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Growing Concerns: The Interactive Effects of Soil Copper and Microplastics on Soybeans

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Abstract

Plastic is a pervasive and persistent environmental pollutant. Widespread use of plastics, such as polyethylene terephthalate (PET), exacerbate this issue. PET plastics can degrade in the environment and generate microplastics, which can then interact with other substances, including adsorption of metal ions. Copper is a metal that is often applied as a fungicide to agricultural fields, and repeated application leads to its accumulation in soil to a level that inhibits plant growth. I investigated the growth of soybean (*Glycine max*) in the presence of PET microplastics and copper ($CuSO_4$). I predicted that increased copper in soil has phytotoxic effects, which would be offset by the presence of soil microplastics. Soybeans grown in pots of soil were treated with 250 mg/kg copper and/or 10 g/kg microplastics (w:w dry soil). Bioavailable soil copper; leaf and root copper concentration; and leaf, stem, and total biomass did not vary among treatments. This was unexpected but is explainable as only ~1.2 % of total copper in the control soil was bioavailable. The low copper bioavailability was attributed to the high concentration of soil organic matter (~40 %) to which copper ions were likely bound. Regardless, there was evidence that microplastics interact with copper: F_v/F_m in the combined treatment was reduced by ~2.0 % relative to the control, and soybean root biomass in the combined treatment was ~35 % greater relative to the control and copper treatments. These effects could be related to the influence of microplastics on soil physiochemical properties.

Introduction

The annual rate of plastic production in 2015 was 380 Mt, with ~60 % of all plastics ever produced ending up in either landfills or the environment (Geyer et al. 2017). In response to the large-scale production and disposal of plastics, studies on plastics—particularly microplastics—have increased in recent years (Xiang et al. 2022). Plastics between 0.3–5 mm in size are classified as microplastics, which exist in both the terrestrial and aquatic environment. There are less studies on terrestrial relative to aquatic microplastics, as it is more difficult to isolate microplastics from soils due to the binding of microplastics to soil particles (Chia et al. 2022). Microplastics have been found to accumulate in agricultural soils (Liu et al. 2018; Zhang et al. 2018), floodplains (Scheurer and Bigalke, 2018), and around waste sites (Chai et al. 2020). In these environments, microplastics can affect soil properties such as bulk density, water holding capacity, microbial activity (Machado et al. 2018), and pH (Medynska-Juraszek and Jadhav, 2022). The soil environment presents a complex matrix of factors that can influence the magnitude and direction of these microplastic induced effects (Yu et al. 2021). This means that further investigations should be performed on terrestrial microplastics due to their interactions within the complex physiochemical soil environment.

Polyethylene terephthalate (PET), which is a common component of plastic beverage bottles, degrades into PET microplastics (Jaiswal et al. 2022). The number of studies on the terrestrial effects of PET microplastics have increased. Chen et al. (2022) reported that rice (*Oryza sativa*) grown in the presence of 0.5 % PET microplastics had ~30 % increased grain yields relative to the control. Lozano and Rillig (2022) reported that wild carrot (*Daucus carota*) grown in the presence of 0.4 % PET microplastic fragments had ~39 % increased root tissue density relative to the control. Machado et al. (2019) reported that spring onion (*Allium fistulosum*) grown in the presence of 2.0 % PET microplastic fragments had no significant change in root tissue density nor biomass. Generally, most microplastics are too large to be taken up into plants, with only nanoplastics and very small microplastics being able to accumulate in tissues (Mateos-Cardenas et al. 2021). Thus, the effects that PET microplastics have on plants are likely related to influences on soil physiochemical properties (Machado et al. 2019) instead of plastic accumulation in the tissues (Mateos-Cardenas et al. 2021). These studies demonstrate that more research must be performed to elucidate the effects of PET microplastics on both soils and plants.

One interaction that has been studied more in the aquatic environment, but is relevant to the terrestrial environment, is adsorption between microplastics and metal ions. Plastics in the aquatic environment have been demonstrated to adsorb metal ions, with plastics used in aquaculture (Mohsen et al. 2022) and beached plastic litter accumulating metals such as iron, lead, aluminum, and copper (Ashton et al. 2010; Turner 2017). The degree of adsorption depends on factors such as the presence of other metals (Turner 2017) and microplastic size (Han et al. 2021), age (Huang et al. 2020), and type (Gao et al. 2019). These aquatic studies have investigated the role of microplastics as metal carriers, which might enhance bioaccumulation in the marine environment if ingested by fish (Foo et al. 2022).

In the context of the terrestrial environment, this adsorption interaction affects the bioavailability of metals in soil. Li et al. (2021) reported that 10 % polyethylene microplastics increased Pb^{2+} bioavailability by ~30 % relative to the control. However, there was no change in bioavailability at 0.1 % or 1.0 % microplastic (Li et al. 2021). Yu et al. (2021) reported that Pb^{2+}, Zn^{2+}, and Cu^{2+} bioavailability decreased in the presence of 28 % polyethylene microplastics in soil. The soil physiochemical environment is complex, and different factors such as soil texture, pH, and organic content can affect the strength of the correlation between microplastic addition and metal bioavailability (Yu et al. 2021). Thus, it is important to understand the effects of microplastics on metal bioavailability in different soils.

One metal of particular interest, copper, is the most bioavailable in its Cu^{2+} form, and its environmental concentrations have increased due to its applications in healthcare (Gollwitzer et al. 2018), industry (Qin et al. 2021), and agriculture (Lamichhane et al. 2018). In agriculture, copper is commonly used in fertilizers to help improve plant growth due to its role as a micronutrient. Sewage sludge amendments are added to soils due to the micronutrients that they contain, such as copper, which can help fertilize crops (Shen et al. 2018). However, they can also contribute both excess copper (Shen et al. 2018) and microplastics to soil (Corradini et al. 2019). Copper is also used as an organic fungicide, with its widespread use in areas such as vineyards (Ballabio et al. 2018). Repeated application of sewage sludge, fertilizers, and fungicides over time may lead to an accumulation of copper and microplastics in agricultural soils. Thus, copper is a relevant metal that can interact with microplastics and plants.

In copper-deficient conditions, some species of plants, such as chickpeas (*Cicer arietinum*), have decreased yield (Bhakuni et al. 2009). This is due to the use of copper in plant metabolism,

such as to produce metalloenzymes (Mydy et al. 2021). Conversely, copper excess can inhibit plant growth, as seen in tomato (*Lycopersicon esculentum*), and bok choy (*Brassica rapa*) (Li et al. 2013). This is caused by the copper-induced overproduction of reactive oxygen species (ROS), such as hydrogen peroxide (Cuypers et al. 2011). At lower concentrations of copper, the plant can maintain its internal balance of ROS (Cuypers et al. 2011), but at higher concentrations of copper, overaccumulation of ROS can damage the plant via lipid peroxidation (i.e., degradation) (Baryla et al. 2000). Plant photosynthetic parameters, such as F_v/F_m, have also been reported to decrease when the plant is grown in higher soil copper concentrations (Burzynski and Klobus, 2004). The toxicity of copper depends on the plant (Li et al. 2013), copper concentration (Kulikova et al. 2011), and physiochemical environment (Bernardi et al. 2022). Due to both copper and microplastics being pervasive and interacting soil contaminants (Medynska-Juraszek and Jadhav, 2022) that affect plants (Li et al. 2013; Machado et al. 2019), further research into their combined effects on plants is necessary.

Soybean (*Glycine max*) was used as the model organism for the study. This is due to its quick growth, large size (easing the harvest of biomass), and the availability of seeds in the lab. Soybeans have decreased total biomass at increasingly higher levels of copper (Kulikova et al. 2011), but their response to PET microplastics is unknown. Soybean is grown agriculturally, meaning that soybean crops might be exposed to excess copper and PET microplastics in soil due to sewage sludge amendments (Shen et al. 2018; Corradini et al. 2019). Implications include potential effects on crops grown among polluted agricultural soils. The response of soybean during this study can provide insight into how copper and PET microplastics affect the growth of other legumes such as chickpeas and lentils (*Lens culinaris*).

In this project, I will investigate the combined effect of copper and PET microplastics in soils on soybean growth. Due to the adsorption of copper to PET microplastics, I hypothesize that PET microplastics will reduce the bioavailability of copper in soil. I predict that soybeans grown in soil treated with an excess concentration of copper will take up less copper and grow larger if there are also microplastics in the soil.

Methods

Microplastic Preparation

Microplastics were created from ProPenn Marathon PET tennis ball containers (Head Penn Racquet Sports, Phoenix, AZ, USA) donated by the University of Western Ontario's Tennis Club. The procedure aimed to minimize airborne and instrumental contamination of microplastics according to recommendations by Dioses-Salinas et al. (2020) for experiments using microplastics. This is important as laboratories often contain numerous plastics. Metal and glass instruments, nitrile gloves, and polyester lab coats were used; the lab coat was cleaned with a lint roller before working with the plastics. Preparation of microplastics was performed in a fume hood. PET bottles were rinsed with reverse osmosis (RO) water to remove debris, then cut into squares using scissors before blending inside a glass blender. Ice was added during blending to prevent overheating. Plastics were then dried in an oven at 60 ^{o}C until dry (three hours) then sieved using a 4 mm metal sieve and placed in a glass beaker covered with aluminum foil. With this method, PET microplastic fragments below 4 mm were obtained for use in the experiment. Fragments, rather than uniform shapes, were chosen because other studies that investigate the action of PET microplastics shredded PET pellets into fragments (Machado et al. 2019). Additionally, microplastic quantification in soils report that fragments are among the largest proportions of microplastic shapes (Chai et al. 2020).

