually stirring with a metal spatula for ~3 mins in a large container before transferring the mixture into each experimental pot. Soil in the control treatment pots (with no MPs) was stirred the same way for equivalent ~3 mins to provide the same disturbance.

All the pots were watered as needed to capacity and rotated weekly throughout the experiment. In addition, plants were fertilized on 2 March, 3 April, and 23 April 2023 with a 24% total N solution (3.5% Ammoniacal; 20.5% Urea; Miracle-Gro Water-Soluble All-Purpose Plant Food, Marysville, OH, USA). A bamboo stake was tied to each plant to prevent them from bending. Plants were harvested on 2 May 2023.

Soil and plant measurements

Post-harvest soil analyses

Soil samples were collected after harvest from each pot and airdried prior to analysis. Soil pH was measured after equilibrating 10±0.05g of soil with 10 mL of deionized water (1:1, w: v) for 15 mins with a digital HQ44OD laboratory single input, multi-parameter meter (Hach Company, Loveland, CO, USA).

Soil IN (NH_4^+-N and NO_3^--N) was determined by extraction using 0.5 M potassium sulfate (K_2SO_4; 1:5 ratio, w: v), shaking for 1h, filtering the supernatant through Whatman filter paper No. 1 (VWR International, LLC, Radnor, PA, USA) and storing the extract at -20°C until analysis. The extractant was analyzed on an EPOCHTM 2 Microplate Spectrophotometer (BioTek Instruments, Inc., Winooski, VT, USA) using the single-reagent method [vanadium III, sulfanilamide and N-(1-naphthyl)-ethylenediamine dihydrochloride] at 540 nm for NO_3^--N and using the salicylate and ammonia cyanurate reagent packets (Hach Company, Loveland, CO, USA) at 595 nm for NH_4^+-N (Doane & Horwáth, 2003; De et al., 2020).

The Solvita CO_2-burst method was used to measure short-term soil respiration using commercially available Solvita CO_2-Burst paddles (Woods End® Laboratories Inc., Mt. Vernon, ME, USA) (Solvita Test Color, 2019; Haney and Haney, 2010). For this, 30 g dry soil with the available Solvita CO_2-Burst gel paddles and 9 mL distilled water was incubated for 24 hr. in a 475 mL incubation jar with CO_2 gasket. After 24 hr. incubation, gel paddles changed color due to evolved CO_2 and the color intensity was measured using a Solvita Digital Color Reader (Woods End® Laboratories Inc., Mt. Vernon, ME, USA; Solvita Test Color, 2019).

Plant measurements

Chlorophyll content or greenness of each plant was measured weekly (26 January to 27 April 2023), using a Soil Plant Analysis Development (SPAD) 502 Plus Chlorophyll Meter (Spectrum Technologies, Inc., Aurora, IL, USA) at mid-length of the uppermost fully expanded leaf (Shen et al., 2017; Zhang et al., 2022).

Chlorophyll fluorescence measurements mid-length on the adaxial leaf surface once every week from 26 January to 27 April 2023 were taken with a pulse-amplitude modulated fluorometer (Mini-PAM; Heinz Walz GmbH, Effeltrich, Germany). Plants were dark adapted for 30 min using dark-leaf clips and minimal fluorescence (Fo) was obtained using a weak modulated LED light source. Maximal fluorescence (Fm) was obtained by exposing the lead to a saturating pulse (>18,000 mol m^{-2} s^{-1} Photosynthetically active radiation (PAR) of actinic light. The ratio of variable to maximal fluorescence (Fv=Fm-Fo) was then calculated. The light adapted quantum yield of photosystem II (ϕPSII) electron transfer (NPSII) was measured in an adjacent area using an open leaf clip held about 0.5 cm above the leaf surface at a 40° angle. A weak modulated light (0.15 mol m^{-2} s^{-1}) and a saturating pulse (>18,000 mol m^{-2} s^{-1} PAR) was used to measure F´o and F´m, respectively and ϕPSII was calculated following Ruhland and Krna (2010).

Plant height and number of leaves (green and dead) were measured on plants every 7 d after germination. Plant heights were measured from the 13th day (12 January 2023) after seeds were transplanted to the 118th day (2 May 2023) using a measuring tape. Number of leaves were counted, with each new leaf marked with a small waterproof black-ink dot placed at the tip of the leaves and recorded. In addition, the number of fruits were counted at harvest on 2 May 2023. Aboveground shoot and fruit biomass were measured after drying them at 60^{o}C for >48 h.

Statistical analyses

Data are presented as mean ± standard error (SE) and all statistical analyses were performed using R statistical package (version 4.0.3; R Core Team, 2021). The pot experiment was a completely randomized design with five replications (considered random), with the different types, sizes, and concentrations of MPs, and time as main fixed factors. Data were checked with the Shapiro-Wilk's normality test, Q−Q plots and Barlett's heterogeneity of variances test using the *ggResidpanel* package (version 0.3.0; Goode and Rey, 2019) prior to analyses, and data were transformed to avoid analysis of variance (ANOVA) assumption violations. Thus, NH_4^+-N and NO_3^--N were square root transformed for ANOVA. A 3-way ANOVA was used to determine the main and interactive effects of types, sizes, and concentrations of MPs on response variables (soil pH, NH_4^+-N, NO_3^--N, total IN, soil respiration, fruit count, fruit biomass, and shoot biomass). A four-way ANOVA was used to determine the main and interactive effects of types, sizes, and concentrations of MPs, and time on response variables (plant height, leave counts, total chlorophyll, light adapted, and dark adapted). Estimated marginal means were computed for the linear mixed-effects models using the *emmeans* package (version 1.5.4; Lenth, 2021) in R, and pairwise mean comparisons were evaluated by individual contrasts between treatments where there was significant interaction or main effect using the Tukey test for multiple comparisons when appropriate. The *multcompView* package (version 0.1-9; Graves et al., 2023) in R was used for visualizations of the results of multiple pairwise comparison test. The significance test for all analyses was done at the 5% significance level ($\alpha = 0.05$).

Results

Effects of MPs on soil pH, inorganic nitrogen, and soil respiration

Soil pH

Soil pH was significantly influenced by MP type, concentration, and interactions of type and size (Table 3). The mean pH significantly increased with increasing concentration of MPs regardless of MP sizes and types (Fig. 1A). At 1% and 5% concentrations, the mean pH was 1.65-3.42% greater than the control treatment with no MP concentrations. The highest mean pH, within each MP size and type, always occurred in the PE+PP treatments (7.12±0.03) followed by PP (7.03±0.03), PE (6.95±0.02), and control (6.86±0.02) treatments. (Fig. 1B). Across all MP concentrations, mean pH within the 0.1-1.0 mm and 2.5 – 3 mm MP sizes was 0.93-4.61% and 1.75-3.05% higher in the soil mixed with MP treatments (6.92-7.18), respectively, compared to the control (6.86±0.02) treatments.

Inorganic nitrogen

Although MP types and concentrations significantly affected soil inorganic N (NH_4^+–N + NO_3^-–N), the 2-way and 3-way interactions between MP types, sizes, and concentrations did not affect soil inorganic N (Table 3). The dominant soil inorganic N form in all the treatments was NO_3^-–N, followed by NH_4^+–N. The proportion of inorganic N in the soil ranged from 0% to 32.3% NH_4^+–N and 67.7% to 100% NO_3^-–N (data not shown). A cross all MP concentrations and sizes, the mean concentration of inorganic N in soil mixed with MP types significantly declined to 15-

55% as compared to the control (Fig. 3A). Among the MP types, PE+PP mix had 44-47% significantly greater inorganic N than the PE and PP treatments; however, no significant difference was observed between PE and PP treatments (Fig 3A). The mean concentration of inorganic N decreased with increasing concentration of MPs regardless of MP sizes and types, albeit insignificant (Fig. 3B). Across the MP types and sizes, the control treatments had 36.8 and 44.8% higher inorganic N than the 1% and 5% MP concentrations, respectively.

Soil Respiration

The MP sizes, concentrations, and their interactions did not show a significant effect on soil respiration (Table 3). Only MP types had a significant effect on soil respiration. Across all MP concentrations and sizes, soil respiration significantly declined over time, being more evident in soil mixed with all MP types compared to the control (Fig. 2). Mean soil respiration in PE, PP, and PE+PP treatments was 30.6%, 33.5%, and 32.6% lower, respectively than in the control treatments ($p<0.05$; Fig. 2).

Effects of MPs on tomato physio-bio chemical characteristic

Plant Height

The plant height across treatments (Table 4) was not significantly affected by MPs type, size, and concentration over time, except during the early /mid stages of development (41-62 days), where plants in MP size 2 - 3.5mm and the control had between 15-25% and 14- 25% reduction in height, compared to size 0.1 – 1mm, respectively, with a noticeable decrease found with the PE + PP size 2 – 3.5 ($p<0.05$; Fig. 4A). Furthermore, Plant height at 1% concentration and control was 4 – 15% and 9 – 25% lower, respectively, compared to the 5% concentration, with a noticeable decrease found with the PE + PP at 1% ($p<0.05$; Fig. 4B)

Number of leaves

Number of leaves was significantly reduced by MPs type, size, and concentration over time (Table 3). During the mid/final stages of development (62-118 days), plants growing in soil with MPs size 0.1 – 1mm and 2 – 3.5 mm had 1 – 6% and 9- 16% less leaves, respectively, compared to the control ($p<0.05$; Fig. 4C). In addition, there was 4 – 11% and 1 – 11% less leaves in 1% and 5% concentration, respectively, compared to the control, with a noticeable decrease found with the PE + PP at 1% ($p<0.05$; Fig. 4D). Among the MP types, plants in the PE + PP, PP, and PE had 13%, 10%, and 10% fewer leaves, respectively, compared to the control ($p<0.05$; Fig. 4C & 4D).

Total Chlorophyll

The size and concentration did not affect chlorophyll concentration, with the exception of concentration on day 27 (Fig. 5A). Plants within MPs 1% and 5% concentration had 11% and 3% reduction, respectively, compared to the control ($p<0.05$; Fig. 5B).

Light and Dark Adapted

Microplastic did not have any significant effects on light adapted yield (5C & 5D). There were no significant MP size effects on Fv/Fm, with the exception of day 27 (Figure 5E), while MP concentration had a notable impact on Fv/Fm specifically on day 118 (Figure 5F). Plants growing in soil with MP size 0.1 – 1mm and control had 5% and 4% reduction in Fv/Fm, respectively, compared to size 2 – 3.5mm ($p<0.05$; Fig. 5E). Within the concentration 5% and control, there was 4% and 7% reduction in Fv/Fm, respectively, compared to the 1% concentration ($p<0.05$; Fig. 5F). In addition, plants in PE + PP, PE, and control had 5%, 4%, and 9% reduction, respectively compared to the control ($p<0.05$; Fig. 5E & 5F).

