PLASTIC IN AGRICULTURAL SOILS AND ITS BIOLOGICAL IMPACTS

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ABSTRACT

Plastic in Agricultural Soils and its Biological Impacts

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Agricultural plastic mulch reduces weeds, yields higher crop quality and quantity, and increases soil temperature, but it can also become a soil pollutant. The impact of agricultural plastic contamination on soil microbial activity remains poorly documented. To better understand how plastic pollution influences soil microbial decomposers, we sampled three farms in Watsonville, CA. Each site is characterized by large amounts of plastic contamination in the form of polyethylene mulch and polyvinyl chloride dripline. The fields contain marked amounts of macro- (and presumably micro-) plastic fragments primarily derived from PVC dripline and polyethylene mulch. We haphazardly collected 6" deep soil samples from the fields (i.e., “bulk PC soil”) to compare with soil which had come directly in contact with the remaining surface mulch and dripline (i.e., “macroplastic fragment associated soil”). If plastic incorporation alters edaphic factors while leaching novel compounds, this may alter microbial decomposer community structure and function. We hypothesized that the soil directly associated with plastic fragments would have reduced microbial biomass and decomposer activities relative to the bulk soil, due to a greater likelihood of toxicity and altered microhabitat. We evaluated a suite of abiotic and biotic characteristics to assess the influence of agricultural plastic contamination on soil decomposers. Our study suggests that prolonged contact with agricultural plastic waste may create a novel habitat in low carbon agricultural soils.

Keywords: biogeochemistry, plastisphere, agriculture, macroplastics, microbes

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1. INTRODUCTION

The quantity of plastic released into terrestrial systems is 4-23 times greater than that released into freshwater systems, yet the implications of plastic pollution have not yet been fully studied nor quantified (Horton et al., 2017). The use of agricultural plastics has grown rapidly and now covers millions of acres of farmland globally. Single-use plastic has become an essential tool for weed management, air and soil temperature and moisture modulation in specialty crop fields, allowing for the efficient, cost-effective production of specialty crops (Lamont, 2017, Sanchez-Hernandez, 2019, Xiong et al., 2020). Polyethylene (PE) plastic is used in greenhouses, walk-in tunnels, irrigation tape, and in the field as plastic mulch. Polyvinyl chloride (PVC) irrigation tubing is a rigid, non-flexible piping also commonly used in agricultural fields (Ding et al., 2020, Yan et al., 2020). Both PE plastic and PVC can help make agricultural processes easier and have become an essential part of modern agriculture. While plastics are extremely beneficial for agricultural productivity, there are many environmental externalities associated with agricultural plastics (Hemphill, 1993). Improper plastic disposal, like tilling or burning, releases environmental toxins and can cause public health hazards (Waring et al., 2018). Additionally, plastic in agricultural fields breaks down to micro- and nano-pieces, so it is essentially impossible to remove plastic completely from the soil even when normative removal techniques are used (Qi et al., Huang et al., 2020).

Plasticulture is extensively used in CA specialty crops. For example, in Monterey County, CA ~10,000 acres of strawberry production use ~ 7.8 million pounds of film plastic (plastic mulch + fumigation tarp) annually, accounting for 28% of the US's strawberry demand. Although a valuable technology for these specialty crops, the rise of plasticulture is of growing concern from an environmental and human health perspective, with significant cost burdens to both farmers and society at large (Brodhagen et al., 2017 and Piehl et al., 2018). Across CA, agriculturalists dispose of over fifty-five thousand tons of plastic per year, with strawberries accounting for ten thousand tons and greenhouses and nurseries contributing an additional eleven plus thousand tons of plastic waste (Moore and Wszelaki, 2016).

Plastic mulch removal from fields is labor-intensive, with costly disposal and limited recycling or reuse potential due to the adhesion of soil particles to the films. Nearly 94% of California strawberry growers report using plastic mulch, but only a third of these producers report recycling the material, with comparable recycling rates found across the spectrum of agricultural products produced with this technology (Hurley, 2008). However, plastic mulches are rarely completely removed from a field, leaving plastic residues that remain in soil for decades to centuries and leach out of the soil, also polluting water systems (Jambeck et al., 2015, Brodhagen et al., 2017). Although growers often use care when removing agricultural plastics from their fields, experimental studies indicate that common methods leave at least 10% of PE film plastic in the field due to fragmentation during removal (Miles, unpublished). PE is resistant to breakdown under normal soil conditions (Scalenghe, 2018) and PE films (0.02-0.16mm) undergo only 0.2% mass loss as CO_2 over a decadal period (Albertsson and Karlsson, 1988, Selke et al., 2015). Other forms of plastic—including those resulting from the application of certain fertilizers (Weithmann et al., 2018), and the PVC found in irrigation systems—may either enter as or be physically weathered into smaller microfragments (de Souza Machado et al., 2018), and are similarly-resistant to decomposition in soil.

These PE and PVC plastic fragments remain in the soil environment for decades to centuries, and can leach into both plant tissue as well as connected watersheds long after their use has ceased (Birch et al., and Li et al., 2020). California is increasing its efforts to regulate plastic pollution in potable water systems through *SB1422 California Safe Drinking Water Act: microplastics*; however, both the extent of plastic pollution in agricultural soils and the influence of agricultural plastic contamination on soil biological activities and physical traits remains poorly documented A recent study suggests that plastic mulch residue accumulation in agricultural soils can have multiple negative impacts on plant growth (e.g., reduced crop yield, plant height and root mass) and soil properties (e.g., lower water infiltration rate, organic matter content, and plant-available phosphorus), which threatens long-term food security if these responses are widespread (Shen et al. 2020).

To better characterize the implications of PE and PVC contamination on agricultural soils, we determined the impacts agricultural plastic contamination has on soil biological and physical properties. We then compared these patterns to the response of soil to a starch-based biodegradable plastic mulch (BDM) alternative.

We sampled three farms on the central coast of CA that were identified as having significant agricultural plastic pollution: in all cases, PE mulch and PVC dripline were tilled into the farms' fields. To this day, there are remaining plastic mulch and PVC microplastic fragments on the soil surface. The fields contain marked amounts of macro- (and presumably micro-) plastic fragments primarily derived from PVC dripline and PE mulch. At the fields, we haphazardly collected soil samples from the field (i.e., "bulk soil") to compare with soil which had come directly in contact with the remaining surface mulch and dripline (i.e., "plastic-influenced soil"). If plastic incorporation alters edaphic factors while leaching novel compounds, this may alter both local abiotic conditions and microbial decomposer community structure and function. In this sense, macro-plastic fragment additions may create novel habitats in the soil environment. We hypothesized that the soil directly associated with plastic fragments would have reduced microbial biomass and decomposer activities relative to the bulk soil, due to a greater likelihood of toxicity and altered microhabitat. Additionally, we assessed the soil of a fourth farm in Santa Cruz County, CA that has used BDM as an alternative to PE in its fields relative to a neighboring field with no BDM usage. We evaluated a suite of abiotic and biotic characteristics to assess the influence of agricultural plastic contamination on soil decomposers and physical properties.

2. LITERATURE REVIEW

Plasticulture in Context

Three hundred million tons of plastic waste are generated annually, much of which is improperly handled and grows our collective environmental pollution burden (Cirino, 2019). Environmental law and limitations for plastic waste disposal are essential for continued environmental and human health. Currently, there are many countries working to ban single-use plastic products (Howard et al., 2019). Yet this problem runs much deeper, as micro- and nanoplastics which can be directly manufactured or a byproduct of macroplatic fragmentation are far more challenging to regulate (Kurniawan et al., 2021). The release of these plastics in the soil environment can be just as detrimental as the presence of macroplastics (Astner et al., 2019). Formalized laws to limit plastic pollution remain rare (Gibbens, 2019) and environmentalists believe that holding large polluter industries accountable is the only solution to the ever-growing plastic problem in the United States (Corkery, 2020).