Soil Preparation

Garden topsoil from a local garden store was used for the experiment because of its low cost and accessibility. President's Choice Black Earth All Purpose Soil (Loblaws, London, ON, Canada) was purchased and sieved at 4 mm using a stainless-steel sieve to reduce the amount of non-soil items such as twigs and stones. After sieving, all the soil was thoroughly hand-mixed to reduce differences between individual soil bags. A 1.0 kg sample of fresh soil was collected after mixing for baseline soil physiochemical assessment. The soil was stored in sealed plastic containers at 4 ^{o}C to prevent the growth of mold and bacteria. Perlite was added to the soil at 10 % (v:v fresh soil) to reduce soil compaction (Matt, 2022). Since half of the soybeans would be grown among microplastics, PET microplastics were hand-mixed for five minutes into half the total volume of soil at 10 g/kg (w:w dry soil).

Many studies that quantify environmental microplastics report concentrations with items/kg (Liu et al. 2018; Li et al. 2022), which makes it difficult to determine an environmentally relevant concentration of microplastic by mass. The PET microplastic concentration for the current experiment was 10 g/kg (w:w dry soil), which was equivalent to 0.4 % (w:w fresh soil), and reflects the concentrations used by both Lozano and Rillig (2022), and Chen et al. (2022). It is relevant to note that 2.0 % (w:w fresh soil) PET microplastics used by Machado et al. (2019) did not influence the growth of their plants; however, this could be due to the size and shape of the microplastics. The present experiment used PET microplastics that were less than 4 mm compared to Machado et al.'s (2019) ~1 mm PET microplastics.

Each plastic pot was lined with window screen at the base to prevent soil from falling out and sat atop a plastic saucer. All pot components (i.e., pot, saucer, screen) were soaked in a 15 % bleach solution (v:v RO water) then washed with RO water to mitigate biological contamination. There were 28 soil pots in total, with 7 allocated to each treatment: 1) control, 2) PET microplastic at 10 g/kg (w:w dry soil) (microplastic treatment), 3) $CuSO_4$ at 250 mg/kg (w:w dry soil) (copper treatment), and 4) $CuSO_4$ (250 mg/kg) + PET microplastic (10 g/kg) (combined treatment). Depending on the treatment, each pot was filled with 750 g of microplastic-amended or control soil. For the copper-containing treatments, the soils were then spiked with $CuSO_4$ at 250 mg/kg (w:w dry soil). This treatment was determined from a prior dose response experiment using soybeans grown in soil pots treated with $CuSO_4$ at 0–300 mg/kg (w:w dry soil); 250 mg/kg was selected based on a sublethal concentration that decreased soybean biomass by at least 30 % relative to the control.

After the soils were spiked with copper and/or microplastic they were moistened with RO water, placed in a cold room at 4 ^{o}C, covered with a sheet of Plexiglas, then left for four weeks. This sitting time let the microplastics, copper, and soil interact; this included adequate diffusion of copper throughout the soil. After this period, pots were removed from the cold room then placed in the growth chamber for three days before planting soybeans to prevent the cold temperature from affecting the seedlings.

Soybean Growth

Soybean seeds were obtained from Environmental Sciences Western in 2017 (Western University, London, ON, Canada). Seeds were soaked in 0.1 % Vitaflo-280 fungicide (Chemtura Canada, Elmira, ON, Canada) for 12 hours then washed with RO water prior to germination in

glass Petri dishes on VWR Grade 415 (VWR International, West Chester, PA, USA) filter paper moistened with RO water. Each of the four treatments received 7 soybean seedlings of similar root length to control for the influence of seedling size on the results. Pots were randomly arranged into rows in a growth chamber set to 25 ^{o}C, 16: 8-hour day: night cycle, and 60 % relative humidity. Average irradiance of the growth chamber was measured (mean = 211.7 $\frac{\mu mol}{m^2 * s}$, SD = 12.4 $\frac{\mu mol}{m^2 * s}$, n = 6) using a Fieldscout Quantum Light Meter (Spectrum Technologies, Haltom City, TX, USA) ~60 cm below the growth chamber lights. After four days of germination, soybean seedlings were planted 5 cm below the soil surface in each pot, buried, then moistened with RO water. Soybeans were watered daily with RO water to maintain ~70 % soil field capacity. The soybeans were measured for photochemical efficiency (F_v/F_m) then harvested for biomass at 27 and 28 days of growth, respectively.

Soil Physiochemical Properties (texture, organic and moisture content, field capacity, pH)

Since physiochemical characteristics of the soil can influence the bioavailability of metals such as copper, and the growth of plants, the following variables were measured prior to experimentation: soil texture, organic content, moisture content, field capacity, and pH. Masses below 221.0 g were weighed using a Sartorius Laboratory Balance LP2205 (Sartorius, Edgewood, NY, USA), while masses above this threshold were weighed using a Starfrit Electronic Kitchen Scale 93016 (Starfrit, Longueuil, QC, Canada).

Soil texture was measured using a density separation procedure as described by Brower and Zar (1990). This method is based on differential sedimentation, with larger particles (e.g., sand) settling out of a suspension faster than smaller particles (e.g., clay) (Taubner et al. 2009). Laser diffraction analysis presents a faster and more systematic method of measuring soil texture (Taubner et al. 2009), but the equipment was not available, so density separation was used (Brower and Zar, 1990). Three 20 g samples of dried soil were individually blended with 200 mL of 1.0 % sodium pyrophosphate (10 g/L) to mitigate particle aggregation then poured into separate 200 mL glass graduated cylinders. The height of the first, second, and third layers to settle in the cylinders were measured using a ruler at the two-minute, two-hour, and twenty-four-hour marks, which gave the relative percentages of sand, silt, and clay.

Soil organic content was measured using a loss-on-ignition (LOI) protocol modified from Dean (1974). LOI has been criticized to slightly overestimate soil organic matter relative to other

organic matter determination methods, such as acid dichromate oxidation (Frogbrook and Oliver, 2001). This is due to the inadvertent burning of elemental carbon and soil carbonates at the high temperatures used during LOI (Ball, 1964). However, both LOI and acid dichromate oxidation are highly correlated in their results (Ball, 1964; Frogbook and Oliver, 2001). This means that LOI is still an accurate method for organic matter determination, made even more attractive due to its rapidness, low cost, and simplicity. Five 0.45 g samples of soil that had been oven-dried at 60 ^{o}C until constant weight were placed into ceramic crucibles, weighed (DW_{60}), then put in a muffle furnace for four hours at 550 ^{o}C to ignite organic matter. After ignition, dry weight was recorded (DW_{550}). DW_{60} and DW_{550} were used to calculate organic content as a percentage (Eq. 1).

$$LOI_{550} = \left(\frac{DW_{60} - DW_{550}}{DW_{60}}\right) \cdot 100$$

Equation 1 | Formula used to determine the organic content percentage (LOI_{550}) of soil samples using the weights of soil dried at 60 ^{o}C (DW_{60}) and 550 ^{o}C (DW_{60}). Equation modified from Dean (1974).

Initial soil moisture was measured by weighing five 10 g samples of fresh soil (FW; fresh weight), then reweighing the samples after oven-drying at 60 ^{o}C (DW; dry weight) (Eq. 2). This moisture (mean = 55.64 %, SD = 0.44 %, n = 5) was used to calculate the volume of $CuSO_4$ solution to achieve 250 mg/kg (w:w dry soil).

$$\text{Moisture Content} = \left(\frac{FW - DW}{FW}\right) \cdot 100$$

Equation 2 | Formula used to determine the initial moisture content of the soil using the weights of fresh soil (FW) and soil dried at 60 ^{o}C (DW).

Soil field capacity was measured by slowly adding RO water in 30 mL increments to four pots filled with 750 g of fresh soil until the soil saturated with water and dripped into the saucer below. The volume of water in the saucer was subtracted from the overall volume of water added to the respective pot. This measurement was used to calculate the amount of water needed to maintain ~70 % field capacity (mean = 621.38 mL/kg, SD = 5.85 mL/kg, n = 4), then rounded to 620 mL/kg for ease of use during soybean watering.

Soil pH was measured using a technique modified from Novozamsky et al. (1993) using $CaCl_2$. This protocol was chosen because, relative to using water as a measurement matrix,

$CaCl_2$ is less affected by soil electrolyte concentration and can provide more consistent measurements (Minasny et al. 2011). One consequence of using $CaCl_2$ versus water would be that plants grow in soil without added $CaCl_2$, so pH-$CaCl_2$ measurements might not be as relevant to the plant. Five 2 g samples of dried soil were placed into Falcon tubes, 20 mL of $CaCl_2$ (0.01 M) was added to each tube, lids were fastened, then tubes were shaken for three hours at 100 rpm using a VWR DS-500E Orbital Shaker (VWR International, Radnor, PA, USA). The tubes were then placed upright for 12 hours to allow the contents to vertically settle, after which a Thermoscientific Orion Star A211 pH Meter (Thermo Fisher Scientific, Beverly, MA, USA) was used to measure the pH-$CaCl_2$ of the supernatant.