Fruit count

Fruit count was not influenced by the type or concentration of MPs. However, it was observed that the size of MPs had a significant effect on fruit count ($p<0.01$; Table 5). Plants with MPs size 2.0–3.5mm produced 10% less fruits compared to those with MPs sized 0.1–1mm ($p<0.05$; Fig. 3).

Fruit Biomass

Fruit biomass was significantly influenced by the type and size of MPs (Table 5). No significant difference was observed among treatments within MPs size 0.1 – 1mm. However, there was a reduction in fruit biomass of the treatments within MPs size 2 – 3.5 mm as compared to the control ($p<0.05$; Fig. 3). Furthermore, plants growing in soils with MPs size 2.0–3.5mm had 20% less fruit biomass compared to those with MPs sized 0.1–1mm. Among the MP types, plants growing in the PP and PE+PP had a 24% and 30% reduction in fruit biomass, compared to the control, respectively ($p<0.05$; Fig. 3).

Shoot Biomass

MPs type or size did not have any impact on the shoot biomass. However, the concentration of MPs had a significant effect ($p<0.05$; Table 4). Specifically, MPs at a 1% concentration produced 9% less biomass than those growing at a 5% ($p<0.05$; Fig. 3).

Discussion

The presence of MPs in soil can detrimentally affect soil health and nutrient availability, potentially disrupting plant growth and ecosystem dynamics (de Souza Machado et al., 2019; Fei et al.,2020; Kim et al., 2023; Liu et al., 2016; Rillig et al., 2021). In this study, the investigation into the effects of MPs on soil and plants revealed that soil properties, including pH, inorganic nitrogen levels, and soil respiration, were influenced by different concentrations, sizes, and types of MPs. The introduction of MPs into soil can elevate its pH through mechanisms such as enhanced soil aeration, improved porosity, and the alteration of soil biota due to the influence on the arrangement of soil particles and air spaces, as well as the leaching of toxic compounds from the MPs (De Souza Machado et al., 2019; Judy et al., 2019; Kim et al., 2020; Waldman and Riling, 2020). In our study, soils in the PE+PP treatments consistently exhibited the highest pH levels (7.12±0.03), followed by PP (7.03±0.03) and PE (6.95±0.02) treatments, as compared to the control (Fig. 1b). Our findings align with Med-Juraszek and Jadhav (2020) and Zhao et al. (2021) who found that the addition of MPs to soil increased pH. Zhao et al. (2021) study also supports this trend, reporting an elevation in soil pH with the addition of MPs in the form of foams and fragments. Studies suggest that PE and PP may alter nitrogen fixation bacteria taxa in soil, leading to increase in pH and influencing the conversion of organic N to NH_4^+, as conversion of organic N to NH_4^+ release $H^{+,}$ which can temporarily decrease pH (Butterly et al., 2010; Fei et al., 2020; You et al., 2015). Consistent with this, our observation of lower CO2 respiration levels in soils with MPs (Fig. 2) indicates reduced microbial activities leading to less H+ release and a concomitant increase in pH. Furthermore, CO_2 can lower soil pH by forming aqueous carbonic acid (H_2CO_3) upon reaction with water as the H^+ present in carbonic acid can render the soil acidic, thus lowering the pH (Ferdush et al. 2023; Zabowski and Sletten, 1991). In addition, there was also an effect of MP concentration on soil pH as the mean pH was 1.65-3.42% higher than soils with no MPs present (Fig. 1a). Several studies have found that higher concentration of MPs may lead to an increase in soil pH (Lozano et al., 2021; Yang et al., 2021; Zhang et al., 2024). For example, the presence of 1% (w/w) and 10% (w/w) polylactic acid (PLA) and high-density polyethylene (HDPE) MPs increased soil pH (Yang et al., 2021). Lozano et al. (2021) also found that 0.4% (w/w) PES MFs increased soil pH. This suggests that the accumulation of MPs may affect soil buffering capacity or microbial activity, resulting in notable changes in pH levels (Lozano et al., 2021; Yang et al., 2021).

Microplastics have been linked to potential adverse effects on soil biota, largely because they can disrupt microbial activity by altering soil pH (de Souza Machado et al., 2019; Fei et al.,2020; Liu et al., 2016). In our study, we observed that soil respiration in PE, PP, and PE+PP treatments was 30.6%, 33.5%, and 32.6% lower, respectively than in the control treatments (Fig.2). Fei et al. (2020) reported that MPs can impede essential metabolic pathways in bacteria, resulting in decreased bacterial activity. Furthermore, MPs offer a substantial surface area for the adsorption of organic compounds and pollutants, potentially affecting the availability of organic matter for microbial decomposition and causing a significant reduction in soil respiration (de Souza Machado et al., 2019; Liu et al., 2016). These findings align with Lozano et al. (2021), who noted a decline in soil respiration with the introduction of MPs fibres under well-watered soil condition. Zhao et al. (2021) also reported a significant reduction in soil respiration after 31 days of incubation in soils containing MPs compared to those without. Additionally, Wang et al. (2023) highlighted that the addition of PE and PBAT MPs into soil resulted in a 7.4% to 19.2% and 27.7% to 30.7%, respectively, decrease in microbial activity compared to the control. The decrease in soil respiration may be attributed to the toxic effects of MPs on microbes, as studies suggest that some MPs may release additives or adsorbed pollutants with toxic effects on soil microorganisms (de Souza Machado et al., 2019; Zhao et al., 2021). This, in turn, can lead to a reduction in microbial activity and, consequently, a decline in soil respiration rates (Fei et al.,2020).

Microplastics may also impact inorganic nitrogen within the soil by altering soil properties and influencing the activities of nitrogen-fixing bacteria, thereby affecting nutrient cycling (Kim et al., 2023; Rillig et al., 2021). Our study revealed a significant reduction in inorganic nitrogen levels in soils containing microplastics (Fig. 3a & b). The observed decline in soil respiration within the MPs treatment could be a contributing factor to the diminished inorganic nitrogen levels (Fig. 2). Microbes play a pivotal role in nitrogen cycling, encompassing processes like nitrification and denitrification, and alterations in microbial activity can influence the transformation of inorganic nitrogen species in the soil (Kim et al., 2023; Liu et al., 2016; Wang et al., 2023; Rillig et al., 2021). We found that both PE and PP caused significant changes in soil inorganic nitrogen levels. This finding is consistent with Shi et al. (2022), who reported a significant decrease in inorganic nitrogen (NO_3^--N) in soils containing PE. It appears that both PE and PP can absorb and accumulate nitrogen-containing compounds, including inorganic nitrogen compounds such as ammonium (NH_4^+-N) and nitrate (NO_3^--N) (de Souza Machado et al., 2019; Wang et al., 2023). This absorption and accumulation may contribute to the observed reduction in inorganic nitrogen levels observed in our study between PE and PP treatments. The concentration of MPs can also affect soil inorganic nitrogen (Yan et al. 2021; Zhang et al. 2024). Yan et al. (2021) found that 1% (w/w) PVC MPs, decrease content of soil available N by 10-13%. Zhang et al. (2024) also found high dose 1.5 % (*w*/w) PP significantly reduced the concentrations of soil NO_3^-—N. Consistent with our study, across the MP types and sizes, the control treatments had 36.8 and 44.8% higher inorganic N than the 1% and 5% MP concentrations, respectively (Fig. 3b). Excessive MPs concentrations may inhibit soil microbial activity due to physical or chemical interactions with microorganisms (Yan et al., 2021; Yang et al., 2021). Furthermore, the observed increase in soil pH with the 1% and 5% concentrations (Fig. 1a) may have contributed to the reduction in inorganic nitrogen within the 1% and 5%. An increase in soil pH can decrease nitrogen content by inhibiting denitrification, immobilizing ammonium ions, and altering microbial activity (Lozano et al., 2021; Yang et al., 2021; Zhang et al., 2024).

Recent findings on the effects of MPs on plant physiology have demonstrated multiple effects on water absorption, nutrient uptake, and gas exchange (Zhao et al., 2021; Qi et al., 2018), possibly through the release of toxic derivatives into the soil (Judy et al., 2019; Lozano et al.,2021). Interestingly, some studies have demonstrated positive MP effects (Meng et al.,2021; Lian et al., 2020) while others have shown deleterious effects (Kalcikova et al.,2017; Qi et al., 2018; Wu et al.,2020). Exact mechanisms that oversee these physiological responses remains unknown. In the present study, we found that both growth and physiological parameters were influenced by MP type, size, and concentration in the soil.

There were no MP type, size, or concentration effects on plant height at harvest (Fig. 4a & b), which is consistent with the result of Shi et al. (2022), indicating that the treatment of PE MPs had no effect on the height of *Lycopersicon esculentum* L. Polechońska et al. (2023) also reported that the addition of MPs had no significant effect on the growth of *E. canadensis* shoot height. The lack of significant impact on plant height on these species was attributed to their ability to tolerate the presence of MPs and the relatively short duration of the experiment (Polechońska et al., 2023; Shi et al., 2022). A longer timeframe might have revealed more pronounced effects. The presence of MPs in the soil rhizosphere may hinder water uptake by plant roots, subsequently impacting plant productivity, including the number of leaves (Cseresnyes et al., 2014; Jiang et al., 2019). While there was no significant effect observed in plant height, there was a notable reduction in the number of leaves across all MPs treatments compared to the control (Fig. 4c & d). The size of these MP particles may also influence their mobility in the soil, and possibly be taken up by roots (Lian et al., 2021; Li et al., 2020). In addition, it appears that large MP particles can block root development, as the large particles consistently has fewer leaves than the control or small particles. Our result showed that plants growing in soils with 0.1 – 1mm and 2 – 3.5 mm MPs had a reduction in number of leaves produced compared to the control (Fig. 4c). To the best of our knowledge, no prior studies have reported the influence of MPs size on leaf production. However, other studies have demonstrated a reduction in leaf number in response to various types and concentrations of MPs. For example, Cui et al. (2022) and Ikhajiagbe et al. (2020) observed a significant reduction in leaf production in *Raphanus sativus* and *Celosia argentea*, respectively, when exposed to soil with varying concentration of MPs. Furthermore, Hassan and Jho (2023) observed a reduction in the number of leaves in lettuce exposed to low-density polyethylene (LDPE) fragments as the concentration increased from 0 to 3%. At a 3% concentration, there was a 16.5% reduction in leaf production compared to a control. Along these lines, we observed a similar reduction in leaf number regardless of the MPs concentration, as compared to the control, consistent with the findings of Hassan and Jho (2023) regarding PVC fragments. This decrease in leaf number could be attributed to larger particles adhering to root surfaces or accumulating within the vascular system, thereby obstructing water and nutrient uptake and ultimately impacting leaf production (Cseresnyes et al., 2014; Cui et al., 2022; Jiang et al., 2019). It is also possible that a reduced inorganic nitrogen in the soil (Fig 3a & b) contributed to the reduction in leaf number we observed.

