Microplastics in the Rhizosphere: Consequences on Root Exudation and Microbial Communities

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Problem Statement

Microplastics have recently become an emerging contaminant in the terrestrial environment. Plastic particles in agricultural soil can alter root-soil-microbial interactions that are crucial for plant growth. Biosolids utilized as nutrient sources and amended in the soil of crops has been reported to contain plastic particles that can potentially be ecotoxic and interfere with the normal functioning of the root. Current articles involving root-soil-microbial interactions lacks research to diagnose microplastic induced stress in plants and link microplastics to impaired functioning. This research will explore the fate and threshold for ecotoxicity in agricultural soils amended with biosolids as a nutrient source. Doing so by investigating potential altered root execution, microbial carbon substrate utilization, and rhizosphere microplastic contamination. This project will provide new insights into the prevalence and severity of distributions to root-soil-microbial interactions by microplastics .

Methods

Soybean, alfalfa and wheat crops were grown in rhizoboxes containing soil amended with artificially created microplastics that reflect reported biosolid microplastic properties but were free of other potentially toxic substances. The test soil used was clay-loam that was obtained from a local farm that had no history of biosolid applications. The rhizoboxes used contained six different soil treatments (1) and were made of metal with a transparent acrylic front to allow for root imaging , they were stored at an angle to encourage root growth against the transparent side of the box. Crop seeds were germinated prior to plantation and planted in a greenhouse atmosphere containing supplemental lighting. Root system development was monitored and imaged weekly. Crops were watered weekly, and greenhouse temperature and humidity were recorded.

Once crops reached maturity rhizoboxes were harvested. Initial above and below ground biomass was recorded prior to drying the crops, after being dried the crops were weighed again to obtain their dry weight. Three successful roots were selected from each rhizobox and the rhizosphere soil surrounding the roots was obtained. The obtained soil was split into three soil samples, Sample 1 was incubated at 25C to be used for MicroResp analysis, Sample 2 was frozen to reduce microbial activity and be used for root exudation analysis, and Sample 3 was stored at room temperature for microplastic analysis.

Treatment	Microplastic Type	Number of MPs
1	None	None
2	Polyethylene	2,000
3	Polyethylene	15,000
4	Polypropylene	2,000
5	Polypropylene	15,000
6	Biosolid	N/A

Figure 1. Microplastic treatment, type & amounts.

The MicroResp analysis is responsible for monitoring the evolution of the CO2 in the soil that is a result of microbial respiration. The soil for the MicroResp analysis was condensed into deep well plates and amended with 15 carbon sources. The rate of which the microbial communities used these carbon sources was recorded colometrically using indicator dye (2) and Biotek Epoch 2 plate reader at 572nm.

The root exudation analysis was conducted using centrifugation to separate the moisture containing root exudates from the soil. Soil moisture was then syringed, filtered, and frozen for HPLC analysis.

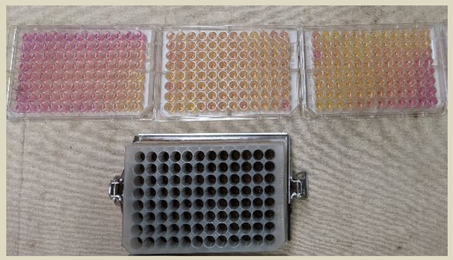

Figure 2. Displays the indicator colouration that resulted from MicroResp incubation

Microplastics analysis will include separation from soil by density separation with Sodium Iodine and digestion to remove surface contaminants. Microplastics filtered onto a glass filter paper will be analyzed by Ramen spectroscopy using a Horiba labRam Solei equipped with 532, 638, 785nm lasers as well as Particle Finder, Q-Scan and Smart Sampling software. The Raman analysis provides data on individual microplastics such as their geometry, surface roughness, etc., in conjunction with Open Specy to identify plastic type.

R Coding Analysis

The programming language R was used for statistical analysis and graphics of the MicroResp data. The soil incubated for the MicroResp analysis was compressed into deep well plates containing 96 wells. Two soil samples were placed into each deep well plate, 48 wells per sample, and 15 carbon substrates were added. Indicator plates were placed on top of the deep well plates and incubated for 24 hours. An initial scan of the indicator plates took place prior to soil incubation, then an additional scan after soil incubation, and the change in Colour due to high or low amounts of microbial activity releasing CO2 was recorded. The range of absorbance change and the average of each treatment and each species is shown below (3).

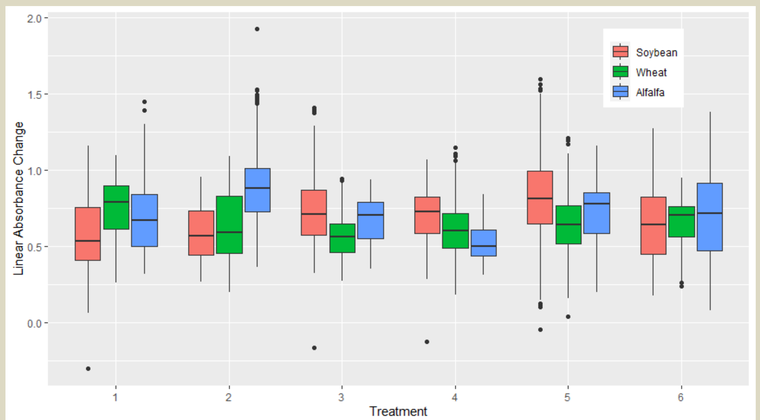

Figure 3. Shows the change CO2 absorbance according to the incubated indicator plates. It displays the results of all 6 treatments and separates the data by species.

Research Contribution

Further knowledge of the ecotoxicity threshold and fate of microplastics is important when already biosolids are being used as nutrient sources in agricultural soils. Microplastics are an emerging contaminant that can be transported from agricultural soils to other areas due to runoff causing further contamination. This research will provide us insight into what amount, type or shape of microplastic should raise concern for the environment.

Results

Further data analysis is required. Preliminary analysis of the weekly root imaging revealed normal root development patterns and increasing root exploration expanding through all the available soil space. Roots were commonly seen in the upper 17 inches of the rhizobox; however, numerous roots grew to the maximum root depth of the box, some even further exceeding the length (4).

Analysis of root exudates is being conducted, initial results have revealed that the ratio of sugars in the exudates was 2.9 : 1.7 : 1 sucrose, glucose, fructose. While the organic acids used are still currently under analysis.

Beginning stages of the MicroResp data analysis suggests a strong microbial respiration response to all the added carbon substrates (2). R coding analysis suggests that for the soybean species an increase in particle number resulted in absorbance increase regardless of particle type (compare 2-3, and 4-5) (3). When fixating on species, all treatments containing microplastic additives where wheat was grown resulted in a lower absorbance level and overall lower microbial activity than the control treatment (3).

Figure 4. Rhizobox images of the same root, the left image is week 2, while the right image is week 6. The increased root depth and root development is shown even in these early stages of root imaging.

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