Soil Copper Bioavailability Experiment

The effect of microplastic on soil metal bioavailability was assessed. Twenty plastic seedling containers were filled with 10 g of fresh soil that had undergone one of four treatments, with five replicate containers per treatment: 1) control, 2) PET microplastic at 10 g/kg (w:w dry soil), 3) $CuSO_4$ at 250 mg/kg (w:w dry soil), and 4) the combined treatment. This was performed separately (i.e., not taken directly from soil in which soybean would be grown) due to the difficulty of separating perlite from soil samples for inductively coupled plasma mass spectrometry (ICP-MS) analysis. The spiked soils were equilibrated for a four-week period at 4 ^{o}C to allow for interactions among the soil, microplastics, and copper to occur.

Bioavailable copper content in the soil was determined using a procedure described by Novozamsky et al. (1993). This procedure uses $CaCl_2$ as an extractant (Novozamsky et al. 1993) and is an accurate measure of the bioavailable metal content that is accessible to a plant (Zhang et al. 2010). Compared to the slightly more accurate NH_4Ac extractant (Zhang et al. 2010), $CaCl_2$ was chosen due to its standard use for experiments in Dr. Macfie's lab. Soil was oven-dried at 60 ^{o}C until constant weight. Dried samples from each container (~1.0 g) were placed into 50 mL plastic centrifuge tubes. 20 mL of $CaCl_2$ (0.01 M) was added to each tube, lids fastened, then shaken for three hours at 200 rpm using a VWR DS-500E Orbital Shaker (VWR International, Radnor, PA, USA). The contents were filtered through a VWR Grade 415 qualitative filter (VWR International, West Chester, PA, USA), then vacuum filtered into a glass Buchner funnel using a 0.45 μm membrane filter (MilliporeSigma, Burlington, MA, USA). The filtered solution was decanted into a clean 50 mL plastic centrifuge tube, with RO water being used to wash solution adsorbed to the interior of the funnel into the tubes. The filtered

supernatant was acidified using 0.4 mL of OmniTrace Ultra Nitric Acid (EMD Millipore Corporation, Billerica, MA, USA) to obtain 2.0 % acid (v:v RO water) in the final solution, brought to a final volume of 20 mL using RO water, then stored at 4 ^{o}C. Three blank samples that did not have soil underwent the same protocol to determine if any of the equipment, water, or solutions used for this extraction were contaminated by copper.

Soybean Photochemical Efficiency (F_v/F_m), Dry Biomass, and Internodal Distance

The day before harvest, an OS30p+ Chlorophyll Fluorometer (Opti-Sciences, Hudson, NH, USA) was used to measure photochemical efficiency using F_v/F_m, a photosynthetic parameter described in Maxwell and Johnson (2000). F_v/F_m is a measurement of the maximum quantum yield of photosystem II (PSII) in the chloroplast, and it is automatically calculated by the chlorophyll fluorometer (Eq. 3). It is important to note that this measurement only indicates the efficiency of PSII biochemistry, and other techniques, such as using a respirometer (i.e., plant inside an enclosed atmosphere monitored using O_2 and CO_2 sensors) are needed to quantify photosynthesis and/or respiration (Bower et al. 1998). A respirometer was not used due to the lack of equipment.

$$\frac{F_v}{F_m} = \frac{(F_m - F_o)}{F_m}$$

Equation 3 | Formula for F_v/F_m. This equation shows the calculation for the ratio between variable and maximum fluorescence, F_v/F_m. F_v, variable fluorescence; F_m, maximum fluorescence yield; F_o, minimum fluorescence yield. These parameters are automatically measured and calculated by the chlorophyll fluorometer.

Generally, F_v/F_m indicates the relative photosynthetic performance of a plant and is usually used as a measurement of photosystem II efficiency (Maxwell and Johnson, 2000). For example, a plant with greater F_v/F_m relative to another might indicate that it is under less stress. The F_v/F_m of the oldest non-senescent leaf was measured across all the soybeans. In this instance, this meant leaves from the second node on the stem, which were exposed to copper for the longest time. The leaves of the plants were dark-adapted for 30 minutes before being measured for F_v/F_m. The dark adaptation is necessary to obtain the minimum fluorescence yield (F_o) (Eq. 3).

To measure biomass, soybeans were first removed from the pots by pressing the sides of the pots and inverting while holding the plant. The roots of the soybean were gently separated

from the soil by washing with RO water. Perlite and microplastics that were stuck among the roots were removed with metal tweezers. A scalpel was used to separate the roots, leaves, and stems. Because I noticed slight etiolation of the plants growing along one edge of the bench in the growth chamber, the internodal distances (i.e., distance between one leaf node and the next) of all the soybean stems were measured using a ruler. All sections were laid flat inside a folded sheet of paper towel and dried in an oven at 60 ^{o}C until constant weight. The dry weights of roots, stems, and leaves for each soybean plant were recorded.

Total Copper Content (soil and soybean)

The concentration of copper in soybean and soil samples was measured using EPA test method SW-846 (US EPA, 2005). More effective acid digestion protocols exist, such as those that use hydrofluoric (HF) acid, which can result in greater extraction efficiencies (around 90–100 %) relative to nitric acid (Sandroni et al. 2003). However, HF is more hazardous to use than nitric acid, especially when coupled to the need for boiling acid for a complete digestion. Thus, hot nitric acid digestion was used. All soil for the total copper content extraction was dried at 60 ^{o}C until constant weight. Five replicate (~0.25 g) samples of oven-dried soils were digested in glass test tubes with 1.0 mL of OmniTrace Ultra Nitric Acid (EMD Millipore Corporation, Billerica, MA, USA) at room temperature overnight, then heated to 95–100 ^{o}C the following day until digestion was completed. Three replicate (~0.50 g) samples of standard reference material (SRM) Montana Soil 2711 (National Institute of Standards and Technology, Gaithersburg, MD, USA) were also processed. Digested samples were filtered using Whatman Grade 230 filter paper (Cytiva, Buckinghamshire, UK) then brought to a final volume of 50 mL with RO water.

Four soybeans from each treatment were randomly selected to be processed for copper content analysis. Following the dried biomass protocol, four soybeans from each treatment were randomly selected to be processed for copper content analysis. Leaves and roots were separated then cut into 1–2 mm pieces with a razor blade. These samples (~0.1 g) were digested in glass test tubes using 1.0 mL of OmniTrace Ultra Nitric Acid (EMD Millipore Corporation, Billerica, MA, USA) at room temperature overnight, then heated to 95–100 ^{o}C the following day until digestion was complete. Three replicates (~0.1 g) of SRM Spinach 1570A (National Institute of Standards and Technology, Gaithersburg, MD, USA), which was pre-ground and did not need to be cut, were also processed. The digested samples were filtered using VWR Grade 415 (VWR International, West Chester, PA, USA) filter paper, then brought to 50 mL with RO water to

achieve 2.0 % acid (v:v RO water). Six blank samples underwent the same protocol to identify if copper was present in the equipment or solvents.

Filtered digested samples and the supernatants that contained bioavailable copper extracts were stored at 4 ^{o}C until analyzed with ICP-MS at the University of Western Ontario's Biotron Analytical Lab (London, ON, Canada) (Agilent 7700x ICP-MS: $R^2 > 0.9980$; Internal standards 45-Sc, 72-Ge, 115-Ln, 159-Tb, 209-Bi).

Statistical Analysis

RStudio (RStudio Team, 2020) was used to perform data analyses and plotting. Plots were generated with the RStudio package 'ggplot2' (Wickham, 2016). A Bartlett's test was first performed to evaluate homogeneity of variance ($p \leq 0.05$) among treatments (Bartlett, 1937). If variance was equal among group means, a one-way analysis of variance (ANOVA) was performed to determine significant differences ($p \leq 0.05$) among the means (Welch, 1951). When ANOVA detected significant main effects, the Tukey's honestly significant different (HSD) post-hoc test was used to determine the means that were significantly different ($p \leq 0.05$) from one another (Tukey, 1949). If variance was unequal among group means, a Welch's t-test was performed to determine significant differences ($p \leq 0.05$) among the means (Welch, 1947) and, when main effects were detected, the Games-Howell post-hoc test was used to determine the means that were significantly different ($p \leq 0.05$) from one another (Games and Howell, 1976).

Results and Discussion

Soil Physiochemical Properties (texture, organic content, pH)

Soil texture was assessed using density separation and determined to be loam according to the percentage of sand (mean = 34.33 %, SD = 3.06 %, n = 3), silt (mean = 49.67 %, SD = 2.89 %, n = 3), and clay (mean = 16.00 %, SD = 4.58 %, n = 3) as classified using the United States' Department of Agriculture (USDA) soil texture triangle (USDA, n.d.). In the context of the present experiment, the soybean roots should perform well in loam relative to finer soils (e.g., clay) due to factors such as decreased penetration resistance (Sato et al. 2015). Dharumarajan and Hegde (2020) used digital mapping on Andhra Pradesh, India to predict that the proportions of loamy soils decreased, and clayey soils increased, at deeper sampling depths.