The reduction in leaf number with increased size of MPs may also explain the reduction on fruit production. Plants exposed to MPs size 2.5–3mm produced 10% less fruits compared to those with MPs sized 0.1–1mm (Fig 6b). Since it appears that larger MPs can interfere with water uptake by plant roots, it is possible that induced water stress impacted flower and fruit development (Wu et al. 2017). To the best of our knowledge, no studies have examined the effect of MP size on fruit production, but other studies have demonstrated reductions in fruit number in response to various types of MPs. For example, Dainelli et al. (2023) found that the addition of MPs to the soil led to a 20% and 38% decline in fruit number for PET and PVC, respectively, in *S. lycopersicum*. In addition to reduced leaf and fruit number in response to MP size, there was a reduction in fruit biomass. Plants growing in soils with MPs sized 2-3.5 mm had 20% lower fruit biomass than those growing in soils with MPs sized 0.1 – 1 mm (Fig.6c). This indicates that the size of MPs can directly influence fruit production and biomass, emphasizing the importance of understanding the specific effects of MPs on plant physiology and yield. Furthermore, in our study, fruit biomass was also influenced by the type of MPs (Fig. 6c). Different types of MPs exert distinct toxic effects on plants due to their diverse polymer compositions and distinct chemical structures (Boots et al., 2019; Chen et al., 2022; de Souza Machado et al., 2019; Gao et al., 2021; Qi et al., 2018). While some MPs may have direct physical effects, others can release chemicals or adsorb pollutants that influence plant health (de Souza Machado et al., 2019; Qiang et al., 2023). Qi et al. (2018) found that *Triticum aestivum* growing in soils with biodegradable macroplastic (Bio-Ma) and Bio-MPs was significantly lower in fruit biomass than in the control. In addition, studies suggest that PE and PP may have surfaces with a higher capacity to absorb certain chemicals compared to other types of MPs (de Souza Machado et al., 2019; Wang et al., 2023). Pignattelli et al. (2020) found that PP and PE inhibited biomass of *Lepidium sativum.* These results agree with our study as we observed a 24% and 30% reduction in fruit biomass for plants growing in PP and PE+PP soils, respectively, compared to the control. The enhanced chemical absorption capacity of PP could lead to decreased nutrient availability in the soil, potentially impacting plant fruit production and, consequently, influencing fruit biomass (de Souza Machado et al., 2019). Our soil inorganic nitrogen analysis (Fig. 3a & b) demonstrated the capacity of PP to reduce nutrient availability.

Microplastic concentration in soils can affect the physiological growth characteristics of plants, including shoot biomass (Qiang et al., 2023; Wang et al., 2023; Zhou et al., 2021; Yuan et al., 2019). Wang et al. (2023) noted a significant decrease in shoot biomass, from 0.82 g to 0.64 g, in *Brassica juncea* exposed to aged PE or aged PP MPs. We found that tomato plants growing at 1% MP concentration produced 9% less biomass than those growing in a 5% concentration (Fig 6a). While we propose that this may be due to the size 0.1 – 1mm of PE + PP, resulting in reduced plant height and leaf count (Fig.4). This was surprising, as most studies have found reductions in shoot biomass with increased soil MP concentrations (Wang et al., 2020; Zhou et al., 2021; Yuan et al., 2019). Low concentrations of MPs may exert minimal effects on plant development (Liu et al., 2023; Song et al., 2023), while high concentrations can lead to stunted growth by intensifying competition for nutrients between MPs and plants (Liu et al., 2023; Song et al., 2023; Zhou et al., 2021), negatively impacting nutrient availability and, consequently, reducing biomass. Liu et al. (2023) observed that MPs above 0.1% significantly reduced shoot biomass. Similarly, Song et al. (2023) found that rice exposed to polylactic acid (PLA) MPs at a concentration of 10% w/w exhibited 25% significant decrease in shoot biomass. Contrary to these findings, our study aligns with Lian et al. (2020) who found that *Triticum aestivum* L exposed to PS NPs concentrations (0.1 mg/L) exhibited 87% increase compared to lower concentrations. Furthermore, Lozano et al. (2021) also discovered a steady increase in shoot biomass as the concentration of PP and polyethersulfone (PES) fibers increases. Although the underlying mechanism remains unknown, studies suggest that the addition of MPs enhances soil aeration and macroporosity which positively impacts plant performance (Lozano et al., 2021; de Souza Machado et al., 2019). Furthermore, the reduction in leaf numbers caused by exposure to MPs (Fig. 4d) correlates with a decrease in shoot biomass across different MPs concentrations.

While not significantly different, soil without MPs shows higher biomass compared to soils with varying concentrations of MPs. This suggests that even subtle reductions in leaf numbers due to MPs exposure can impact shoot biomass, highlighting the potential influence of MPs on plant growth and productivity (Cseresnyes et al., 2014; Jiang et al., 2019).

Chlorophyll is critically important to plant development as it plays a central role in the process of photosynthesis (Wang et al., 2020). Despite the negative effects observed in plant physiological parameters such as leaf number, fruit count, fruit biomass, and shoot biomass in this study, the examination of biochemical processes such as chlorophyll and photosynthesis does not appear to account for some of the reduction in plant physiological parameters. The impact of MPs on chlorophyll content was found to be insignificant (Fig.5a), consistent with previous literature. Pignatelli et al. (2020), Wang et al. (2020), and Singh and Singh (2022) observed no significant effect on the chlorophyll content of *Lepidium sativum*, *Zea mays* L. var. Wannuoyihao, and *Trigonella foenum*-graecum L, respectively, when exposed to soil with PE, PP, PVC, and LDPE. The scare evidence that MPs affect chlorophyll production may be attributed to plants developing tolerance mechanisms, such as activation of defense mechanism antioxidative systems, enhanced detoxification process, and modification of cell wall composition to alleviate any potential deleterious effects. (Pignatelli et al., 2020; Wang et al., 2020). The presence of MPs/NPs in soil can disrupt plant photosynthetic efficiency (Colzi et al., 2022; Dong et al., 2020; Yu et al., 2020; Ren et al., 2021; Liao et al., 2019) by diminishing the photosynthetic activity of PSII reaction centers, due to the physical obstruction of sunlight to the chloroplasts (Meng et al.,2021, Wang et al.,2020; Wu et al., 2019;). In our study, there was no significant impact observed on light-adapted yield as a result of both the size and concentration of MPs (Table 3; Fig 5c and d), which is consistent with results of several studies reporting no effects on whole-leaf gas exchange (Botyanszka et al., 2022; Lian et al., 2020; Shi et al., 2022). Interestingly, in this present study *Fv/Fm* showed a significant increase in all treatments with MPs, as compared to the control (Fig. 4c). Along these lines, Zhang et al. (2023) found that PES-MPs and PP-MPs increase *Fv/Fm* of *Glycine max* during the flowering stage. While the exact mechanism remains unclear, previous research suggests that MPs may enhance photosynthetic parameters by increasing aminolaevulinic acid necessary for chlorophyll synthesis (Pignattelli et al., 2020). Although the current study did not investigate this factor, future research should explore the correlation with increased *Fv/Fm*. Lastly, recent evidence suggests that the negative effects on *Fv/Fm* are dose dependent (Ansari et al.2021; Zhao et al.,2019). Likewise, despite observing an increase within the MPs treatment compared to the control, a notable decrease in *Fv/Fm* was found within the 5% concentration compared to the 1% concentration within PE, PP, and PE + PP treatments. In line with our findings, Ansari et al. (2021) found a significant decrease in Fv/Fm in the microalga *Acutodesmus obliquus* when the concentration of MPs exceeded 100 mg L^{-1} in HDPE, PP, and PVC treatments. This was attributed to decrease in light penetration through the cultures at higher concentration, due to the attachment of the MPs to the cell surface (Ansari et al.,2021; Zhao et al.,2019). Overall, our study provides insights into the complex interactions between MPs, soil properties, and plant physiology, emphasizing the need for further research to fully understand the implications of MPs on terrestrial ecosystems. Further investigations into the mechanisms underlying these effects and their long-term consequences are warranted to develop effective strategies for mitigating the impacts of MPs on soil and plant health.

Conclusion

The study examines how plastic pollutants (PE, PP, PP + PE) impact soil health and plant growth. We found that MPs alter soil conditions, affecting nutrient availability and microbial activity, which in turn influences plant physiology. Microplastics elevate soil pH, reduce respiration, and lower nitrogen levels, disrupting nutrient cycling. Furthermore, MPs affect plant parameters like leaf and fruit production, and biomass. Larger MPs obstruct water and nutrient transport, reducing leaf and fruit production. Microplastics also impact plant parameters like leaf, fruit and shoot biomass, with varying effects based on concentration, type, and size. Despite not affecting chlorophyll content and photosynthetic efficiency significantly, MPs increase *Fv/Fm* ratio, suggesting complex interactions with plant physiology. Overall, the study emphasizes the intricate relationship between MPs, soil health, and plant growth, urging further research to better understand the mechanisms through which MPs interact with soil and plants. Understanding these interactions is vital for advancing sustainable agricultural practices. Further investigation into the effects of various MP size, type, and concentration on soil and plant health is essential, with potential implications for human health.

Literature Cited

Ansari, F. A., Ratha, S. K., Renuka, N., Ramanna, L., Gupta, S. K., Rawat, I., & Bux, F. (2021). Effect of microplastics on growth and biochemical composition of microalga Acutodesmus obliquus. *Algal Research*, *56*, 102296. DOI:10.1016/j.algal.2021.102296

Azeem, I., Adeel, M., Ahmad, M. A., Shakoor, N., Jiangcuo, G. D., Azeem, K., Ishfaq, M., Shakoor, A., Ayaz, M., Xu, M., & Rui, Y. (2021). Uptake and Accumulation of Nano/Microplastics in Plants: A Critical Review. *Nanomaterials (2079-4991)*, *11*(11), 2935. DOI:10.3390/nano11112935

Bakaraki Turan, N., Sari Erkan, H., & Onkal Engin, G. (2021). Microplastics in wastewater treatment plants: Occurrence, fate and identification. *Process Safety & Environmental Protection: Transactions of the Institution of Chemical Engineers Part B*, *146*, 77–84. DOI:10.1016/j.psep.2020.08.039

Blake, G., & Hartge, K. 1986. Bulk density. In: A. Klute (Ed.), Methods of soil analysis. Part 1. Physical and mineralogical methods (pp. 363–375). Madison, WI: ASA and SSSA.