The success of the packaging, transportation, agricultural, and technological industries are highly dependent on plastic; this directly contributes to the overall plastic pollution issues faced globally. Although industry reliance on plastic continues to grow and plastic production is projected to triple by 2050, there is still little regulation in place regarding plastic waste (Volcovici, 2021). Regulations are largely not centered around the usage of plastic waste, but rather the implications of various disposal methods for plastic waste. The agricultural industry is of particular concern, as each step of the farming process uses plastic in some form. The Federal Clean Air Act, the Clean Water Act, and the Insecticide, Fungicide, and Rodenticide Act are all examples of environmental agencies which touch on plastic disposal in agriculture, but they do not set specific limits of plastic use. Additionally, these laws are reactive rather than proactive, and usually are brought into action once damage has already occurred to the environment. These acts are all forms of federal law, and not state law. Until recently, this meant that major crop producing states, like California and Iowa, often abided by the same laws as non-crop producing states (USDA ERS).

Regulations and legislation on plastic waste in California

California is working towards a 75% reduction in plastic waste by 2030, yet this will only be possible if large industries can commit to alternative methods (Singh, 2019). This is challenging for many reasons: there is little data on alternatives; alternative farming methods can be more expensive; it is hard for farmers to change methods that have been efficient for seasons (Brodhagen et al., 2017). To combat plastic issues on a state level, a group of eight California lawmakers introduced the 2021 Legislative Plastics and Waste Reduction (Carpenter, 2021). This legislative package is a collection of twelve bills that aim to combat the state's growing plastic waste problem in various sectors, from food service to technology. While this legislation has not yet been voted on, it is a step in the right direction.

Economics and Plasticulture

Plasticulture is loosely defined as plastic used in agriculture. Plastic markets are as large of a commodity as corn and oil are in the United States; plastic and corn prices are positively correlated, a relationship which is strengthened by the 2007 Energy Independence and Security Act (EISA) (Jiang et al. 2017). Plastic is part of the "green technology market”, which is an umbrella term for products which are designed to be more environmentally friendly through the use of science and technology (Kenton, 2020).

Types of Plastic in Agriculture

Plastic in agricultural settings is perhaps the most beneficial technological advancement for horticulture, yet the agriculture industry is one of the largest drivers of plastic pollution, as plastic is used in majority of agricultural processes (Wittwer, 1993). The demand for agricultural plastic is most prevalent in the U.S. but is closely followed by China and the Middle East (Sintim & Flury, 2017). These agricultural uses include: greenhouse construction, drip-irrigation systems, and plastic mulch to cover the soil prior to planting. Plastic mulch is used to control the soil temperature, preserve moisture, retard weeds, and deter insect invaders (Grant, 2018). On average, plastic mulch will increase soil temperatures by about 2°C (Anifantis et al., 2012). Plastic mulch reduces pesticide use and can allow for earlier planting and can increase crop yield and quality (Sintim et al., 2017). Most irrigation systems are comprised of PVC Biodegradable mulch is made from biodegradable starch-based polymers (Halley et al., 2001), while PE mulch is comprised of polymers which do not readily break down (Garrison et al., 2016). While agricultural plastics are of noted utility, improper disposal methods include burning and tilling, which can release micro- and nanoplastics into the air, groundwater, and soil. Conventional plastics contain toxic chemicals including: phthalates, heavy metals, bisphenol A, brominated flame retardants, nonylphenol, polychlorinated biphenylethers, dichlorodiphenyldichloroethylene, phenanthrene, and more (Okunola A Alabi et al., 2019). These chemicals can create public health and environmental hazards when they are released.

Polyethylene mulch

Plastic mulch is primarily composed of PE, a petroleum-based synthetic product that is comprised of decomposition-resistant polymers. The cost of PE mulch removal is about $250 per hectare and can take about 16 hours per hectare to properly remove using both hand labor and machinery (Cowen et al., 2016). Since removal is both expensive and labor-intensive, PE is often left behind in soils and hardly ever recycled, ultimately contributing to plastic pollution. In fact, recycling rate of plastic mulch are estimated to be below 30% (Plastics Europe, 2008).

Polyvinyl chloride

PVC fragments are among the most identified microplastics in the soil environment (Ding et al., 2020). PVC is used in various sectors of agriculture: drip irrigation, sprinkler systems, and hydroponic gardens. The main concern surrounding PVC is its high leaching potential, as PVC use is most often associated with irrigation processes.

Biodegradable mulch

Biodegradable alternatives to PE mulch have been sought to reduce the plastic waste and pollution burden associated with conventional agricultural plastics. Biodegradation is a naturally occurring process involving organic chemicals in the environment. These molecules are converted to simpler compounds, are mineralized, and are finally redistributed through natural biogeochemical cycles (Chandra and Rustgi, 1998). BDM is comprised of mixtures of polymers from different additives which creates distinct chemical and physical properties from mulches with pure polymers (Brodhagen et al., 2015).

The biodegradation process of BDM occurs in two steps: disintegration/weathering followed by biodegradation which occurs in the soil itself (Cowan et al., 2016). BDM appears to be an ecologically sustainable alternative to PE mulch that can be tilled into the soil at the end of the season, which may ultimately lower its costs relative to traditional plasticulture.

BDM biodegradation takes longer than a year to reach completion under certain field conditions (Ghimire et al., 2020). Notably, after 8 years, 20% of the original BDM mass can still be present in the soil, leading to concerns about the efficacy of its breakdown under field conditions (Miles et al., 2017). There is little knowledge regarding the long-term implications of BDM presence in the soil environment. As farmers continue to use BDM at a rate which is faster than its degradation process, it is important to understand whether or not prior exposure to BDM will increase the rate of *in situ* biodegradation.

Current Opinions Regarding Biodegradable Mulch

In order to sustain crop production, global agriculture methods must become more sustainable and climate-friendly. While BDM appears to hold promise to jointly address environmental and economic externalities associated with conventional plasticulture practices, the efficacy, degradability, and ease of use of BDM remains equivocal.

BDM is not currently popular among farmers due to its cost and the lack of research on biodegradable mulch’s performance (Dentzman and Goldberger, 2020). This knowledge gap creates a clear challenge: from an agriculturalist perspective, if something works well, why change it? Although survey data indicates that consumers are willing to pay over 10% more for strawberries that are grown using BDM (Chen et al., 2018), the artificially low cost of externalities associated with plasticulture reduces the incentive for growers to adopt this technology (Zhang and Ghimire, 2017).

Some argue that BDM is not necessarily a better alternative to PE mulch, and that far more extensive research must be conducted. BDM may not fully biodegrade on its own, which could potentially be just as problematic for the soil environment as standard plastic (Shen et al., 2020). Further, BDM disposed of in a landfill can increase methane production under anaerobic conditions.

Aesthetics of an unclean field

The visual appearance of farms is a newly contested aspect of sustainable farming (Dentzman and Goldberger, 2020). Negative aesthetics related to more environmentally friendly ways of farming can delay or prevent adoption of such practices (Carlson, 1976). The aesthetics of BDM may create a challenge in their adoption, even though black biodegradable mulch looks identical to black PE mulch and its presence is relatively temporary. The hesitation is due to the scraps that remain post-harvest, as it creates a "messy" appearance in the field (Dentzman and Goldberger, 2020).