Organic content was measured using loss-on-ignition (mean = 39.84 %, SD = 1.47 %, n = 5). As a concentration, this would be ~398 g/kg organic matter (w:w fresh soil). To contrast, organic matter concentrations averaged 520.4 g/kg in processed peat from Cravinhos, Brazil

(Marques et al. 2020), between 437–593 g/kg from a coastal peatland in Maludam National Park, Malaysia (Sangok et al. 2020), and between 19–24.6 g/kg across agricultural soils subjected to different tillage treatments in Ariss, Canada (Man et al. 2021). This highlights the variability of soil organic matter content depending on the environment. So, the experimental soil might be more representative of what is naturally found in peatlands but is still relevant as microplastics are present in wetland environments (Scheurer and Bigalke, 2018). The reason for the higher organic matter in this experimental soil might be due to the topsoil being store-bought, with the first ingredient on the bags' packaging being "humus". Humus is decayed organic matter of which there are several types (e.g., mull, mor) that each interact with metals, such as copper, to varying extents (Lasota et al. 2020). PET microplastics have been reported to interact with and affect the structure of soil organic matter; this interaction was attributed to the hydrophobicity of microplastics affecting the water molecule bridges present among organic matter (Fojt et al. 2022).

Soil pH-$CaCl_2$ was measured (mean = 6.88, SD = 0.07, n = 5). To contrast, pH among environments with higher soil organic matter was 5.1 (pH-H_2O) and 4.1 (pH-KCl) in processed peat from Cravinhos, Brazil (Marques et al. 2020), and between 3.5–4.5 (pH-H_2O) from a coastal peatland in Maludam National Park, Malaysia (Sangok et al. 2020). This experimental soil had a near-neutral pH, which is uncharacteristic when compared to other soils with similar levels of organic matter (Marques et al. 2020; Sangok et al. 2020). Soil pH can be artificially raised by the application of lime (Ca^{2+}), which can be used to adjust the pH of agricultural soils to become more basic (Nyamaizi et al. 2022). The list of ingredients for the purchased soil did not list lime in the ingredients, but there might have been a pH-adjusting amendment added to the soil to make it suitable for use in small-scale gardening.

Soil Total and Bioavailable Copper

Total and bioavailable soil copper concentrations were measured using hot acid digestion and $CaCl_2$, respectively, followed by ICP-MS. The concentration of total copper in the soil was 26.7 ± 2.16 mg/kg (mean ± SD). Only ~1.2 % of copper in the control soil sample was bioavailable (Table 1). Bernardi et al. (2022) assessed the effects of copper fungicides applied in vineyard soils and reported that the total copper in soil ranged from 25–75 mg/kg, with 5–15 mg/kg being bioavailable. The contrast between Bernardi et al. (2022) and my experiment may be attributed to interactions with soil organic matter; their vineyard soil was 1–3 % organic,

whereas my experimental soil was ~40 % organic. This is important to consider since organic matter forms complexes with ionic Cu^{2+} (Karlsson et al. 2006) and can reduce overall copper bioavailability to organisms such as plants (McGrath et al. 1988). Hypothetically, as the amount of soil organic matter increases, the fraction of bioavailable copper will decrease.

There was no significant difference among any of the treatments in the mean concentration of bioavailable copper in the soil (Table 1). These results were unexpected, especially since the hypothesis was that microplastics would decrease the bioavailability of copper in the soil. XinYue et al. (2023) reported that soil treated with polystyrene microplastics and copper did not have significant differences in copper bioavailability for polystyrene treatments between 0–50 mg/kg, but a 75 % significant increase in copper bioavailability was found in the 50–100 mg/kg polystyrene treatments. The concentration range of microplastics in XinYue et al. (2023) was lower than our 10 g/kg, yet they found a significant effect on copper bioavailability; this could likely be attributed to the specific microplastic polymer used, or the size (which was not mentioned in their study).

The extraction efficiency for the soil SRM was ~70 % (Table 3). This indicated that ~30 % of the copper in the soil SRM was not quantified, which is a result of the chosen acid digestion. Hseu (2004) used a similar hot nitric acid digestion and reported that a sewage sludge SRM yielded a copper extraction efficiency of 73 %. This could mean that the extraction efficiency of ~70 % for nitric acid digestion was expected. Regardless, the extraction efficiencies for the SRM digestions were accounted for when determining the copper concentration in soil samples.

Internodal Distance, Soybean Photochemical Efficiency (F_v/F_m), and Dry Biomass

Internode distance was measured to determine if some plants were etiolated. There was no significant difference among internode distances among the experimental treatments (Fig. 1; Table 2). However, the internodal distances between nodes 1–2 differed among the rows on the shelf and the distances between nodes 4–5 was marginally significant among rows (Table 2). Upon application of Tukey's HSD test, it was discovered that soybeans growing at the edge of the shelf (row 3) had a marginal significantly greater length compared to soybeans at the wall (row 1), of the growth chamber (Fig. 1; Table 2) This was attributed to possible etiolation of the soybeans, with etiolated soybeans growing taller to reach light. Although all rows were aligned directly under the growing lights, there were no lights above the walkway adjacent to row 3. So,

the decreased irradiance from the aisle might have induced the row 3 soybeans to elongate towards the area of greater irradiance. A photograph highlighting the difference in height among the rows, and the growth chamber pot arrangement, is included (Fig. 2). Thus, five of the soybeans (i.e., pot numbers 3, 13, 20, 22, and 28) were considered outliers and left out of the biomass and F_v/F_m data analysis. The complete data sets can be seen in supplementary figures S1 and S2.

F_v/F_m was measured using a chlorophyll fluorometer on day 27 of growth. A Welch's one-way t-test showed that F_v/F_m varied among treatments ($F(3) = 5.923$, $p = 0.0195$) (Fig. 3). The Games-Howell post hoc test for significance indicated that the average soybean F_v/F_m was ~2.0 % greater ($p = 0.047$) for the control plants (mean = 0.756, SEM = 0.002, n = 6) compared to plants in the combined treatment (mean = 0.741, SEM = 0.004, n = 4). A ~2.0 % greater F_v/F_m is a relatively minor magnitude of effect and may not be biologically relevant. The average F_v/F_m for plants from the microplastic (mean = 0.7465, SEM = 0.007, n = 6) or copper (mean = 0.7420, SEM = 0.009, n = 6) treatments were not significantly different from each other nor the other treatments.

The lack of difference in F_v/F_m for the copper-treated plants relative to the control was unexpected (Fig. 3), as F_v/F_m had been reported to be lowered in the presence of excess copper in water hyacinth (*Eichhornia crassipes*) (Jin et al. 2021), wheat (*Triticum aestivum*) (Dai et al. 2016), and cucumber (*Cucumis sativus* L.) (Burzynski and Klobus, 2004). Other plants such as the coastal shrub *Limoniastrum monopetalum* are more tolerant, having little to no decrease in F_v/F_m when exposed to heightened copper concentrations that would otherwise cause F_v/F_m reductions in other plants (Cambrollé et al. 2013). However, these studies were performed hydroponically and not in soil, so the copper concentrations that decreased F_v/F_m might affect F_v/F_m less when tested in different soils. This is due to the ability for soil organic matter to bind free copper ions, reducing its overall bioavailability, and thus uptake and potential toxicity, to the plant (Karlsson et al. 2006). The lack of copper-induced changes in F_v/F_m is likely related to the ~40 % organic matter in the soil, which would adsorb most of the added copper.

The decreased F_v/F_m in plants from the combined treatment was unexpected (Fig. 3), as these plants were expected to have the greatest F_v/F_m, not the lowest. Higher concentrations of microplastics such as 250 g/kg (w:w dry soil) decreased the photosynthetic parameters of tobacco (*Nicotiana tabacum*) (Teng et al. 2022). Although I used a relatively lower microplastic

concentration of 10 g/kg in soil, Ren et al. (2021) found that 10 mg/kg microplastics in soil negatively affected the photosynthetic parameters of flowering Chinese cabbage (*Brassica rapa*). However, the effects of microplastics may be more related to size than to concentration, as smaller size means a more homogenous distribution throughout the soil matrix (Ren et al. 2021). Thus, the indirect effects of microplastics on the plant, such as by changing soil properties that affect plant growth (Machado et al. 2019), might be more prominent with smaller than larger microplastics.

Dried biomass was measured by harvesting the soybeans after 28 days and weighing the roots, stems, and leaves. Dried biomass of the total, stem, or leaf means (Fig. 4A, 4C, 4D, respectively) did not vary with treatment. However, there was a significant difference in root biomass among the treatments ($F(3,18) = [4.733]$, $p = 0.0132$) (Fig. 4B); the average root dried biomass was ~35 % greater ($p = 0.023$) for the combined treatment plants (mean = 0.983 g, SEM = 0.067 g, n = 4) relative to the plants from the control treatment (mean = 0.729 g, SEM = 0.045 g, n = 6). Since above ground biomass (i.e., stem or leaf) was not affected by the treatments, but the below ground biomass was affected, this indicates that something in the soil could explain these results.

Microplastics may have indirectly influenced the growth of roots by directly affecting soil physical properties. Machado et al. (2019) reported that microplastics influenced physiochemical properties of the soil, both with and without the presence of plants. For example, the application of PET microplastics to soil decreased soil bulk density, relative to the control, by ~13 % or ~5.0 % in the absence or presence of spring onion (*Allium fistulosum*) (Machado et al. 2019). Additionally, Ingraddia et al. (2022) reported that polyester microplastics significantly decreased soil bulk density by ~9.0 % in vertisol-type soil, but no significant difference was found in entisol-type soil. This indicates that the type of soil, and presence of plants, can modulate the size of the effect that microplastics have on soil properties. It is also important to note that these other experiments used different sizes and concentrations of microplastics, which would also influence the effects of microplastics on soil physical properties (Machado et al. 2019; Ingraddia et al. 2022).