Boots, B., Russell, C.W., & Green, D.S. (2019). Effects of Microplastics in Soil Ecosystems: Above and Below Ground. *Environmental Science & Technology*, *53*(19), 11496-11506 DOI:10.1021/acs.est.9b03304

Bosker, T., Bouwman, L. J., Brun, N. R., Behrens, P., & Vijver, M. G. (2019). Microplastics accumulate on pores in seed capsule and delay germination and root growth of the terrestrial vascular plant Lepidium sativum. *Chemosphere*, *226*, 774–781. DOI:10.1016/j.chemosphere.2019.03.163

Botyanszká1, L., Šurda, P., Vitková, J., Lichner, L., Igaz, D. (2022). Effect of microplastics on silty loam soil properties and radish growth. *Journal of Hydrology and Hydromechanics, 3*, 321-329. DOI:10.2478/johh-2022-0018

Butterly, C., Baldock, J., Tang CaiXian, Gilkes, R. J., & N. Prakongkep. (2010). Chemical mechanisms of soil pH change by agricultural residues. 43–46.

Cambardella, C. A., Gajda, A. M., Doran, J. W., Wienhold, B. J., & Kettler, T. A. (2001). Estimation of particulate and total organic matter by weight loss-on-ignition. In R. Lal, J. Kimble, R. Follett, & B. Stewart (Eds.), Assessment methods for soil carbon (advances in soil science) (pp. 349–359). CRC Press.

Chatterjee, S., and Sharma, S. (2019). Microplastics in our oceans and marine health. Field Actions Science Reports. J. Field Actions 2019, 54–61.

Chen, G., Li, Y., Liu, S., Junaid, M., & Wang, J. (2022). Effects of micro (nano) plastics on higher plants and the rhizosphere environment. *Science of the Total Environment*, *807*, 150841. DOI:10.1016/j.scitotenv.2021.150841

Cheng, M., Wang, H., Fan, J., Xiang, Y., Liu, X., Liao, Z., Abdelghany, A. E., Zhang, F., & Li, Z. (2022). Evaluation of AquaCrop model for greenhouse cherry tomato with plastic film mulch under various water and nitrogen supplies. *Agricultural Water Management*, *274*, 107949. DOI:10.1016/j.agwat.2022.107949

Choi YR, Kim YN, Yoon JH, et al. (2021). Plastic contamination of forest, urban, and agricultural soils: a case study of Yeoju City in the Republic of Korea. *J Soils Sediments 21*, 1962–1973. DOI:10.1007/s11368-020-02759-0

Colzi, I., Renna, L., Bianchi, E., Castellani, M. B., Coppi, A., Pignattelli, S., Loppi, S., & Gonnelli, C. (2022). Impact of microplastics on growth, photosynthesis and essential elements in Cucurbita pepo L. *Journal of Hazardous Materials*, *423*, 127238. DOI:10.1016/j.jhazmat.2021.127238

Cseresnyés, I., Takács, T., Füzy, A., & Rajkai, K. (2014). Simultaneous monitoring of electrical capacitance and water uptake activity of plant root system. *International Agrophysics*, *28*(4), 537–541. DOI:10.2478/intag-2014-0044

Cui, M., Yu, S., Yu, Y., Chen, X., & Li, J. (2022). Responses of cherry radish to different types of microplastics in the presence of oxytetracycline. *Plant Physiology & Biochemistry*, *191*, 1–9. DOI:10.1016/j.plaphy.2022.09.012

Dainelli, M., Pignattelli, S., Bazihizina, N., Falsini, S., Papini, A., Baccelli, I., Mancuso, S., Coppi, A., Castellani, M. B., Colzi, I., & Gonnelli, C. (2023). Can microplastics threaten plant productivity and fruit quality? Insights from Micro-Tom and Micro-PET/PVC. *Science of the Total Environment, 895*, 165119. DOI:10.1016/j.scitotenv.2023.165119

De, M., Riopel, J. A., Cihacek, L. J., Lawrinenko, M., Baldwin-Kordick, R., Hall, S. J., & McDaniel, M. D. (2020). Soil Health Recovery after grassland reestablishment on cropland: The effects of time and topographic position. *Soil Science Society of America Journal*, *84*(2), 568–586. DOI:10.1002/saj2.20007

de Souza Machado, A. A., Lau, C. W., Kloas, W., Bergmann, J., Bachelier, J. B., Faltin, E., Becker, R., Görlich, A. S., & and Rillig, M. C. (2019). Microplastics Can Change Soil Properties and Affect Plant Performance. *Environmental Science & Technology 53*(10), 6044-6052. DOI:10.1021/acs.est.9b01339

de Souza Machado, A. A., Lau, C. W., Till, J., Kloas, W., Lehmann, A., Becker, R., & Rilling, M. C. (2018). Impacts of Microplastics on the Soil Biophysical Environment. *Environmental Science & Technology, 52*, 9656-9665. DOI:10.1021/acs.est.8b02212

Doane, T. A., & Horwath, W. R. (2003). Spectrophotometric determination of nitrate with a single reagent. *Analytical Letters, 36*, 2713– 2722. DOI:10.1081/AL-120024647

Dong, Y., Gao, M., Song, Z., & Qiu, W. (2020). Microplastic particles increase arsenic toxicity to rice seedlings. *Environmental Pollution*, *259*, 113892. DOI:10.1016/j.envpol.2019.113892

Doran, J., Kettler, T., & Tsivou, M. (1997) Field and Laboratory Solvita Soil Test Evaluation. University of Nebraska USDA-AR

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., & Basra, S. M. (2009). Plant drought stress: Effects, mechanisms and management. *Sustainable Agriculture*, 153–188. DOI:10.1007/978-90-481-2666-8_12

Fei, Y., Huang, S., Zhang, H., Tong, Y., Wen, D., & Xia, X. (2020). Response of Soil Enzyme Activities and Bacterial Communities to the Accumulation of Microplastics in an Acid Cropped Soil. *Sci. Total Environ, 707*, 135634. DOI:10.1016/j.scitotenv.2019.135634

Ferdush, J., Paul, V., Varco, J., Jones, K., & Sasidharan, S. M. (2023). Consequences of elevated CO2 on soil acidification, cation depletion, and inorganic carbon: A column-based experimental investigation. *Soil & Tillage Research, 234*, 105839. DOI:10.1016/j.still.2023.105839

Fuller, S., & Gautam, A. (2016). A procedure for measuring microplastics using pressurized fluid extraction. *Environmental Science & Technology*, *50*(11), 5774–5780. DOI:10.1021/acs.est.6b00816

Gao, M., Liu, Y., Dong, Y., & Song, Z. (2021a). Effect of polyethylene particles on dibutyl phthalate toxicity in lettuce (Lactuca sativa L.). *Journal of Hazardous Materials*, *401*, 123422. DOI:10.1016/j.jhazmat.2020.123422

Gao, M., Liu, Y., & Song, Z. (2019). Effects of polyethylene microplastic on the phytotoxicity of di-n-butyl phthalate in lettuce (Lactuca sativa L. var. ramosa Hort). *Chemosphere*, *237*, 124482. DOI:10.1016/j.chemosphere.2019.124482

Gardner,W. H. (1986).Water content. In A. Klute (Ed.), Methods of soil analysis. Part 1. Physical and mineralogical methods (pp. 493–544). Madison, WI: SSSA and ASA.

Giorgetti, L., Spanò, C., Muccifora, S., Bottega, S., Barbieri, F., Bellani, L., & Ruffini Castiglione, M. (2020). Exploring the interaction between polystyrene nanoplastics and Allium cepa during germination: Internalization in root cells, induction of toxicity and oxidative stress. *Plant Physiology & Biochemistry*, *149*, 170–177. DOI:10.1016/j.plaphy.2020.02.014

Goode, K., & Rey, K. (2019). ggResidpanel: Panels and interactive versions of diagnostic plots using ‘ggplot2’.Rpackage version 0.3.0. https://CRAN.Rproject.org/package=ggResidpanel

Graves, S., Piepho, H. P., Selzer, L., & Dorai-Raj, S. (2023). multcompView: Visualizations of Paired Comparisons. R package version 0.1-9. https://CRAN.R-project.org/package=multcompView

Guo, J.-J., Huang, X.-P., Xiang, L., Wang, Y.-Z., Li, Y.-W., Li, H., Cai, Q.-Y., Mo, C.-H., & Wong, M.-H. (2020). Source, migration and toxicology of microplastics in soil. *Environment International, 137*, 105263. DOI:10.1016/j.envint.2019.105263

Haney, R. L., & Haney, E. B. (2010). Simple and rapid laboratory method for rewetting dry soil for incubations. *Communications in Soil Science and Plant Analysis, 41*, 1493–1501. DOI:10.1080/00103624.2010.482171

Hasan, M. M., & Jho, E. H. (2023). Effect of different types and shapes of microplastics on the growth of lettuce. *Chemosphere,* 339, 139660. DOI:10.1016/j.chemosphere.2023.139660

Horton, A.A., Walton, A., Spurgeon, D.J., Lahive, E., & Svendsen, C. (2017). Microplastics in freshwater and terrestrial environments: evaluating the current understanding to identify the knowledge gaps and future research priorities. *Sci. Total Environ, 586*, 127–141. DOI:10.1016/j.scitotenv.2017.01.190

Hu, J., He, D., Zhang, X., Li, X., Chen, Y., Wei, G., Zhang, Y., Ok, Y.S., Luo, Y., 2022. National-scale distribution of micro(meso)plastics in farmland soils across China: implications for environmental impacts. *J. Hazard Mater, 424*(Pt A), 127283. DOI:10.1016/j.jhazmat.2021.127283

Ikhajiagbe, B., Omoregie, G. O., Adama, S. O., & Esheya, K. U. (2023). *Growth Responses of* Celosia Argentea*l. in Soils Polluted with Microplastics*. DOI:10.1101/2023.01.07.523084

Jiang, X., Chen,H., Liao,Y., Ye, Z., Li,M., Klobučar, G.(2019). Ecotoxicity and genotoxicity of polystyrene microplastics on higher plant Vicia faba. *Environmental Pollution, 250*, 831-838. DOI:10.1016/j.envpol.2019.04.055

John, J., Nandhini, A. R., Velayudhaperumal Chellam, P., & Sillanpää, M. (2022). Microplastics in mangroves and coral reef ecosystems: a review. *Environmental Chemistry Letters*, *20*(1), 397–416. DOI:10.1007/s10311-021-01326-4

Judy, J. D., Williams, M., Gregg, A., Oliver, D., Kumar, A., Kookana, R., & Kirby, J. K. (2019). Microplastics in municipal mixed-waste organic outputs induce minimal short to long-term toxicity in key terrestrial biota. *Environmental pollution (Barking, Essex : 1987)*, *252*(Pt A), 522–531. DOI:10.1016/j.envpol.2019.05.027

Kalčíková, G., Žgajnar Gotvajn, A., Kladnik, A., & Jemec, A. (2017). Impact of polyethylene microbeads on the floating freshwater plant duckweed Lemna minor. *Environmental Pollution*, *230*, 1108–1115. DOI:10.1016/j.envpol.2017.07.050

Kim, K., Song, I.-G., Yoon, H., & Park, J.-W. (2023). Sub-micron microplastics affect nitrogen cycling by altering microbial abundance and activities in a soil-legume system. *Journal of Hazardous Materials*, *460*, 132504. DOI:10.1016/j.jhazmat.2023.132504