Biodegradable mulch as a polyethylene mulch alternative

Biodegradable mulch can be a suitable, sustainable alternative for PE mulch and hoop houses (Hemphill, 1993). Biodegradable mulch deteriorates far quicker than PE mulch, thus limiting any additional maintenance post-harvest (Bandopadhyay et al., 2018 and Zhang et al., 2020). BDM is considered to have promise as a more sustainable alternative to PE mulch that provides comparable growing advantages to conventional plastics (Bandopadhyay et al., 2018). BDM is comparable to PE mulch at field conditions, as both type of mulch resulted in similar weed control and fruit yield (Zhang et al., 2021). Incentives for adopting biodegradable plastic alternatives in agriculture are reduced waste, environmental benefits, and economic gains if plastic disposal and waste regulation costs continue to increase (Brodhagen et al., 2015). If farmers adopt BDM, they have the potential to significantly reduce non-biodegradable PE mulch waste from their farms, thus addressing serious environmental and human health concerns (Dentzman, 2020).

Commercial farmers have been using PE mulch since the early 1950s, and the upfront cost of BDM is perhaps the greatest barrier to adoption (Goldberger et al., 2015). The long-term impacts of biodegradable mulch are not yet known. More persistent plastic mulch particles can accumulate overtime in agroecosystems and remain in the system for decades, which is worrisome as there is not sufficient data regarding the physical and biological impacts of BDM over time (Brodhagen et al., 2017).

Soil Properties and Plasticulture

Polyethylene

When PE mulch remains in the soil it can interfere with the root development of the subsequent crop (Kasirajan et al., 2012). PE mulch significantly impacts the soil environment, as residual soil plastic film fragments (RPFF) significantly alter soil water distribution and water infiltration. Water followed a path along the RPFF; this is perhaps because the RPFF are hydrophobic. In addition to the hydrological impacts of PE mulch, RPFF also impacts the crops ability to take in nutrients. Lower amounts of K, N, and P are reported in soils containing PE mulch (Hou et al., 2019 and Steinmetz et al., 2016). Remaining fragments also reduce the rooting zone of maize growing in agricultural systems (Jiang et al., 2017). The gravimetric water content, bulk density, and total porosity in 0-20 cm were all significantly impacted by the addition of plastic as well (Jiang et al., 2017).

PVC

High PVC contamination (>1%) had a significant impact on available nitrogen and phosphorous content. PVC increased the available phosphorous content, thus disturbing P cycling. On the other hand, it decreased the concentration of nitrate (NO^{3-}) (Yan et al., 2020).

BDM

Mulch is designed to conserve soil moisture. Because soil hydrological properties are strong regulators of decomposer activity and plant productivity, it is useful tofe know the effects BDM has on soil moisture properties once it is incorporated into the soil (Jiang et al., 2017). BDM was modeled with variable-flux boundary conditions. Infiltration and evaporation were affected by mulch treatments. The deterioration of BDM enhanced the evaporation in the soil environment and reduced runoff, which has both positive and negative implications (Saglam et al., 2017). Additionally, water infiltration in soils increased by 10-12% more than soils without mulch. BDM reduced 4-7 kg ha^{-1} of nitrate leached into groundwater, which addresses concerns regarding the leaching of the material (Sintim et al., 2020). BDM degradation should ideally result in CO_2, H_2O, microbial biomass, and soil organic matter, which may contribute to improving soil quality (English 2019 and Serrano-Ruiz et al., 2021).

When *in situ* degradation of four types of BDM (2 commercially available starch-based films, 1 experimental spunbond polyactic acid mulch, & 1 commercially available cellulose paper mulch) was monitored in three different regions, the percent of area remaining (PMAR) of the mulch did not differ significantly at any location, providing evidence that climate and location do not influence the rate of decomposition of biodegradable mulch (Li et. al, 2014).When compared to mulchless soil, soil with BDM increased soil aggregate stability anywhere from 6-16% (Sintim *et al.,* 2020).

Biological Impacts of Plastic in Soils

Microbial Activity

Microbial life is one of the most important aspects of the soil environment. Microbes have many benefits: they help raise crop yields, enhance nutrient uptake, improve plants’ resistance to pests and diseases, and are the largest biological reservoirs of nitrogen (N) and phosphorus (P), which are plant essential nutrients (Harman et al., 2019).

Microbes and Plastic

Although plastic in soils and the impact plastic has on microbial communities has not yet been thoroughly studied, PE mulch has been shown to accelerate C:N metabolism. In turn, this will eventually deplete soil organic matter (SOM) and will allow for the release of greenhouse gases (Steinmetz et al., 2016). Microbes have a potential for accelerating degradation of used biodegradable plastics at field conditions. Biodegradable mulch is not harmful to the soil microbe community and can even help solve the plastic waste problem (Koitabashi et al., 2012).

The presence of fungi was found in soils after BDM had been sitting in the soil post-tilling for a total of 6 months. The fungi were isolated from the soil and the microbial communities' ability to colonize and degrade the same mulches in pure culture was observed. The majority of these fungi were in the family *Trichocomaceae*, which includes beneficial fungi like *Penicillium* and *Aspergillus*. Overall, no isolate substantially degraded any mulch, which means that perhaps biodegradable mulch is not able to decompose as easily as it is marketed. They also determined that the structure of soil microbial communities was significantly affected by geographical location, and not by specific mulch treatments, which could have resulted in skewed data regarding the rates of BDM decomposition (Acosta-Martínez et al., 2014)

BDM tends to influence soil microbial communities in two ways: First as a surface barrier before soil incorporation and second as a direct input of physical fragments after soil incorporation. The first instance indirectly affects the soil microclimate and atmosphere. On the other hand, the second instance adds carbon, microorganisms, additives, as well as adherent chemicals to the soil environment and to the atmosphere. They even found that BDM can result in enhanced microbial activity and can enrich certain fungal taxa, which is very beneficial information (Bandopadhyay et al., 2018).

3. METHODS

Site descriptions

To characterize the consequences of plasticulture on agricultural soil systems, we periodically surveyed four farms on the California Central Coast from January-July of 2021. These sites are in regions characterized by significant agricultural plastic use. The conventional plastic contaminated farms (Sites 1-3) are located in Watsonville, CA (36.910233, -121.756897), with a mean annual temperature (MAT) between 47-68 F and mean annual precipitation (MAP) of 23.5 inches, which falls predominantly during the winter wet season. These sample sites were selected to reflect sites that have extensive conventional plastic pollution. Site 4 is located in Davenport, CA (37.052683, -122.226323) and is characterized by long-term BDM use in some of its fields.

Site 1 is an active farm which rotates celery, fava beans, romaine, squash, pumpkins, peppers, tomatoes, brussels sprouts, and straw flowers in Monterey County where numerous fields within a ranch (195 acres) were contaminated with agricultural plastic in 2016. Site 1 is classified as a Santa Ynez fine sandy loam soil with 15 to 30 percent slopes. It is a moderately well drained alluvial soil derived from igneous and sedimentary rock. Site 2 is a fallowed strawberry field (about 50 acres) within a 107 acre parcel managed by the Elkhorn Slough Foundation set on an Arnold loamy sand with 9 to 20 percent slopes. It is a somewhat excessively drained soil from residuum weathered from sandstone. Site 2 was a strawberry farm for four decades, but since 2016 is managed the Elkhorn Slough Foundation (*Sandhill Farm*, 2017). The Elkhorn Slough is essential for our environment as it helps sequester carbon, filters and purifies water, and serves as a home for various forms of wildlife. Site 3 is a fallowed field (13 acres) that was contaminated with agricultural plastics in 2018 when tenants tilled remaining plastic into the soil. Sites 2 and 3 are comprised of standing plant biomass juxtaposed with macroplastic fragments. It is classified as an Arnold loamy sand with 15 to 50 percent slopes. It is a somewhat excessively drained soil from residuum weathered from sandstone. All three sites are now contaminated with various forms of conventional plastic: dripline, surface mulch, and miscellaneous pieces of litter.