In the context of my experiment, there might have also been a decrease in soil bulk density due to microplastic addition, which aligns with previous studies (Machado et al. 2019; Ingraddia et al. 2022). Soil density can influence root growth, as seen by Zhou et al. (2021), who

reported that wheat root density was negatively correlated with soil density. Additionally, Popova et al. (2016) reported that corn (*Zea mays*) root length and depth into the soil were higher in less dense soils. Thus, denser soils could restrain the growth of roots, which might help explain the difference in root biomass between treatments with or without microplastics. This is further supported by Fig. 4, in which the magnitudes of the root biomass for the control and copper treatments (i.e., no microplastics added) were ~14 % lower relative to the microplastic treatment, albeit not a statistically significant difference. Thus, future studies that assess the effects of microplastic in soils could also measure changes to soil physiochemical properties, such as density.

Soil biota can also influence the effects of microplastics. For example, Lehmann et al. (2019) reported that soil treated with polyester microplastics in the presence of microbes increased the number of new soil aggregates by seven-folds relative to the microplastic treatment without microbes. Conversely, microplastics can also influence soil biota. Ng et al. (2021) reported that the Shannon index score (i.e., biodiversity measurement) decreased by ~35 % in the presence of PET microplastics relative to the control. These studies demonstrate that there are important interactions between microplastics and soil biota, which could also provide a direction for future studies.

pH is important to consider because it interacts with soybean growth. Rogovska et al. (2007) found that soybean growth was negatively affected by higher pH, among soil with pH ranging between 5.5–8.0. The near-neutral pH of the experimental soil, at 6.88, falls within a pH range in Rogovska et al. (2007) that caused a 75 % soybean yield (number of soybean grains (seeds) per unit area). This corroborates with Anthony et al. (2012), who reported a negative correlation between soybean mass and soil pH. Ferreira et al. (2016) examined the effects of pH on the nodulation of hydroponically grown calopo (*Calopogonium mucunoides*), which is a legume, much like soybean. They found that calopo grown at pH 7.0 had a ~44 % and ~99 % decrease in the number of nodules and activity of nitrogenase (i.e., enzyme that fixes atmospheric nitrogen into useable ammonia), respectively, relative to calopo grown at pH 4.0 (Ferreira et al. 2016). These effects of pH on yield are likely related to nutrient availability, with many elements becoming less soluble at higher pH (Neina 2019). Copper is also less available at higher pH (Chaignon et al. 2009), meaning that our experimental soil pH further reduced the

availability of copper alongside the higher organic matter content. So, the soybeans in my experiment might have been negatively affected by the near-neutral pH.

It is important to reiterate that the copper treatment concentration was expected to decrease soybean biomass by at least 30 % relative to the control, which was verified during a prior dose response experiment that assessed the effects of sequentially greater soil copper concentrations on soybean biomass (Figure 6). The difference in biomass results cannot be attributed to the properties of the bulk soil; if the high organic content of the soil was responsible, then the greater than 30 % reduction in growth at 250 mg/kg copper during the dose response experiment would not have been observed, as the exact same bulk soil was used for both experiments. However, differences in soil equilibration conditions between the dose response and present experiment following $CuSO_4$ application could explain this finding.

In both experiments, $CuSO_4$ was applied to the soil using water to ensure a homogenous copper distribution, with a sheet of glass above the pots to reduce the rate of evapotranspiration. During the dose response experiment, $CuSO_4$ equilibration was performed in a 25 ^oC growth chamber, where the soil dried between watering sessions. During the present experiment, $CuSO_4$ equilibration was performed in a 4 ^oC cold room, where the soil was consistently wet due to the colder temperature; soil moisture increases among colder soils (Reich et al. 2018).

Soils that are consistently wet, such as equilibrated soil from the present experiment, will have faster copper diffusion and movement relative to drier soils (Xu et al. 2013). The dose response soil might not have been properly equilibrated due to the dry periods. One month of wet equilibration is necessary to diffuse copper homogenously throughout the volume of soil in the pots. Although the dose response equilibration occurred over two months, diffusion would have slowed or stopped during the dry periods, and the copper in that soil may not have reached equilibration. Consequently, there might have been a heterogenous distribution of copper in the pot, which would cause soybeans during the dose response to experience inconsistent magnitudes of biomass inhibition. This is supported by the biomass results during the dose response experiments (Fig. 6), where the standard errors among treatments are high relative to the present experiment (Fig. 4). Thus, the reason why copper at 250 mg/kg had no effect during the present experiment, whereas it had an effect during the dose response, can likely be attributed to the relationship between copper mobility in water and the difference in equilibration conditions between the dose response and present experiment.

Concentration of Copper in Soybean

Soybean copper concentration in the roots and leaves was measured using hot nitric acid digestion followed by ICP-MS. The extraction efficiencies for the plant SRM used in our experiment demonstrated that the copper digestion for the plants worked as intended (Table 3). This is because of the high extraction efficiency, ~95.5 %, indicating that the soybean copper content could be accurately quantified using our acid digestion methodology. Copper concentration in the roots was nine-folds greater than in the leaves, which is consistent with previous studies on the translocation and accumulation of copper in plants such as tomato (*Solanum lycopersicum*) (Ryan et al. 2013) and soybean (Xiao et al. 2022). There was no significant difference in copper content in both roots and leaves among the treatments (Fig. 5). This was unexpected, as it was believed that the copper treatment would result in a greater copper content. Xiao et al. (2022) reported that copper content in soybean roots positively correlated with soil copper concentration. Of course, the concentration of soil copper needed to observe this effect would depend on the soil itself. The heightened levels of soil organic matter (~40 %) in this experiment could have bound most of the copper applied to the soil. In less organic soils, the proportion of bioavailable soil copper as a percentage of total copper is expected to be greater (Bernardi et al. 2022). Additionally, there was no difference in soil copper bioavailability among treatments, meaning that the soybean roots were exposed to similar concentrations of copper (Table 1).

Since the amount of copper in the roots was not different among the treatments (Fig. 5), these results cannot explain the increased root biomass (Fig. 4). If copper positively affected the root biomass, I would expect that the root copper concentration would be significantly greater for the copper treatment relative to the control treatment. This is because the addition of copper in copper-deficient conditions can stimulate plant growth, due to its role as a micronutrient, as demonstrated in *A. thaliana* (Ishka and Vatamaniuk, 2020), rice, bean (*Phaseolus vulgaris*), corn, wheat (Fageria, 2001), and chickpea (Bhankuni et al. 2009). However, this effect would not be isolated to the roots, as seen in the present experiment, but would affect the entire plant. As previously discussed, the action of microplastics might be more likely to explain this increase in

root biomass, especially since microplastics affect the soil matrix that the roots grow in (Machado et al. 2019).

Conclusion

In conclusion, these results demonstrate that soybean root growth was stimulated in soils treated with both PET microplastic and copper. Although this cannot be directly attributed to the action of copper, the microplastics likely affected the soil in a way that facilitated root growth. This is further supported as neither copper content in the roots, nor soil copper bioavailability, was significantly different among the treatments. Additionally, only below ground biomass was affected by the treatments, further indicating that physiochemical differences among the treatment soils are responsible. However, without directly measuring the physiochemical properties of the soil before and after the treatments, it is unknown as to the exact properties that influenced root growth in this experiment. This research also contributes to the growing body of knowledge of terrestrial microplastics and is relevant because the present experiment's concentration of PET microplastics was found to affect plant root biomass, while other studies that used greater concentrations of PET did not find effects on roots (Machado et al. 2019).

Future directions could include using different soils, microplastic sizes and types, plants, and metals. Further research must be done on the interactive effects of soil contaminants, especially because no contaminant exists alone in the environment; interactions always occur. Understanding these contaminant interactions in the context of terrestrial biota is important, as these have downstream effects on important industries such as agriculture. Additionally, plastics are a persistent and ubiquitous environmental contaminant that permeate both water and soils, meaning that they will be relevant for potentially hundreds of years despite gradual phase-out.

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Tables

Table 1 | Bioavailable copper (Cu) content extracted using nitric acid digestion according to Novozamsky et al. (1993). Mean **(**± standard error) bioavailable copper content in the soils was measured. Bioavailable copper did not differ among treatment groups (Tukey's HSD test, $p \geq 0.05$, $n = 5$).

Treatment	Concentration of microplastics (g/kg)	Concentration of Cu (mg/kg)	
	Added	Added	Bioavailable
Control	0	0	0.32 ± 0.11
Cu	0	250	0.46 ± 0.12
Microplastic	10	0	0.47 ± 0.22
Microplastic and Cu	10	250	0.62 ± 0.32

Table 2 | Statistical results for internodal distance according to internode number before adjustment for etiolation. Row number (i.e., 1 = nearest to wall, 2 = center, 3 = nearest to aisle) or treatment (i.e., control, copper, microplastic, microplastic and copper) was used as the independent variable for the statistical tests to determine if plants in the third row were etiolated. Asterisks (*) represent significant differences for the respective test ($p \leq 0.05$).