Kim, S. W., Waldman, W. R., Kim, T.-Y., & Riling, M. C. (2020). Effects of Different Microplastics on Nematodes in the Soil Environment: Tracking the Extractable Additives Using an Ecotoxicological Approach. *Environ. Sci. Technol. 54*(21), 13868–13878. DOI:10.1021/acs.est.0c04641

Kleunen, M., Brumer, A., Gutbrod, L., & Zhang, Z. (2020). A microplastic used as infill material in artificial sport turfs reduces plant growth. *Plants, People, Planet*, *2*(2), 157–166. DOI:10.1002/ppp3.10071

Leistenschneider, C., Burkhardt-Holm, P., Mani, T., Primpke, S., Taubner, H., & Gerdts, G. (2021). Microplastics in the Weddell Sea (Antarctica): A Forensic Approach for Discrimination between Environmental and Vessel-Induced Microplastics. *Environmental science & technology*, *55*(23), 15900–15911. DOI:10.1021/acs.est.1c05207

Lenth, R. V. 2021. emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.5.5-1. https://cran.r-project.org/web/packages/emmeans/index.html

Li, Y., Liu, X., Shinde, S., Wang, J., and Zhang, P. (2021). Impacts of Micro- and Nanoplastics on Photosynthesis Activities of Photoautotrophs: A Mini-Review. *Front Microbiol.* DOI:10.3389/fmicb.2021.773226

Li, Z., Li, Q., Li, R., Zhao, Y., Geng, J., & Wang, G. (2020). Physiological responses of lettuce (Lactuca sativa L.) to microplastic pollution. *Environmental Science and Pollution Research*, *27*(24), 30306–30314. DOI:10.1007/s11356-020-09349-0

Liao, Y. C., Nazygul, J., Li, M., Wang, X. L., & Jiang, L. J. (2019). *Huan jing ke xue= Huanjing kexue*, *40*(10), 4661–4667. DOI:10.13227/j.hjkx.201903113

Lian, J., Liu, W., Meng, L., Wu, J., Chao, L., Zeb, A., & Sun, Y. (2021). Foliar-applied polystyrene nanoplastics (psnps) reduce the growth and nutritional quality of lettuce (Lactuca sativa L.). *Environmental Pollution*, *280*, 116978. DOI:10.1016/j.envpol.2021.116978

Lian, J., Wu, J., Xiong, H., Zeb, A., Yang, T., Su, X., Su, L., & Liu, W. (2020). Impact of polystyrene nanoplastics (psnps) on seed germination and seedling growth of wheat (triticum aestivum L.). *Journal of Hazardous Materials, 385*, 121620. DOI:10.1016/j.jhazmat.2019.121620

Liu, H., Yue, L., Zhao, Y., Li, J., Fu, Y., Deng, H., Feng, D., Li, Q., Yu, H., Zhang, Y., & Ge, G. (2022) Changes in bacterial community structures in soil caused by migration and aging of microplastics. *Science of The Total Environment*, 848, 157790. DOI:10.1016/j.scitotenv.2022.157790

Liu, R., Liang, J., Yang, Y., Jiang, H., & Tian, X. (2023). Effect of polylactic acid microplastics on soil properties, soil microbials and plant growth. *Chemosphere, 329*, 138504. DOI:10.1016/j.chemosphere.2023.138504

Liu, Y., Liu, S., Wan, S., Wang, J., Luan, J., & Wang, H. (2016). Differential responses of soil respiration to soil warming and experimental throughfall reduction in a transitional oak forest in central China. *Agricultural & Forest Meteorology, 226*, 186–198. DOI:10.1016/j.agrformet.2016.06.003

Lozano, Y. M., Aguilar, T. C. A., Onandia, G., Maaß, S., Zhao, T., Rillig, M. C., & Macinnis, N. C. (2021). Effects of microplastics and drought on soil ecosystem functions and multifunctionality. Journal of Applied Ecology, 58(5), 988–996. DOI:10.1111/1365-2664.13839

Ma, J., Wu, Y., Xu, C.-L., Luo, Z., Yu, R., Hu, G., & Yan, Y. (2023). Inhibitory effect of polyethylene microplastics on roxarsone degradation in soils. *Journal of Hazardous Materials, 454*, 131483. DOI:10.1016/j.jhazmat.2023.131483

Mariano S, Tacconi S, Fidaleo M, Rossi M, Dini L. (2021). Micro and nanoplastics identification: classic methods and innovative detection techniques. *Front Toxicol, 3*(2), 636640. DOI:10.3389/ftox.2021.636640

Medynska-Juraszek, A., & Jadhav, B. (2022). Influence of Different Microplastic Forms on pH and Mobility of Cu2+ and Pb2+ in Soil. *Molecules, 27*, 1744. DOI:10.3390/molecules27051744

Meng, F., Yang, X., Riksen, M., Xu, M., & Geissen, V. (2021). Response of common bean (phaseolus vulgaris L.) growth to soil contaminated with microplastics. *Science of The Total Environment, 755*, 142516. DOI:10.1016/j.scitotenv.2020.142516

Mori, M., Amato, M., Di Mola, I., Caputo, R., Quaglietta Chiaranda, ` F., & Di Tommaso, T.2008. Productive behavior of “cherry”-type tomato irrigated with saline water in relation to nitrogen fertilisation. *Eur. J. Agron. 29*, 135–143. DOI:10.1016/j.eja.2008.05.001

Nannipieri, P., & Eldor, P. (2009). The chemical and functional characterization of soil N and its biotic components. *Soil Biology & Biochemistry, 41*(12), 2357–2369. DOI:10.1016/j.soilbio.2009.07.013

Nelson, D. W., & Sommers, L. E. (1996). Total carbon, organic carbon, and organic matter. In D. L. Sparks (Ed.), Methods of soil analysis. Part 3. Chemical methods, SSSA book ser. 5 (pp. 961–1010). SSSA.

Ng, E.-L., Huerta Lwanga, E., Eldridge, S. M., Johnston, P., Hu, H.-W., Geissen, V., & Chen, D. (2018). An overview of microplastic and nanoplastic pollution in Agroecosystems. *Science of The Total Environment*, *627*, 1377–1388. DOI:10.1016/j.scitotenv.2018.01.341

Nizzetto, L., Futter, M., & Langaas, S. (2016). Are agricultural soils dumps for microplastics of urban origin? *Environmental Science & Technology*, *50*(20), 10777–10779. DOI:10.1021/acs.est.6b04140

Pignattelli, S., Broccoli, A., & Renzi, M. (2020). Physiological responses of garden cress (L. sativum) to different types of microplastics. *Science of the Total Environment*, *727*, 138609. DOI:10.1016/j.scitotenv.2020.138609

Polechońska, L., Rozman, U., Sokołowska, K., & Kalčíková, G. (2023). The bioadhesion and effects of microplastics and natural particles on growth, cell viability, physiology, and elemental content of an aquatic macrophyte elodea canadensis. *Science of The Total Environment*, *902*, 166023. DOI:10.1016/j.scitotenv.2023.166023

Qi, Y., Yang, X., Pelaez, A. M., Huerta Lwanga, E., Beriot, N., Gertsen, H., Garbeva, P., & Geissen, V. (2018). Macro- and micro- plastics in soil-plant system: Effects of plastic mulch film residues on wheat (triticum aestivum) growth. *Science of The Total Environment*, *645*, 1048–1056. DOI:10.1016/j.scitotenv.2018.07.229

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing. https://www.R-project.org/

Ren, X., Tang, J., Wang, L., & Liu, Q. (2021). Microplastics in soil-plant system: effects of nano/microplastics on plant photosynthesis, rhizosphere microbes and soil properties in soil with different residues. *Plant & Soil*, *462*(1/2), 561–576. DOI:10.1007/s11104-021-04869-1

Rillig, M. C., Leifheit, E., & Lehmann, J. (2021). Microplastic effects on carbon cycling processes in soils. *PLoS Biology*, *19*(3), 1–9. DOI:10.1371/journal.pbio.3001130

Rong, L., Zhao, L., Zhao, L., Cheng, Z., Yao, Y., Yuan, C., Wang, L., & Sun, H. (2021). LDPE microplastics affect soil microbial communities and nitrogen cycling. *Science of the Total Environment*, *773*, 145640. DOI:10.1016/j.scitotenv.2021.145640

Ruhland, C. T., & Krna, M. A. (2010). Effects of salinity and temperature on Deschampsia antarctica. *Polar Biology*, *33*(7), 1007–1012. DOI:10.1007/s00300-010-0764-5

Saboor, F. H., Hadian-Ghazvini, S., & Torkashvand, M. (2022). Microplastics in Aquatic Environments: Recent Advances in Separation Techniques. *Periodica Polytechnica: Chemical Engineering*, *66*(2), 167–181. DOI:10.3311/PPch.18930

Solvita Test Color. (2019). SOP 2019 Rev. 1. DCR Models 701.2 + https://solvita.com/product/solvita-soil-co2-burst-manual/

Song, B., Shang, S., Cai, F. M., Liu, Z., Fang, J., li, N., Adams, J. M., & Razavi, B. S. (2023). Microbial resistance in rhizosphere hotspots under biodegradable and conventional microplastic amendment: Community and functional sensitivity. Soil Biology & Biochemistry, 180, 108989. DOI:10.1016/j.soilbio.2023.108989

Sun, Y., Duan, C., Cao, N., Ding, C., Huang, Y., & Wang, J. (2022). Biodegradable and conventional microplastics exhibit distinct microbiome, functionality, and metabolome changes in soil. *Journal of Hazardous Materials*, *424*, 127282. DOI:10.1016/j.jhazmat.2021.127282

Shen Yuan, Goron, T. L., Liying Huang, Lilian Wu, & Fei Wang. (2017). Rice Leaf Lateral Asymmetry in the Relationship between SPAD and Area-Based Nitrogen Concentration. *Symmetry (20738994)*, *9*(6), 83. DOI:10.3390/sym9060083

Shi, J., Wang, J., Lv, J., Wang, Z., Peng, Y., & Wang, X. (2022). Microplastic presence significantly alters soil nitrogen transformation and decreases nitrogen bioavailability under contrasting temperatures. *Journal of Environmental Management*, *317*, 115473. DOI:10.1016/j.jenvman.2022.115473

Shi, R., Liu, W., Lian, Y., Wang, Q., Zeb, A., & Tang, J. (2022b). Phytotoxicity of polystyrene, polyethylene and polypropylene microplastics on tomato (Lycopersicon esculentum L.). *Journal of Environmental Management*, 317115441. DOI:10.1016/j.jenvman.2022.115441

Shi, R., Liu, W., Lian, Y., Zeb, A., & Wang, Q. (2023). Type-dependent effects of microplastics on tomato (Lycopersicon esculentum L.): Focus on root exudates and metabolic reprogramming. *Science of the Total Environment*, *858*, 160025. DOI:10.1016/j.scitotenv.2022.160025