Site 4 is a productive organic farm that has used BDM for nearly a decade in some of its fields but removes the material to compost offsite. It is located in Davenport, CA in Santa Cruz County (MAT = 57 ºF, MAP = 30 inches that falls primarily during the winter). Site 4 is classified as a Soquel loam with 2 to 9 percent slopes.

Experimental design

From January through July 2021, the study sites were sampled to assess existing plastic pollution levels and its potential impacts on a suite of biotic and abiotic edaphic properties (Gavlak et al., 2005). We used a transect design to estimate surface soil agricultural microplastic contamination at Sites 1 and 2. Due to time limitations and growth of vegetation, a transect survey of surface plastic contamination for Sites 3 and 4 will be completed in Fall 2021.

In February and March, 2021, surface microplastic fragment contamination was assessed at Sites 1 and 2. At Site 1, a 100 ft long transect was placed alongside the plastic-contaminated field, with a 100 ft perpendicular transect run every 20 feet. Every 10 ft point along the perpendicular transect, a $1m^2$ quadrat was centered and surface plastic was collected. We found 434.8 grams total surface plastic fragments that included both PE mulch and PVC tubing with an average of 6.6 ± 1.58 g macrofragments per $1m^2$ quadrat placed. At Site 2, a 180 ft. long transect was placed alongside the plastic-contaminated field, with a 100 ft perpendicular transect run every 20 feet. At each 10 ft point along the perpendicular 100 ft transect, a $1m^2$ quadrat was placed and surface plastic was collected. We found 95.8 grams total surface plastic fragments that reflected both PE mulch and PVC tubing with an average of 0.9 ± 0.69 g macrofragments per $1m^2$ quadrat placed.

Three 5 cm deep cores were randomly taken along the perpendicular transects at each site; these cores were homogenized at the transect level and were considered 'bulk' soil from the contaminated field and became the bulk PC soil. We also collected samples plastic associated soils: those in direct contact with buried PVC dripline ('dripline associated soil') and the other in direct contact with buried PE mulch ('mulch associated soil'). Equal numbers of soil subsamples were derived from the dripline-associated and mulch-associated plastic fraction (N = 10 at Site 1 and N = 8 at Site 2) to compare to the bulk soil replicates (N = 10 at Site 1 and N = 10 and Site 2). In June and July, 2021, we resampled these sites and added a third plastic-contaminated study field (Site 3). For this sampling effort, we used a block design, whereby 8 haphazardly placed blocks separated by at least 3 m (10 x 30 m at Site 1 and 10 x 20 m at Sites 2 and 3) were established in each site and all visible PVC dripline and PE mulch were collected from the quadrat area, as well as 8 bulk soil samples (top 5 cm) per site. Dripline associated, plastic mulch associated, and bulk soils within each quadrat were pooled to represent a field replicate (N = 8). During the March 2021 sampling of Site 4, there was an extensive cover crop present, and we were unable to find any remaining surface plastic. We then haphazardly sampled the top 5 cm of bulk soil (N = 6) from a block within a field that had no BDM use and from a neighboring block that had long-term BDM incorporation (N = 6); both blocks had rosemary planted with conventional plastic in 2005.

Soil biotic conditions

Microbial decomposer biomass

Substrate induced respiration (SIR) was used to estimate soil microbial biomass (Beare et al., 2002). SIR is a method of estimating active microbial biomass by providing a labile carbon (C) source of autolyzed yeast to drive a maximum potential respiration rate. Ten mL of 12g/L yeast extract was added to 6 grams of fresh soil (or 5 mL yeast extract to 3 g fresh soil when soil was limiting) in a half-pint size mason jar fitted with a gas tight septum (blue butyl rubber septum, Bellco Glass). Each jar shook at 240 rpm for fifteen minutes. A bench top infrared gas analyzer (IRGA, LI-COR 820) was used to measure CO_2 (ppm) at the initiation of the incubation period, two hours and four hours into the incubation. SIR is calculated as the slope of CO_2 (ppm) production over time per g dry weight soil.

Soil respiration

We measured the respiration at field conditions for the blocked design samples (Insam, 1990). Three grams of fresh soil were added to a half-pint size mason jar fitted with a gas tight septum (blue butyl rubber septum, (Bellco Glass). Respiration was measured three times over a 24 hour period (roughly 8 hours apart). The soils were kept at room temperature throughout the 24 hour period.

Decomposer community-level physiological profiling

BIOLOG EcoPlates were used to assess how plastic incorporation into agricultural soil impacts microbial community substrate use potential as an index of community-level physiological profile (Stefanowicz, 2006). Fresh soil samples were diluted with phosphate-buffered saline (PBS) to a concentration of 10^{-3} (g soil/ml PBS) using a serial dilution technique, with each dilution vortexed for 30 seconds100µl of the 10^{-3} soil extract dilution was pipetted into each well of the 96 well EcoPlate in triplicate (3 analytical replicates). A negative control (no soil) plate was included. Change in absorption over time was measured at 590nm (absorbance peak of tetrazolium) to evaluate color development plus turbidity and 750 nm to measure turbidity of dilutions is due to clay and humic particles in soil colloidal suspension (Sofo and Ricciuti, 2019**)**. Plates were measured at the same time for up to 6 consecutive days to measure the microbial growth on a Teacan Infinite M Nano Plus platereader.

Soil abiotic conditions

Total inorganic nitrogen via nutrient extractions (NO_3^-, NH_4^+)

Extractable N was assessed by adding 20 mls of 0.5M K_2SO_4 to 4 g of fresh soil (2 g for blocked samples) and shaking the slurry for one hour at 250 rpm (Hawkes Lab, NCSU). While the soils were shaking, Whatman 1 filter papers were folded and placed into funnels, which were then placed into 50 mul Falcon tubes. The soils settled for 5 minutes after shaking and were vacuum filtered into the 50 ml Falcon tubes using Whatman 1 filter papers.

Eight standard concentrations (10, 5, 2.5, 1, 0.75, 0.5, 0.25, 0 ppm NO_3^-) were prepared from the K_2SO_4 stock solution to create working standards (Doane and Horwath, 2003). The working standards as well as the soil samples mixed with nitrite reagent were pipetted into a 96 well plate. The plate sat covered with foil overnight and was read on a platereader at 540 nm (Teacan Infinite M Nano Plus platereader). Soil extractable NO_3^- was calculated using eq. 1:

1. $[NO_3^-]\ (\mu g/g\ soil) = \frac{[NO_3^-]\ in\ ppm \times extract\ volume\ in\ mL}{dry\ soil\ weight\ in\ g}$

Eight standard concentrations (5, 2.5, 1, 0.75, 0.5, 0.25, 0.1, 0 ppm $NH4^+$) were prepared from the K_2SO_4 stock solution to create working standards. The working standards as well as the soil samples mixed with ammonium reagent A and ammonium reagent B were pipetted into a 96 well plate. The plate sat covered with foil for one hour and was read on the Teacan Infinite M Nano Plus platereader at 650 nm. Soil extractable $NH4^+$ was calculated using eq. 2:

2. $$[NH_4^+]\ (\mu g/g\ soil) = \frac{[NH_4^+]\ in\ ppm \times extract\ volume\ in\ mL}{dry\ soil\ weight\ in\ g}$$

The individual amounts of nitrate (NO_3^-) and ammonium (NH_4^+) (µg/kg soil) were summed together to calculate the total inorganic nitrogen (TIN) content in the soil.