[Row number] X [Internode distance]						
Internode number	Test	Source	df	F	p	Post-hoc
1–2	1-way ANOVA	dose	1	6.166	**0.02 ***	Tukey HSD: [Row 1]-[Row 3] p = 0.0576 [Row 1]-[Row 2] p = 0.389 [Row 2]-[Row 3] p = 0.469
		error	25			
2–3	1-way ANOVA	dose	1	1.821	0.189	
		error	25			
3–4	1-way ANOVA	dose	1	2.135	0.156	
		error	25			
4–5	1-way ANOVA	dose	1	4.195	0.051	
		error	25			
5–6	1-way ANOVA	dose	1	1.480	0.235	
		error	25			
[Treatment] X [Internode distance]						
Internode number	Test	Source	df	F	p	Post-hoc
1–2	1-way ANOVA	dose	3	0.159	0.923	
		error	23			
2–3	1-way Welch t-test	dose	3	0.638	0.606	
		error	10.987			
3–4	1-way ANOVA	dose	3	0.639	0.598	
		error	23			
4–5	1-way ANOVA	dose	3	0.702	0.56	
		error	23			
5–6	1-way ANOVA	dose	3	0.869	0.471	
		error	23			

Table 3 | Copper (Cu) extraction results for standard reference materials and blanks. Standard reference materials—Montana Soil 2711 and Spinach 1570a—were purchased from the National Institute of Standards and Technology. Blank samples measured background copper contamination from items such as equipment or solutions used during digestion. Samples underwent hot nitric acid digestion according to Novozamsky et al. (1993) then measured using inductively coupled plasma mass spectrometry in a 2.0 % nitric acid: water matrix. Non-applicable results denoted (N/A).

		Concentration of Cu (mg/kg)		
Sample	n	Total	Extracted	Extraction Efficiency (%)
Montana Soil 2711	3	114 ± 2	80.98 ± 3.04	71.04 % ± 0.03 %
Spinach 1570a	3	12.2 ± 0.6	11.65 ± 0.34	95.49 % ± 0.03 %
		Concentration of Cu (μg/L)		
Sample	n	Total	Extracted	Extraction Efficiency (%)
Blanks	6	N/A	0.00081	N/A

Figures

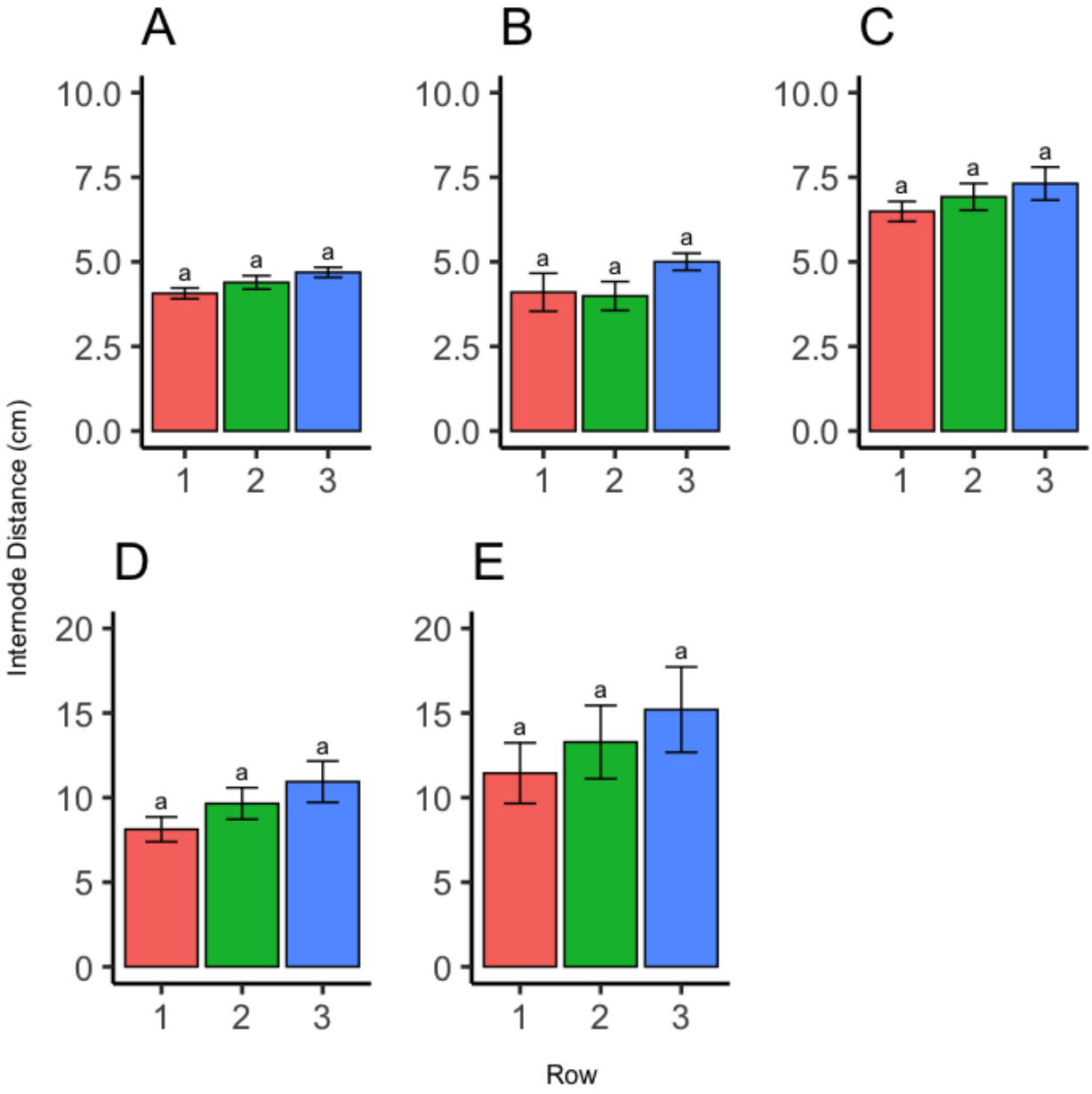


Figure 1 | Internodal distance (cm) of soybeans according to row number measured on day 28 of growth before adjustment for row 3 outliers. Rows 1, 2, and 3 represent the growth chamber shelf wall, center, and edge, respectively. Distance between stem leaf nodes (A) 1 and 2, (B) 2 and 3, (C) 3 and 4, (D) 4 and 5, (E) 5 and 6. Vertical standard error bars are shown. Different letters above the means represent significant differences among rows (Tukey's HSD test, $p \leq 0.05$).

Figure 2 | Photograph highlighting the etiolation of soybeans in the growth chamber. Rows 1 (right), 2 (middle), and 3 (left). Soybeans had grown for 24 days when this photograph was taken. Note the lack of lights adjacent to row 3, and the white wall adjacent to row 1 that could reflect light evenly onto all sides of row 1 plants.

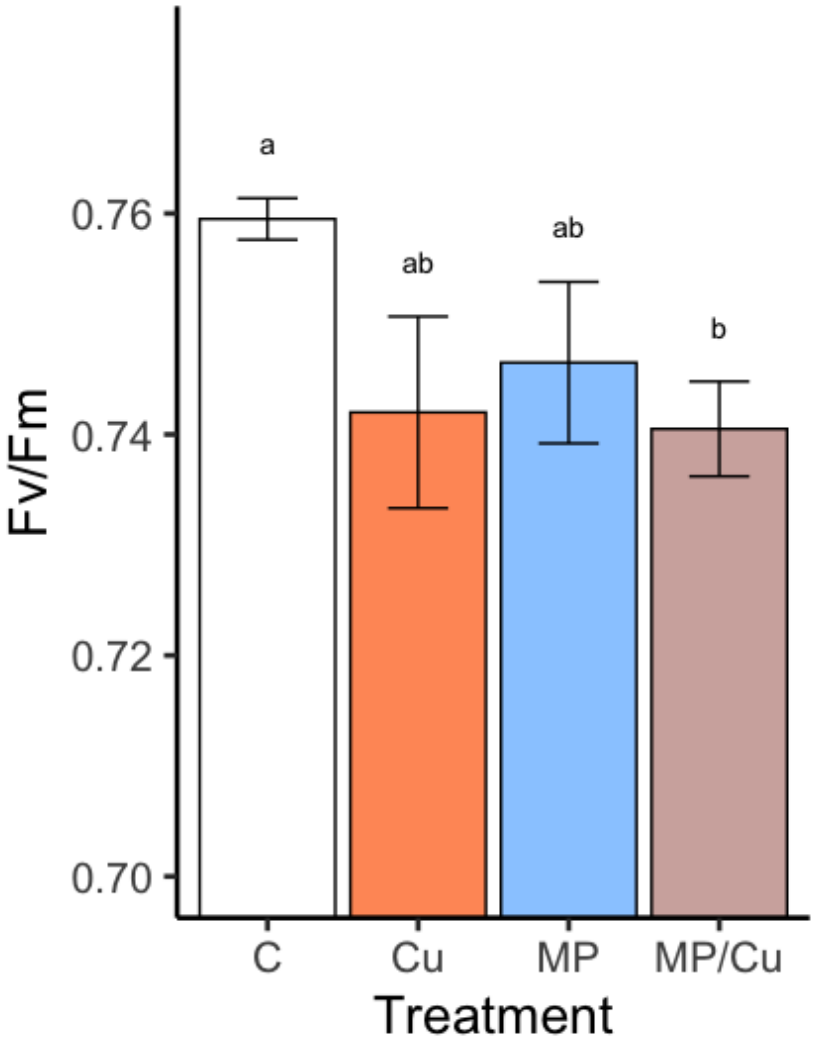


Figure 3 | Soybean F_v/F_m values measured using a chlorophyll fluorometer after 27 days of growth. Measurements from a single leaf growing from the second soybean stem leaf node across all soybeans. Treatments were control (C), copper (Cu; 250 mg/kg), microplastic (MP; 10 g/kg), and combined microplastic (10 g/kg) and copper (250 mg/kg) (MP/Cu). Vertical standard error bars are shown. Different letters above the means represent significant differences among treatments (Games-Howell test, $p \leq 0.05$).