Singh, B., & Singh, K. (2022). Microplastics contamination in soil affects growth and root nodulation of fenugreek (trigonella foenum-Graecum L.) and 16 S rrna sequencing of Rhizosphere soil. *Journal of Hazardous Materials Advances*, *7*, 100146. DOI:10.1016/j.hazadv.2022.100146

Teng, L., Zhu, Y., Li, H., Song, X., & Shi, L. (2022). The phytotoxicity of microplastics to the photosynthetic performance and transcriptome profiling of Nicotiana tabacum seedlings. *Ecotoxicology and Environmental Safety*, *231*, 113155. DOI:10.1016/j.ecoenv.2021.113155

Waldman, W. R., and Rillig, M. C. (2020). Microplastic Research Should Embrace the Complexity of Secondary Particles. *Environ. Sci. Technol. 54*(13), 7751–7753. DOI:10.1021/acs.est.0c02194

Wang, B., Wang, P., Zhao, s., Shi, H., Zhu, Y., Teng, Y., Jiang, G., & Liu, S. (2023b). Combined effects of microplastics and cadmium on the soil-plant system: Phytotoxicity, Cd accumulation and microbial activity. *Environmental Pollution* 333, 121960. DOI:10.1016/j.envpol.2023.121960

Wang, F., Zhang, X., Zhang, S., Zhang, S., & Sun, Y. (2020). Interactions of microplastics and cadmium on plant growth and arbuscular mycorrhizal fungal communities in an agricultural soil. *Chemosphere*, *254*, 126791. DOI:10.1016/j.chemosphere.2020.126791

Wellburn, A. R. (1995). The Spectral Determination of Chlorophylls a and b, as well as Total Carotenoids, Using Various Solvents with Spectrophotometers of Different Resolution. *Journal of Plant Physiology*, *144*(3), 307-313. DOI:10.1016/S0176-1617(11)81192-2

Wu, P., Wu, C., & Zhou, B. (2017). Drought stress induces flowering and enhances carbohydrate accumulation in Averrhoa carambola. *Horticultural Plant Journal*, *3*(2), 60–66. DOI:10.1016/j.hpj.2017.07.008

Wu, X., Liu, Y., Yin, S., Xiao, K., Xiong, Q., Bian, S., Liang, S., Hou, H., Hu, J., & Yang, J. (2020). Metabolomics revealing the response of rice (Oryza sativa L.) exposed to polystyrene microplastics. *Environmental Pollution*, *266*, 115159. DOI:10.1016/j.envpol.2020.115159

Wu, Y., Guo, P., Zhang, X., Zhang, Y., Xie, S., & Deng, J. (2019). Effect of microplastics exposure on the photosynthesis system of freshwater algae. *Journal of Hazardous Materials*, *374*, 219–227. DOI:10.1016/j.jhazmat.2019.04.039

Xin, J. J., Shi, H. B., Li, X. Y., Liang, J. C., Liu, R. M., & Wang, Z. C. (2014). Effects of plastic film residue on growth and yield of maize. *J. Irrig. Drain*, *33*, 52-54.

Ya, H., Jiang, B., Xing, Y., Zhang, T., Lv, M., & Wang, X. (2021). Recent advances on ecological effects of microplastics on soil environment. *Science of the Total Environment*, *798*, 149338. DOI:10.1016/j.scitotenv.2021.149338

Yan, Y., Chen, Z., Zhu, F., Zhu, C., Wang, C., & Gu, C. (2020). Effect of polyvinyl chloride microplastics on bacterial community and nutrient status in two agricultural soils. *Bulletin of Environmental Contamination and Toxicology*, *107*(4), 602–609. DOI:10.1007/s00128-020-02900-2

Yang, W., Cheng, P., Adams, C. A., Zhang, S., Sun, Y., Yu, H., & Wang, F. (2021). Effects of microplastics on plant growth and arbuscular mycorrhizal fungal communities in a soil spiked with zno nanoparticles. *Soil Biology and Biochemistry*, *155*, 108179. DOI:10.1016/j.soilbio.2021.108179

You, J., Liu, X., Zhang, B., Xie, Z., Hou, Z., &Yang, Z. (2015). Seasonal Changes in Soil Acidity and Related Properties in Ginseng Artificial Bed Soils under a Plastic Shade. *J. Ginseng Res. 39*(1), 81–88. DOI:10.1016/j.jgr.2014.08.002

Yu, H., Zhang, X., Hu, J., Peng, J., & Qu, J. (2020). Ecotoxicity of polystyrene microplastics to submerged carnivorous Utricularia vulgaris plants in freshwater ecosystems. *Environmental Pollution*, *265*, 114830. DOI:10.1016/j.envpol.2020.114830

Yuan, W., Zhou, Y., Liu, X., & Wang, J. (2019). New perspective on the nanoplastics disrupting the reproduction of an endangered fern in artificial freshwater. *Environmental Science & Technology*, *53*(21), 12715-12724. DOI:10.1021/acs.est.9b02882

Zabowski, D., & Sletten, R. S. (1991). Carbon dioxide degassing effects on the ph of Spodosol Soil Solutions. *Soil Science Society of America Journal*, *55*(5), 1456–1461. DOI:10.2136/sssaj1991.03615995005500050043x

Zhang, G.S., Liu, Y.F., (2018). The distribution of microplastics in soil aggregate fractions in southwestern China. *Sci. Total Environ. 642*, 12–20. DOI:10.1016/j.scitotenv.2018.06.004

Zhang, Y., Tian, X., Huang, P., Yu, X., Xiang, Q., Zhang, L., Gao, X., Chen, Q., & Gu, Y. (2023). Biochemical and transcriptomic responses of buckwheat to polyethylene microplastics. *Science of the Total Environment, 899*, 165587. DOI:10.1016/j.scitotenv.2023.165587

Zhang, Y., Cai, C., Gu, Y., Shi, Y., & Gao, X. (2022). Microplastics in plant-soil ecosystems: A meta-analysis. *Environmental Pollution*, *308*, 119718. DOI:10.1016/j.envpol.2022.119718

Zhang, Z., Wang, W., Liu, J., & Wu, H. (2024). Discrepant responses of bacterial community and enzyme activities to conventional and biodegradable microplastics in paddy soil. *Science of the Total Environment*, *909*, 168513. DOI:10.1016/j.scitotenv.2023.168513

Zhao, T., Lozano, Y. M., & Rillig, M. C. (2021). Microplastics increase soil ph and decrease microbial activities as a function of microplastic shape, polymer type, and exposure time. *Frontiers in Environmental Science*, *9*. DOI:10.3389/fenvs.2021.675803

Zhao, T., Tan, L., Huang, W., & Wang, J. (2019). The interactions between micro polyvinyl chloride (mpvc) and marine dinoflagellate Karenia Mikimotoi: The inhibition of growth, chlorophyll and photosynthetic efficiency. *Environmental Pollution*, *247*, 883–889. DOI:10.1016/j.envpol.2019.01.114

Zhou, J., Gui, H., Banfield, C. C., Wen, Y., Zang, H., Dippold, M. A., ... & Jones, D. L. (2021). The microplastisphere: biodegradable microplastics addition alters soil microbial community structure and function. *Soil Biology and Biochemistry*, *156*, 108211. DOI:10.1016/j.soilbio.2021.108211

Zhou, W. (2024). Responses of lettuce (Lactuca sativa L.) growth and soil properties to conventional non-biodegradable and new biodegradable microplastics. *Environmental pollution (Barking, Essex: 1987)*, *341*, 122897. DOI:10.1016/j.envpol.2023.122897

Table 1: Overview of differing combinations of microplastic sizes, concentrations, and types used in this experiment (n = 5).

Size	Concentration	Type	Replicate
0.1mm – 1mm	1%	PE	5
		PP	5
		PE + PP	5
	5%	PE	5
		PP	5
		PE + PP	5
2.5mm - 3mm	1%	PE	5
		PP	5
		PE + PP	5
	5%	PE	5
		PP	5
		PE + PP	5
—	—	Control	5

Abbreviations: PE, polyethylene; PP, polypropylene; and PE+PP, the mixture of both PE and PP

Table 2: Background physical, chemical, and biological characteristics of the soil used in this experiment (n = 5; mean ± standard error).

Soil properties	Value
Sand (%)	8.3 ± 0.61
Silt (%)	77.6 ± 0.51
Clay (%)	14.0 ± 0.18
Texture	Silt loam
Gravimetric Moisture Content (GMC, g g^{-1})	0.36 ± 0.02
Bulk Density (g cm^{3})	1.01 ± 0.05
Total Porosity (%)	62.0 ± 2.24
Water Field Pore Spaces (WFPS, %)	57.8 ± 3.97
Maximum Water Holding Capacity (MWHC, g g^{-1})	0.67 ± 0.04
pH	7.1 ± 0.04
Electrical Conductivity (EC, mmho cm^{-1})	0.17 ± 0.01
Cation Exchange Capacity (CEC, me $100g^{-1}$)	27.0 ± 0.39
Soil Organic Matter (SOM, %)	7.3 ± 0.02
Total Carbon (TC, g kg^{-1})	37.2 ± 0.07
Total Nitrogen (TN, g kg^{-1})	3.1 ± 0.002
C:N	12.1 ± 0.02
Ammoniacal Nitrogen (NH_4^+–N, mg kg^{-1})	5.6 ± 0.11
Nitrate Nitrogen (NO_3^-–N, mg kg^{-1})	9.9 ± 0.14
Mehlich P-III (P, mg kg^{-1})	84.6 ± 0.93
Potassium (K, mg kg^{-1})	334.2 ± 7.9
Calcium (Ca, mg kg^{-1})	3903.2 ± 51.1
Magnesium (Mg, mg kg^{-1})	792.4 ± 13.9
Sulfate-S (SO4-S, mg kg^{-1})	12.3 ± 0.28
Zinc (Zn, mg kg^{-1})	4.09 ± 0.04
Iron (Fe, mg kg^{-1})	62.1 ± 0.45
Manganese (Mn, mg kg^{-1})	18.2 ± 0.13
Copper (Cu, mg kg^{-1})	2.30 ± 0.02
Sodium (Na, mg kg^{-1})	13.4 ± 0.68

Table 3: Summary of three-way ANOVA on the effect of adding different microplastic size, concentration, and types and their interactions to measured soil variables.

ANOVA Factor	Df	pH		Total IN		Respiration	
		F Value	P Value	F Value	P Value	F Value	P Value
Type (T)	2	17.4	**<0.001**	40.9	**<0.001**	4.26	**<0.01**
Size (S)	1	1.10	NS	0.01	NS	1.01	NS
Concentration (C)	1	26.7	**<0.001**	4.50	**<0.05**	0.06	NS
T × S	2	4.01	**<0.05**	2.28	NS	0.06	NS
T × C	2	1.50	NS	1.68	NS	0.18	NS
S × C	1	0.003	NS	1.99	NS	0.04	NS
T × S × C	2	0.68	NS	0.54	NS	0.09	NS

*The significance for all analyses was set at an alpha (α) of 0.05 (significant values are shown in bold).