Permanganate Oxidizable Carbon (POXC):

To determine whether conventional or biodegradable plastic incorporation into the soil affects the readily oxidizable C fraction, POXC analyses were conducted following Weil et al. (2003). In brief, four standard concentrations (0.005, 0.01, 0.015, and 0.02M) were prepared from the $KMNO_4$ stock solution to create working standards. 2.5 g dry soil was weighed into 50 mL falcon tubes. There were two tubes per sample. One tube had the soil, water, and POXC reagent while the other had a diluted reagent. The dilution was added to the soil after shaking for two minutes and settling for 10 minutes (Culman et al., 2012). Next, the substrate was pipetted into a 96 well plate and analyzed by reading absorbance at 550 nm (Teacan Infinite M Nano Plus platereader). POXC was calculated using eq. 3.

3. $$POXC\ \left(\frac{mg}{kg}soil\right) = \left[0.02\frac{mol}{L} - \ (a + b\ *\ Abs)\right] * \left(9000\ mg\frac{C}{}mol\right) * \left(0.02\ L\frac{solution}{Wt}\right)$$

Water Holding Capacity:

Water holding capacity (WHC) was determined by weighing ~20 g of air dried soil into a small PVC tube, covered with Nylon, and placing the tube in 2 cm of water overnight until saturated. The soil was then placed into a tin and the wet weight was recorded. The soil was placed in the oven for 24 hours @ 105 °C and dry mass was recorded when the soil reached a constant mass with drying (Domeignoz-Horta, 2018). Soil WHC was calculated using eq. 4.

4. $$WHC = \frac{g\ soil\ Saturated - g\ soil\ Dry}{g\ soil\ Dry}$$

WHC was done on bulk PC soil as well as macroplastic fragments separated by surface mulch and dripline for all transect/quadrat samples. For the blocking design, WHC was only recorded for the bulk soil due to a lack of soil.

Statistical analysis

For the unblocked soil samples, an ANOVA and a Tukey post-hoc test were done for each method using R (R Core Team, 2021). The three different types of plastic association (bulk PC soil, dripline, and surface mulch) were all compared to each other. T tests were done for Site 4.

For the blocked soils, statistical analyses were done using “Block” nested within “Site” as a random variable. The emmeans (Length, 2021) and lme4 (Bates et al., 2015) packages were used to perform ANOVAs for each method.

All plots were made using the ggplot2 function on R (Wickham, 2016). Significant effects were reported when p-values were less than or equal to 0.05.

4. RESULTS

Soil biotic conditions

Decomposer community-level physiological profiling (CLPP)

CLPP was measured using Biolog Ecoplates. The richness, Shannon diversity, substrate preferences, and average well-color development (AWCD) were obtained based on spectrometer readings of the color development of all 96 wells during a 5 day period. Pairwise Tukey tests were done to determine the statistical significance of the previously stated variables by plastic association.

There were no significant differences for richness, Shannon diversity, and AWCD among plastic association types for both Site 2 and Site 4. The preferences for the following substrates were measured: amine, amino acid, carbohydrate, carboxylic acid, phenolic compounds, polymer. At Site 2, the surface mulch decomposers showed a significant reduced preference (P=0.056) for the carbohydrate group (Fig. 1A). At Site 4, the no BDM community showed a significant preference (P=0.045) for the amine group (Fig 1B).

This experiment was replicated after the initial visits to Site 2 and 4, yet the plates became contaminated on the third day of reading; we were not able to obtain any legitimate results due to the contamination of the wells.

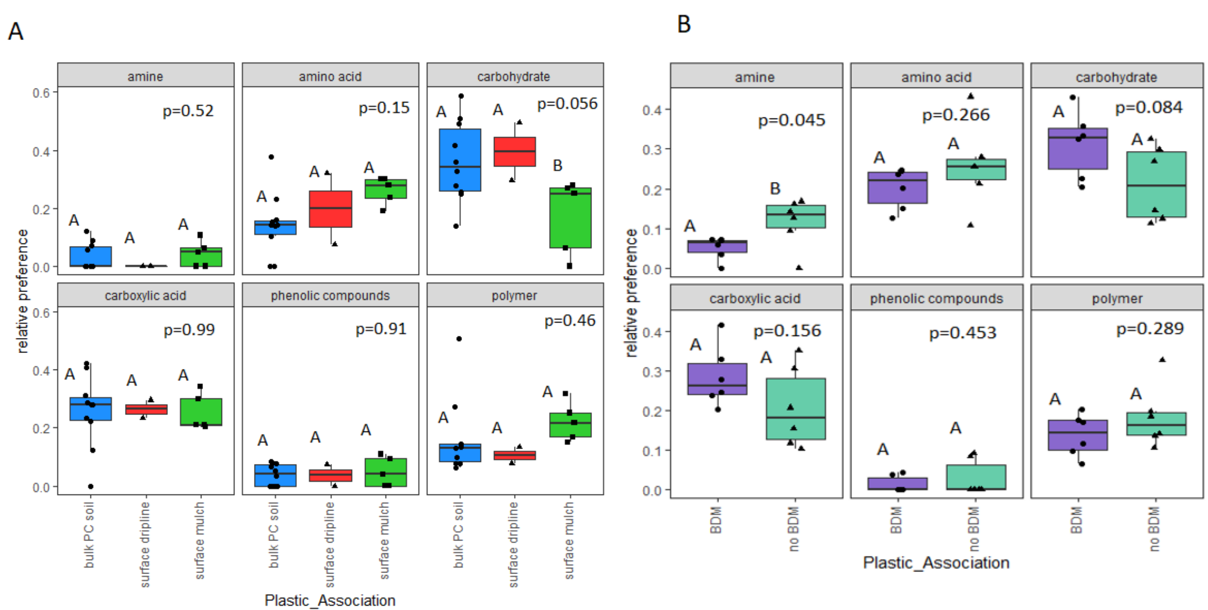

Figure 1: Substrate preferences from Site 2 (A) and Site 4 (B). The surface mulch community from Site 2 showed a significant reduced preference for carbohydrates (P=0.056). The no BDM community from Site 4 showed a significant preference for the amine group (P=0.045). The letters above each boxplot indicate the Tukey pairwise connecting letters among treatments.

Microbial decomposer biomass

We collected substrate-induced respiration (SIR) measurements over a four-hour time period to measure microbial decomposer biomass. To see if moisture had a contributing effect on respiration measurements, the gravimetric moisture content (θg) was obtained from each site. A pairwise Tukey test was done to determine the statistical significance of decomposer biomass by plastic association. Sites 1-3 followed the same pattern: microbial decomposer biomass was higher in soils directly associated with surface macroplastic fragments (Fig. 2 A-F). This pattern was also evident when the soils were analyzed using a blocking design (Fig. 4). The significance of this relationship varied by Site and Date (Table 1). There was no significant relationship ($P=0.3141$) between the presence of biodegradable mulch and microbial biomass at Site 4 (Fig. 3).

We were unable to find a significant relationship between gravimetric moisture content and microbial decomposer biomass at Sites 2, 3 and 4. Tomatoes and squash were planted during our second visit to Site 1, and the site had been freshly irrigated. We believe this significant difference in gravimetric moisture content between the bulk soil and the soil associated with macroplastic fragments ($P=0.0000000$) is responsible for the lack of significance in microbial biomass; this set of measurements for microbial biomass are the only ones which are insignificant.