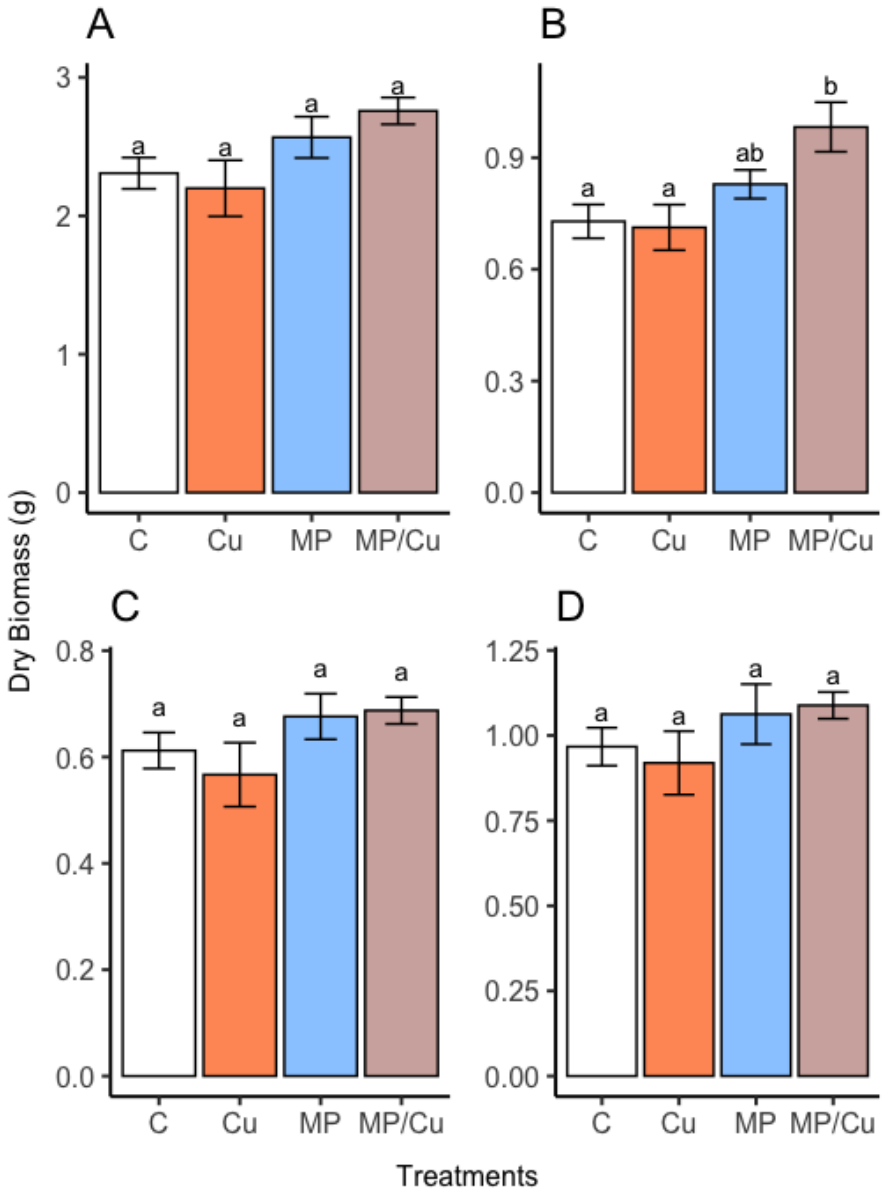


Figure 4 | Dry biomass (g) of soybeans harvested on day 28 of growth. Dry biomass of (A) total plant, (B) root, (C) stem, (D) and leaf. Treatments were control (C), copper (Cu; 250 mg/kg), microplastic (MP; 10 g/kg), and combined microplastic (10 g/kg) and copper (250 mg/kg) (MP/Cu). Vertical standard error bars are shown. Different letters above the means represent significant differences among treatments (Tukey's HSD test, $p \leq 0.05$).

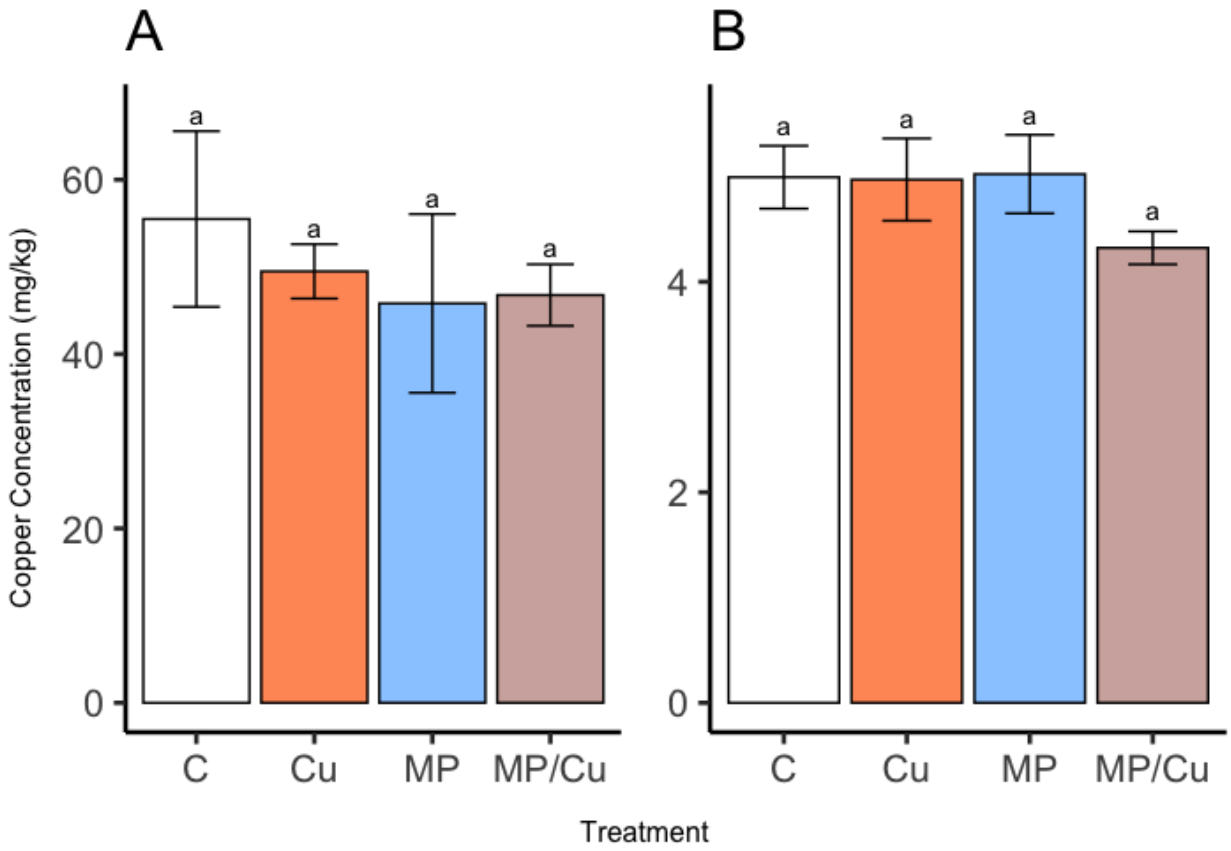


Figure 5 | Total copper concentration (mg/kg) in soybean roots or leaves extracted using hot nitric acid digestion followed by inductively coupled plasma mass spectrometry. Copper concentration of soybean (A) root and (B) leaf. Treatments were control (C), copper (Cu; 250 mg/kg), microplastic (MP; 10 g/kg), and combined microplastic (10 g/kg) and copper (250 mg/kg) (MP/Cu). Vertical standard error bars are shown. Different letters above the means represent significant differences among treatments (Tukey's HSD test, $p \leq 0.05$).