Abbreviations: Df, the degree of freedom; IN, inorganic nitrogen; NH_4^+–N, ammoniacal nitrogen; NO_3^-–N, nitrate nitrogen.

Table 4: Summary of four-way ANOVA on the effect of adding different microplastic size, concentration, and types and their interactions to measured plant variables.

ANOVA Factor	Plant height		Leaf count		SPADMETER		Light adapted		Dark adapted	
	F Value	P Value	F Value	P Value	F Value	P Value	F Value	P Value	F Value	P Value
Type (T)	10.3	**<0.001**	23.4	**<0.001**	1.56	NS	1.43	NS	2.21	NS
Size (S)	59.4	**<0.001**	276	**<0.001**	2.08	NS	1.21	NS	2.10	NS
Concentration (C)	13.7	**<0.001**	37.5	**<0.001**	4.15	**<0.05**	4.52	**<0.05**	1.38	NS
Time (t)	1672.01	**<0.001**	1590	**<0.001**	185	**<0.001**	36.9	**<0.001**	3.76	**<0.01**
T × S	17.6	**<0.001**	6.20	**<0.01**	0.03	NS	0.12	NS	0.29	NS
T × C	3.57	**<0.05**	24.3	**<0.001**	1.91	NS	0.12	NS	0.53	NS
S × C	43.6	**<0.001**	63.8	**<0.001**	9.15	**<0.01**	0.0001	NS	3.03	NS
T × t	1.32	NS	0.81	NS	1.79	**<0.01**	1.76	**<0.01**	1.35	NS
S × t	8.77	**<0.001**	4.27	**<0.001**	3.20	**<0.001**	1.32	NS	2.10	NS
C × t	1.30	NS	1.25	NS	2.53	**<0.01**	1.43	NS	1.88	NS
T × S × C	27.4	**<0.001**	14.1	**<0.001**	0.11	NS	0.75	NS	3.27	**<0.05**
T × S × t	1.87	**<0.01**	0.65	NS	1.49	NS	0.89	NS	1.81	NS
T × C × t	0.65	NS	1.38	NS	2.16	**<0.001**	0.91	NS	1.32	NS
S × C × t	4.64	**<0.001**	0.33	NS	1.25	NS	1.07	NS	2.43	**<0.05**
T × S × C × t	0.42	NS	0.53	NS	1.66	**<0.05**	1.03	NS	1.77	NS

*The significance for all analyses was set at an alpha (α) of 0.05 (significant values shown in bold).

Table 5: Summary of three-way ANOVA on the effect of adding different microplastic size, concentration, and types and their interactions to measured plant variables.

ANOVA Factor	Df	Shoot biomass		Fruit Biomass		Fruit count	
		F Value	P Value	F Value	P Value	F Value	P Value
Type (T)	2	2.01	NS	3.86	**<0.05**	0.84	NS
Size (S)	1	2.94	NS	14.3	**<0.001**	7.43	**<0.01**
Concentration (C)	1	6.24	**<0.05**	0.33	NS	0.04	NS
T × S	2	0.67	NS	0.19	NS	0.05	NS
T × C	2	0.09	NS	2.56	NS	1.70	NS
S × C	1	1.99	NS	0.97	NS	0.01	NS
T × S × C	2	0.80	NS	0.09	NS	0.30	NS

*The significance for all analyses was set at an alpha (α) of 0.05 (significant values are shown in bold).

Abbreviations: Df, the degree of freedom.

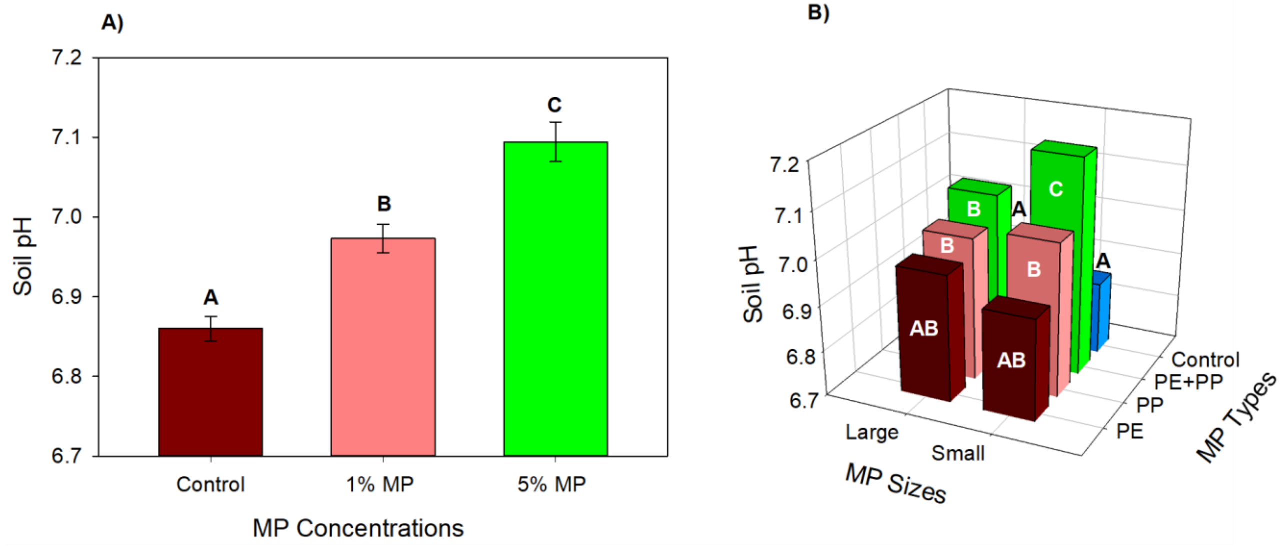

Fig. 1. (**A**) The effects of microplastic (MP) concentrations (1% and 5%) on soil pH. Means and standard error bars are shown. Significance between MP concentrations are indicated by different letters ($p < 0.05$). (**B**) The interaction effects of MP types (PE: polyethylene, PP: polypropylene, and PE+PP: the mixture of both PE and PP) and sizes (small: 0.1-1.0 mm and large: 2.0-3.5 mm) on soil pH. The significant ($p < 0.05$) difference among MP types within each MP size indicated with different letters.

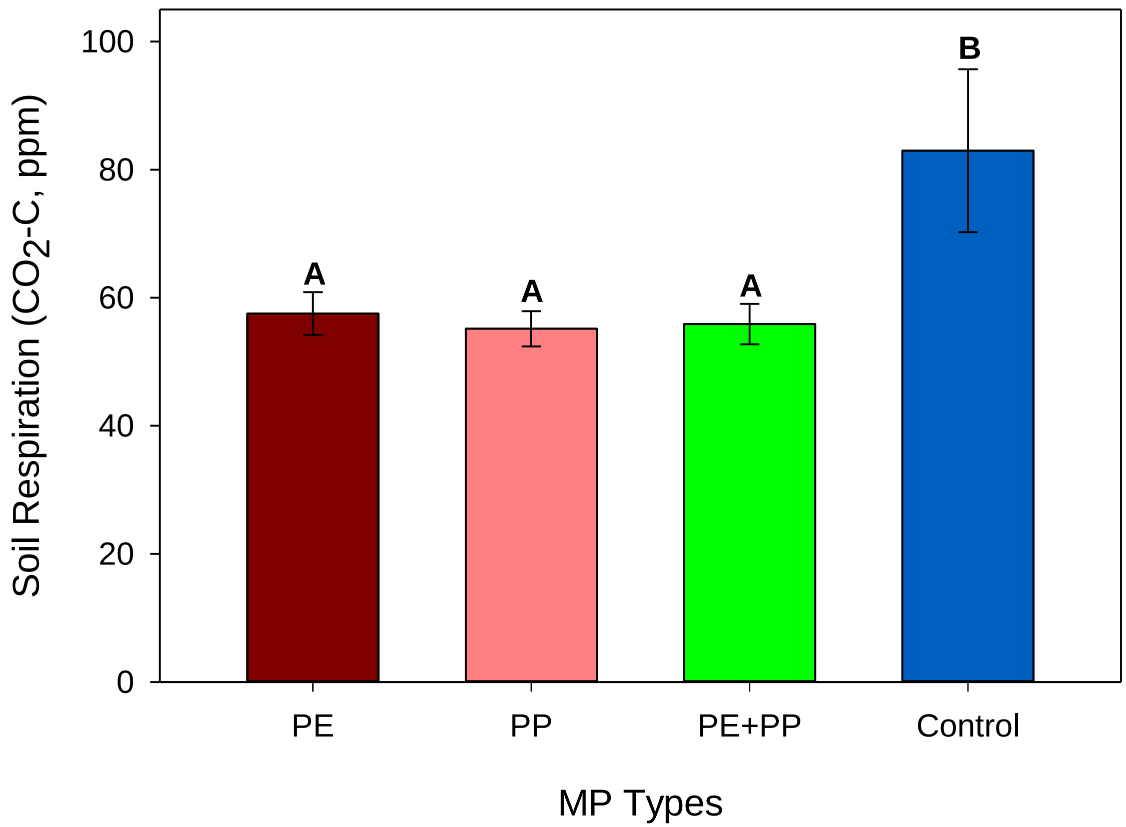

Fig. 2. The effects of MP types on soil respiration. Means and standard error bars are shown. Significance between MP types are indicated by different letters ($p < 0.05$).

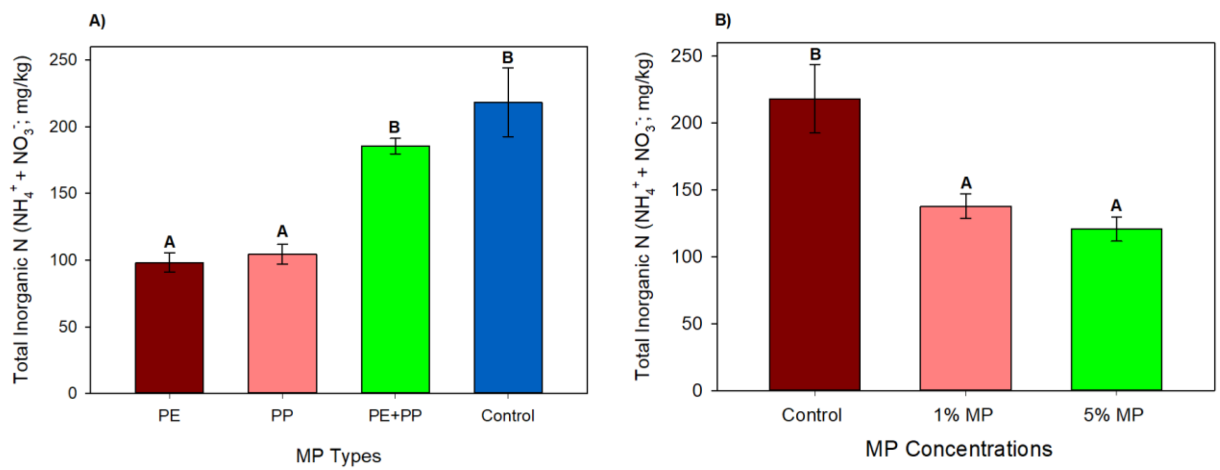

Fig. 3. The effects of microplastic (MP) (**A**) types (PE: polyethylene, PP: polypropylene, and PE+PP: the mixture of both PE and PP) and (**B**) concentrations (1% and 5%) on inorganic nitrogen (N). The significant difference ($p < 0.05$) among MP types and concentrations are indicated by different letters.