Table 1: Microbial decomposer biomass p-values from Sites 1-3. Significant values are presented in bold.

Site/Date	Bulk PC soil-dripline	Bulk PC soil-surface mulch	Dripline-surface mulch
Site 1/March 2021	**P=0.0000000**	**P=0.0000000**	P=0.0802094
Site 1/June 2021	P=0.6314	P=0.2064	P=0.6686
Site 2/February 2021	**P=0.0247843**	**P=0.0000000**	**P=0.0000041**
Site 2/March 2021	**P=0.0000000**	**P=0.0000000**	P=0.0802094
Site 2/July 2021	**P=0.0007**	**P=0.0167**	P=0.1231
Site 3/June 2021	**P=0.0281**	**P=0.0463**	P=0.9620
Sites 1-3 (block)	**P=<0.0001**	**P=<0.0001**	P=0.9984

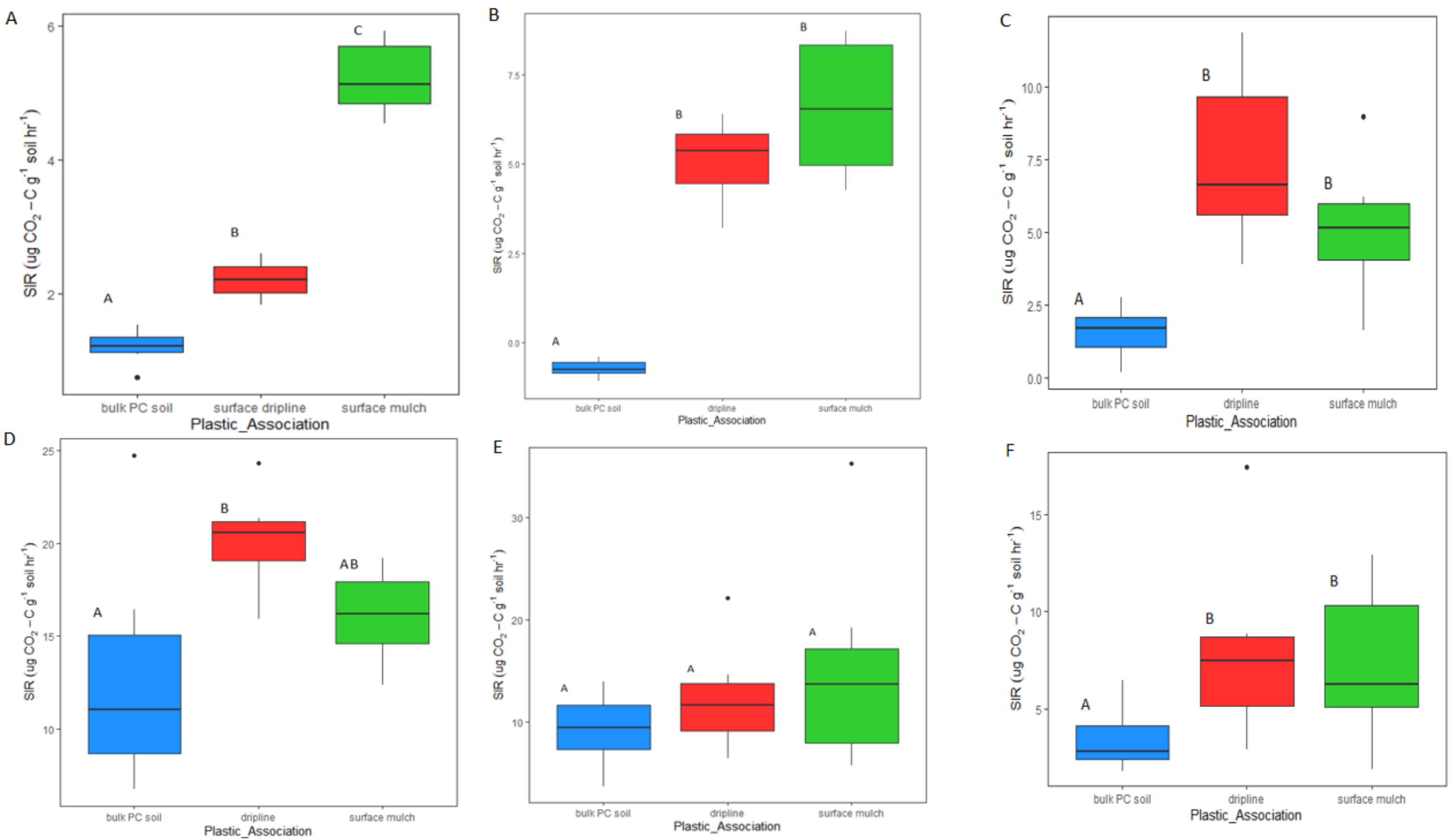

Figure 2: Microbial biomass for Sites 1 (D&E), 2 (A, B, & C) and 3 (F). The letters above each boxplot indicate the Tukey pairwise connecting letters among treatments.

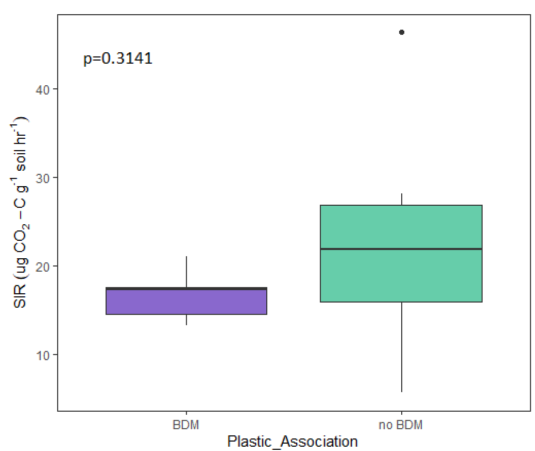

Figure 3: Microbial biomass for Site 4. No significant difference was found between BDM and no BDM.

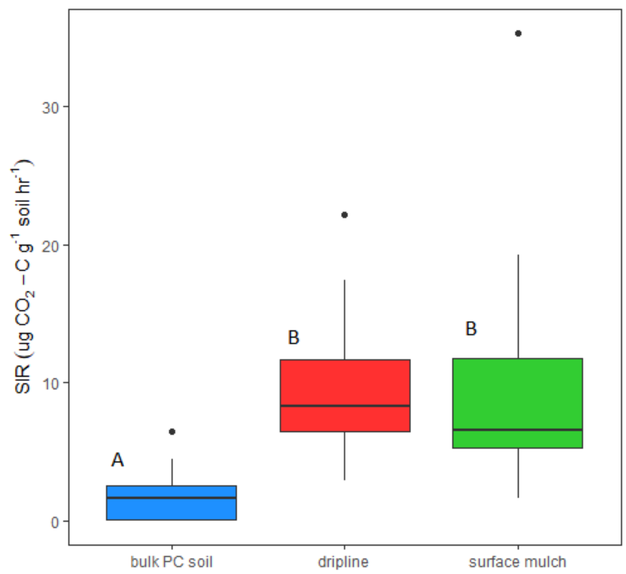

Figure 4: Microbial biomass for Sites 1-3 with a blocked design. The letters above each boxplot indicate the Tukey pairwise connecting letters among treatments.