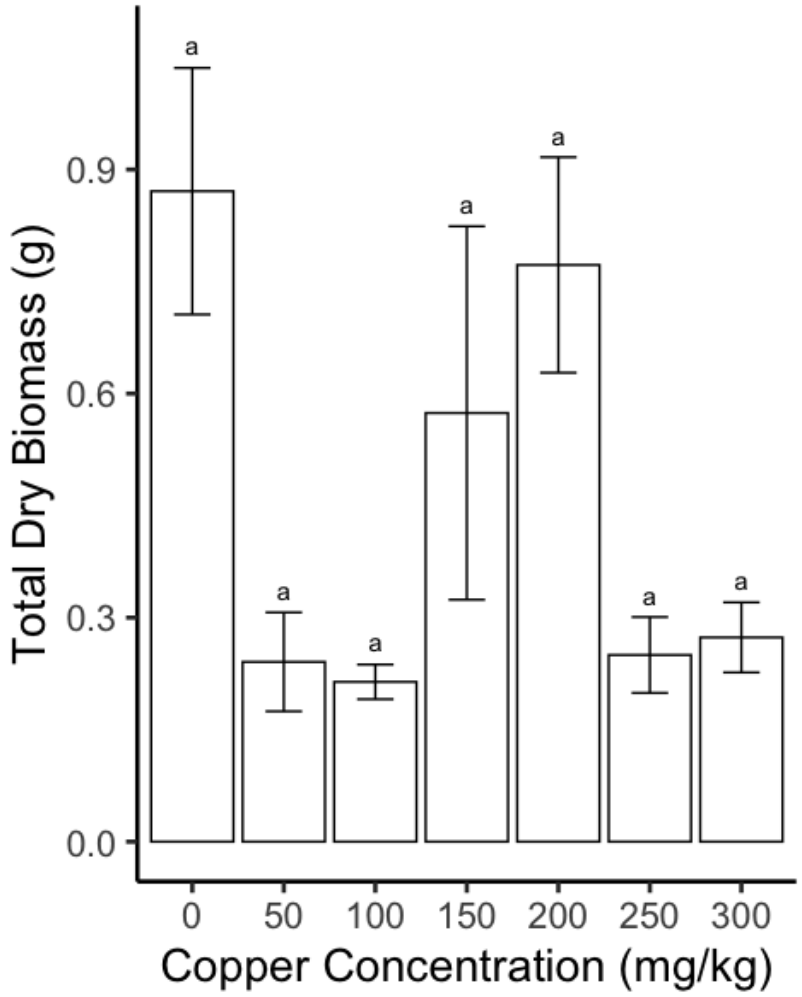
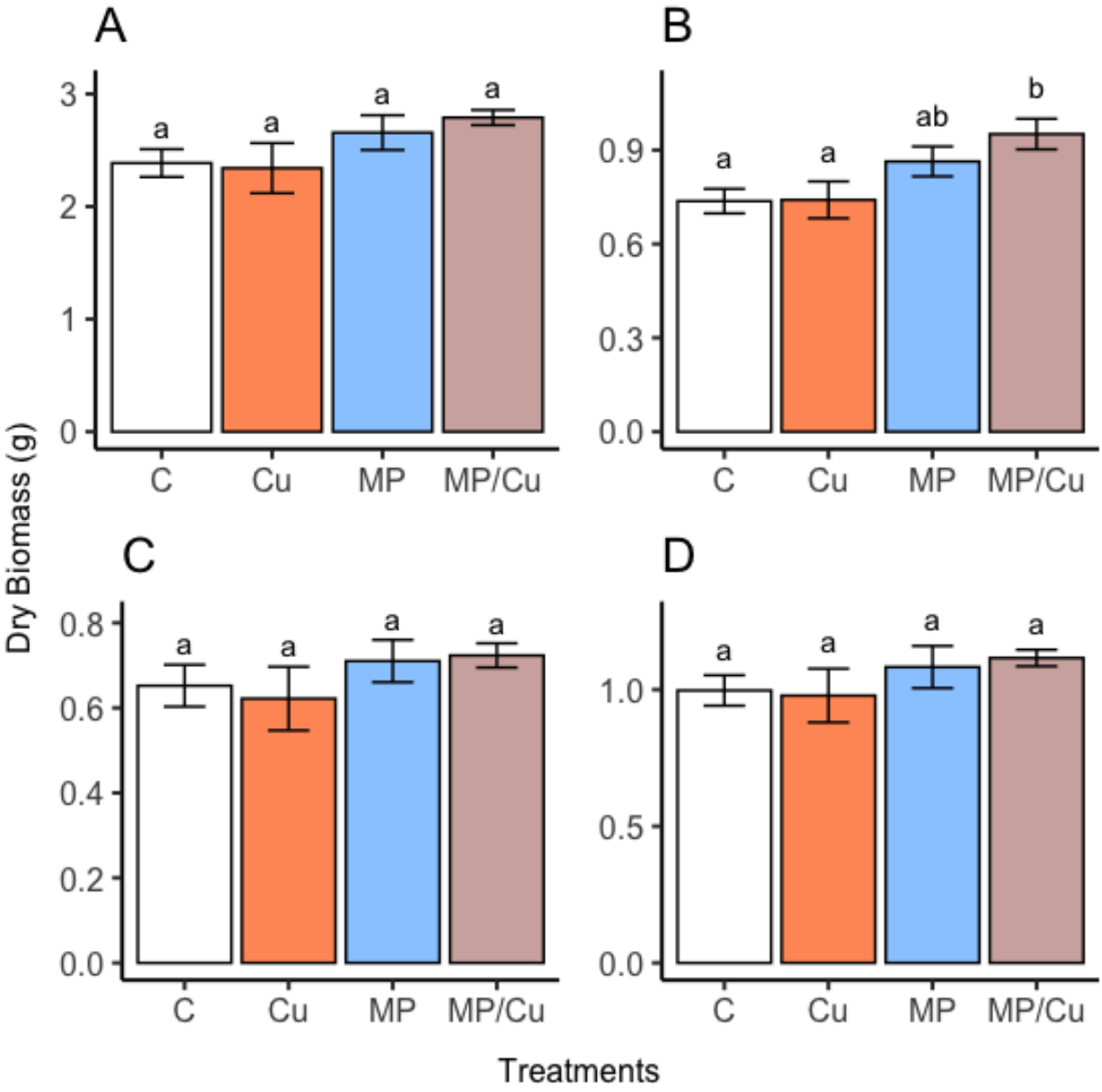
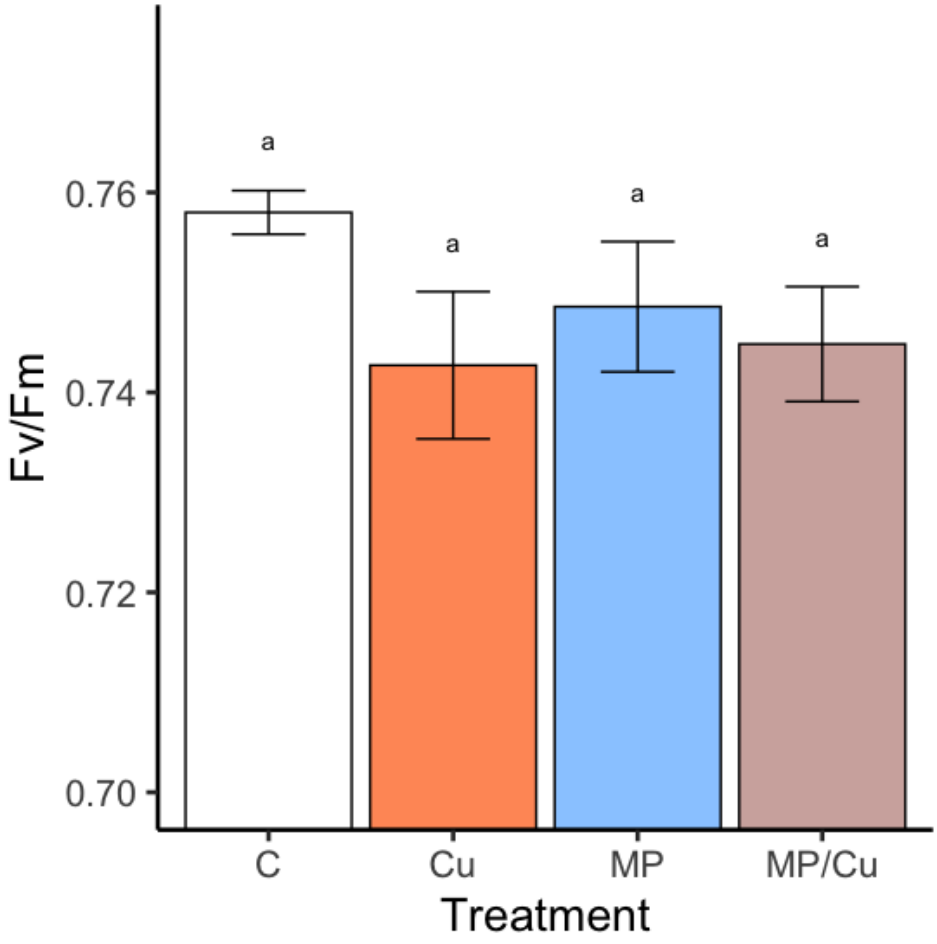


Figure 6 | Total dry biomass (g) of soybeans harvested on day 21 of growth during the prior dose response experiment across a range of soil copper concentrations. Four soybeans were grown at each concentration then measured for total dry biomass. Vertical standard error bars are shown. Different letters above the means represent significant differences among treatments (Tukey's HSD test, $p \leq 0.05$).

Supplementary Figures



Supplementary Figure S1 | Dry biomass (g) of soybeans harvested on day 28 of growth before adjusting for etiolated plants. Dry biomass of (A) total plant, (B) root, (C) stem, (D) and leaf. Treatments were control (C), copper (Cu; 250 mg/kg), microplastic (MP; 10 g/kg), and combined microplastic (10 g/kg) and copper (250 mg/kg) (MP/Cu). Vertical standard error bars are shown. Different letters above the means represent significant differences among treatments (Tukey's HSD test, $p \leq 0.05$).



Supplementary Figure S2 | Soybean F_v/F_m values measured using a chlorophyll fluorometer after 27 days of growth before adjusting for etiolated plants. Measurements from a single leaf growing from the second soybean stem leaf node across all soybeans. Treatments were control (C), copper (Cu; 250 mg/kg), microplastic (MP; 10 g/kg), and combined microplastic (10 g/kg) and copper (250 mg/kg) (MP/Cu). Vertical standard error bars are shown. Different letters above the means represent significant differences among treatments (Tukey's HSD test, $p \leq 0.05$).