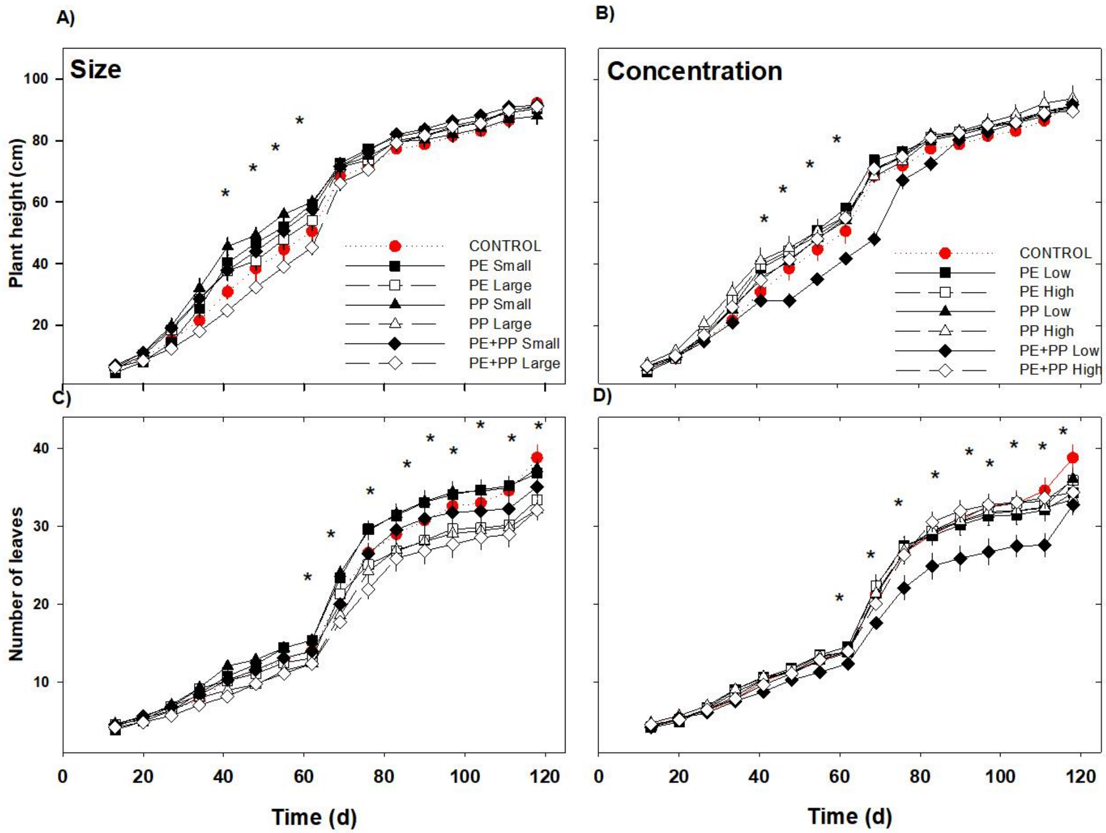

Fig. 4. (**A** & **B**) Showing the effects of microplastic (MP) types (PE: polyethylene, PP: polypropylene, and PE+PP: the mixture of both PE and PP), size (0.1-1mm and 2.0– 3.5mm) and concentrations (1% and 5%) on Plant height for 118 days. The significant difference ($p < 0.05$) among MP types within each MP size and concentration indicated with *. (**C** & **D**) Showing the effects of microplastic (MP) types (PE: polyethylene, PP: polypropylene, and PE+PP: the mixture of both PE and PP), size (0.1-1mm and 2.0– 3.5mm) and concentrations (1% and 5%) on Number of leaves. The significant difference ($p < 0.05$) among MP types within each MP size concentration indicated with *. Means and standard error bars are shown (n = 5)

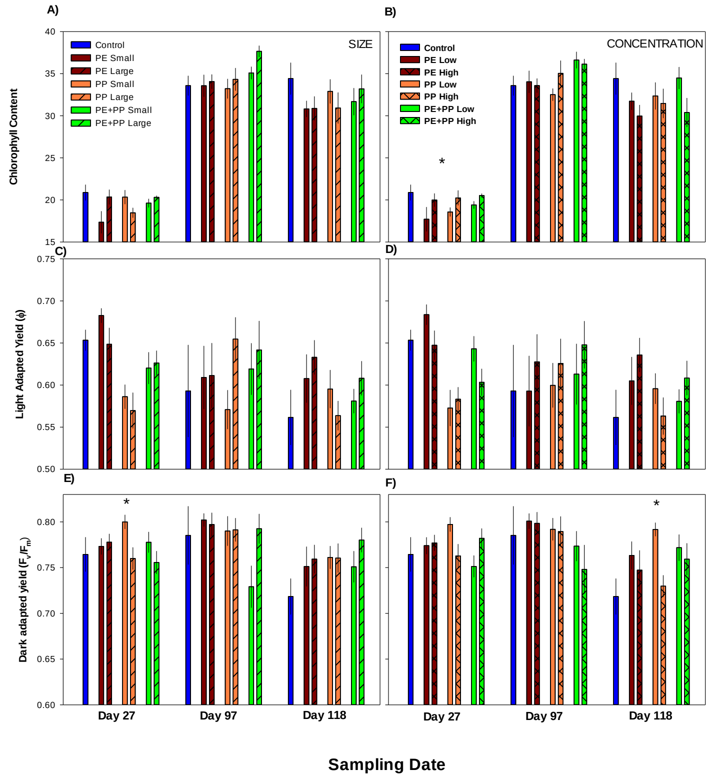

Fig. 5. (**A** & **B**) Showing the effects of microplastic (MP) types (PE: polyethylene, PP: polypropylene, and PE+PP: the mixture of both PE and PP), size (0.1-1mm and 2.0– 3.5mm) and concentrations (1% and 5%) on Chlorophyll content (SPAD Meter). The significant difference ($p < 0.05$) among MP types within each MP size and concentration indicated with *. (**C** & **D**) Showing the effects of microplastic (MP) types (PE: polyethylene, PP: polypropylene, and PE+PP: the mixture of both PE and PP), size (0.1-1mm and 2.0– 3.5mm) and concentrations (1% and 5%) on Light Adapted yield. The significant difference ($p < 0.05$) among MP types within each MP size and concentration indicated with *. (**E** & **F**) Showing the effects of microplastic (MP) types (PE: polyethylene,

PP: polypropylene, and PE+PP: the mixture of both PE and PP), size (0.1-1mm and 2.0– 3.5mm) and concentrations (1% and 5%) on Dark Adapted yield. The significant difference (*p* < *0.05*) among MP types within each MP size and concentration indicated with *. Means and standard error bars are shown (n = 5)

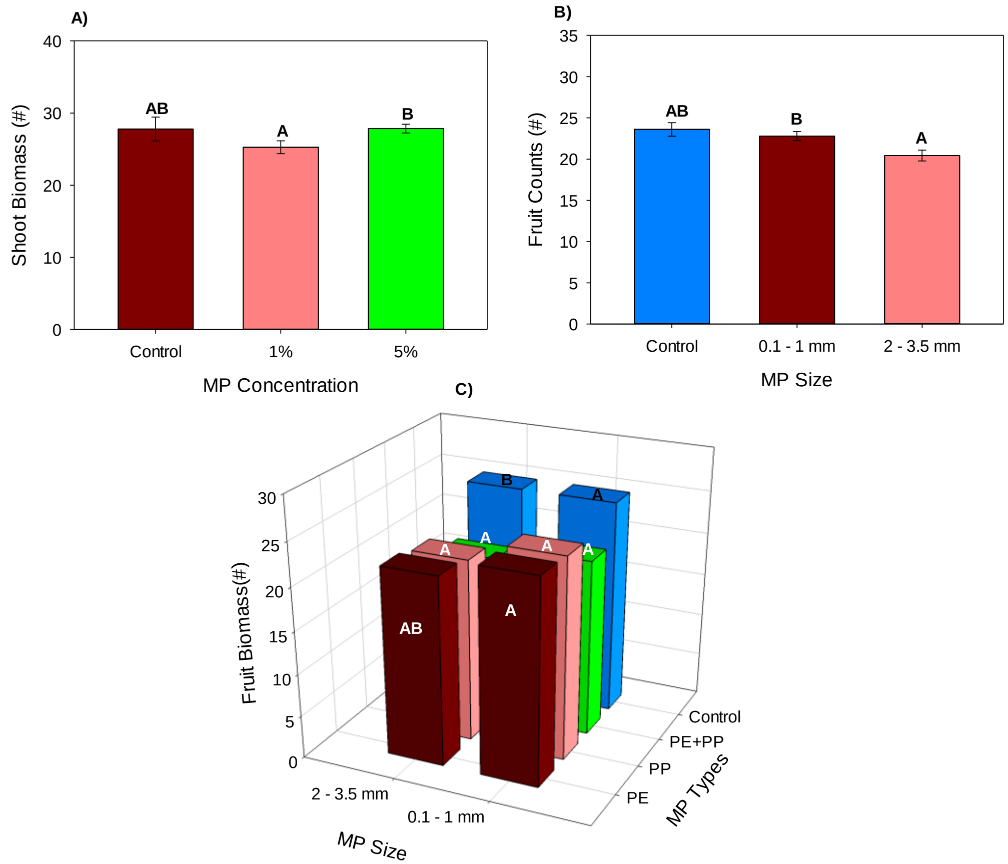

Fig 6. (**A**) The effect of microplastic (MP) concentrations (1% and 5%) on shoot biomass. Means and standard error bars are shown. Significance ($p < 0.05$) between MP concentration indicated by different letters. (**B**) The effects of MP sizes (small: 0.1-1.0 mm and large: 2-3.5 mm) on fruit counts. Means and standard error bars are shown. Significance ($p < 0.05$) between MP sizes indicated by different letters. (**C**) The effect of MP types (PE: polyethylene, PP: polypropylene, and PE+PP: the mixture of both PE and PP) and sizes on fruit biomass. The significant difference ($p < 0.05$) among MP types within each MP size indicated with different letters.