Soil basal respiration

The soil respiration was measured at Sites 1, 2 and 3. The results were analyzed using a blocking design; block was nested within site. At Site 1 (Fig. 5A) the soil respiration was significantly higher in the bulk PC soil than in the dripline and surface mulch soils (P=0.0015, 0.0017 respectively). At Site 2 (Fig. 5B), there were no significant differences in respiration measurements among the three types of soils. At Site 3, respiration was significantly higher in the surface mulch soil than the bulk PC soil and the dripline soil (P=0.0016, 0.0066 respectively). When all sites were analyzed together, respiration measurements did not follow the same pattern as SIR measurements (Fig. 6). The dripline community's respiration was significantly lower than the bulk PC soil (P=0.0206). This is evidence for less efficient microbial communities from the surface mulch and dripline samples.

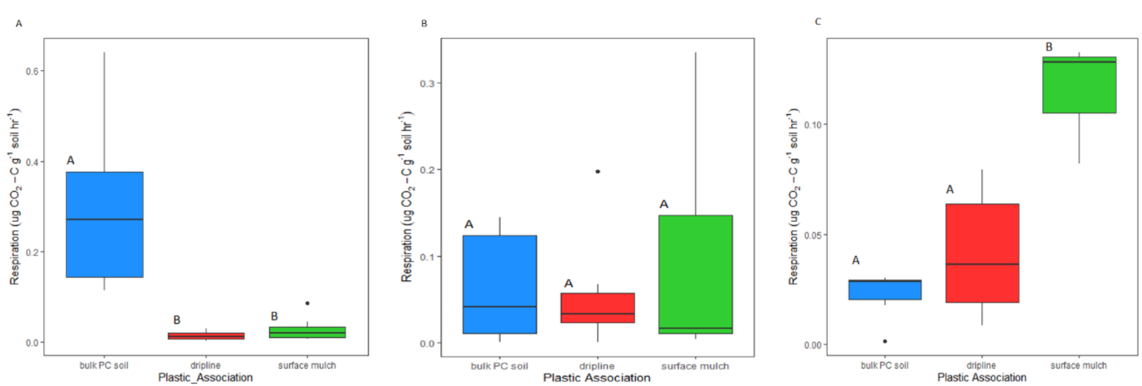

Figure 5: Soil basal respiration measurements for Site 1 (A), Site 2 (B) and Site 3(C). The letters above each boxplot indicate the Tukey pairwise connecting letters among treatments.

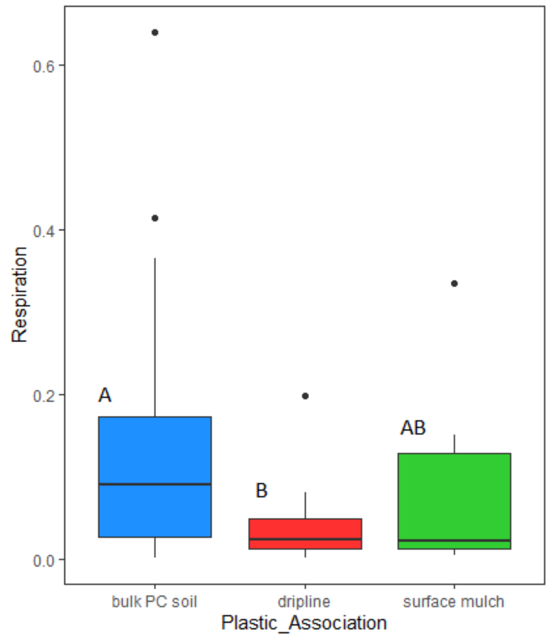

Figure 6: Soil respiration for Sites 1-3 with a blocked design. The letters above each boxplot indicate the Tukey pairwise connecting letters among treatments.

Soil abiotic conditions

Total inorganic nitrogen content

The concentrations of ammonium and nitrate were summed together to obtain the total inorganic nitrogen content (TIN) within each soil. At Sites 1-3 TIN was higher in the soils directly associated with macroplastic fragments. The relationship between TIN concentrations and plastic association over the three soil types were determined using a pairwise Tukey test; the significance of this pattern varied at each site. Blocks were used as a random variable in the ANOVA analyses. At Site 1 (Fig. 7A), TIN in the dripline soil was significantly higher than in the bulk PC soil (P=0.0273). While the surface mulch appeared higher, this relationship was not significant (P=0.0995). When the two plastic associated soils were compared to each other, there was not a significant difference between them (P=0.7652). At Site 2 (Fig. 7B), there were no significant differences among plastic associations. At Site 3 (Fig. 7C), the TIN concentrations in both the dripline soil and the surface mulch soil were significantly higher than in the bulk PC soil (P=0.0001 and P=0.0013 respectively). The difference in TIN between the two plastic associated soils was insignificant (P=0.4747). At Site 4, a t test was performed to determine the difference between TIN in the BDM field and the no BDM field (Fig. 8). Similarly, TIN was significantly higher in the field which contained BDM (P=0.006176). When TIN was analyzed using block nested within site as a random variable, both the dripline and the surface mulch values were significantly higher than the bulk PC soil (P=0.0001 and P=0.0006 respectively). There was no significant difference between dripline and surface mulch (P=0.6536, Fig. 9).

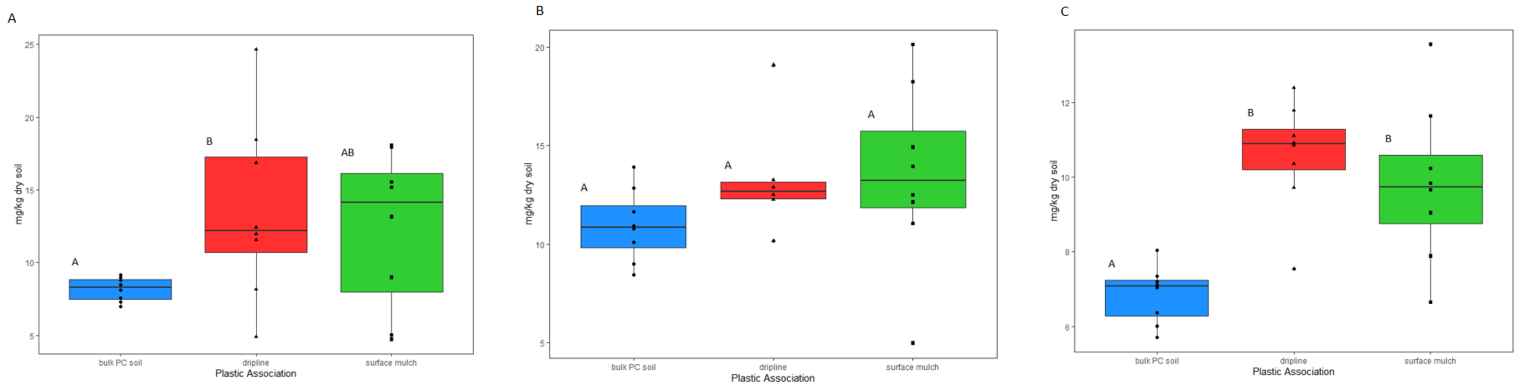

Figure 7: Total inorganic nitrogen for Site 1 (A), Site 2 (B), and Site 3 (C). The letters above each boxplot indicate the Tukey pairwise connecting letters among treatments.

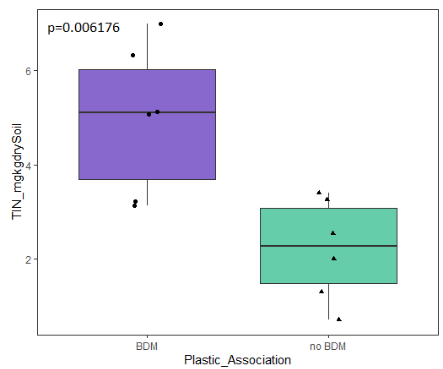

Figure 8: Total inorganic nitrogen content at Site 4. The TIN content was significantly higher in the BDM soil (P=0.006176).

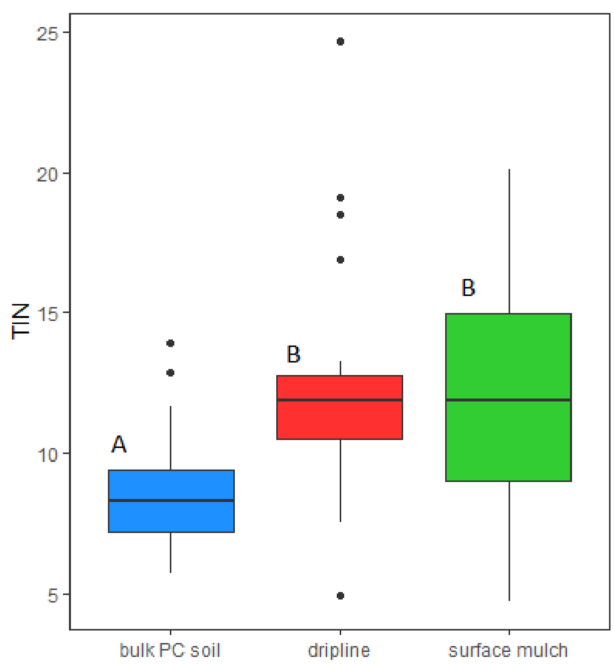

Figure 9: TIN for Sites 1-3 with a blocked design. The letters above each boxplot indicate the Tukey pairwise connecting letters among treatments.

Active carbon content-POXC

POXC was performed to determine the amount of active carbon in the soils. At Sites 1-3 POXC was higher in the soils directly associated with macroplastic fragments. The relationship between the amount of POXC and plastic association over the three soil types were determined using a pairwise Tukey test; the significance of this pattern varied at each site. Blocks were used as a random variable during the ANOVA analyses. During the first visit to Site 1 (Fig. 10A) there were no significant differences in POXC among the three types of soil. However, during the second visit to Site 1 (Fig. 10D) the POXC in both the dripline soil and the surface mulch soil was significantly higher than in the bulk PC soil ($P=0.0113$ and $P=0.0370$ respectively). There was no significant difference between the two macroplastic fragment soils ($P=0.8119$). During the first visit to Site 2 (Fig. 10B) POXC was significantly higher in the dripline soil than in the bulk PC soil ($P=0.0283788$). POXC in the surface mulch as not significantly different than the bulk PC soil and the dripline soil ($P=0.1538337$ and $P=0.7251326$ respectively). During the second visit to Site 2 (Fig. 10E) there were no significant differences in POXC among the three types of soil. At Site 3, POXC was significantly higher in the dripline soil than in the bulk PC soil ($P=0.0318$). POXC in the surface mulch was not significantly different than the bulk PC soil and the dripline soil ($P=0.7717$ and $P=0.1122$ respectively). When POXC was analyzed using block nested within site as a random variable (Fig. 12), the dripline values were significantly higher than the bulk PC soil ($P=0.0001$). There was no significant difference between the bulk PC soil and the surface mulch ($P=0.0611$) and the dripline and the surface mulch ($P=0.0836$).

At Site 4, a t test was performed to determine the difference between POXC in the BDM field and the no BDM field (Fig. 11). There was not a significant difference in POXC between the two fields ($P=0.2145$).

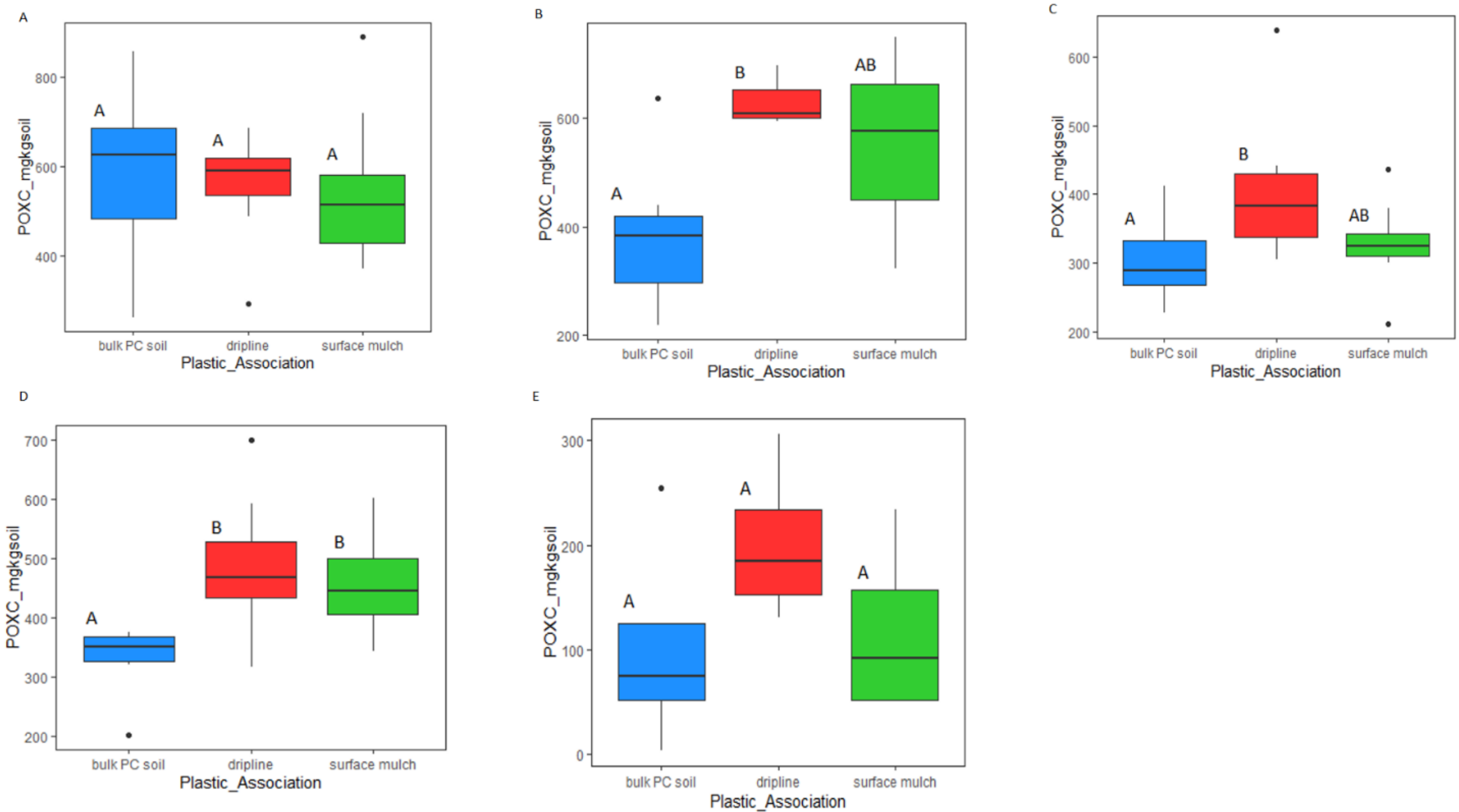

Figure 10: POXC (mg/kg dry soil) for Site 1 (A & D), Site 2 (B & E) and Site 3 (C). The letters above each boxplot indicate the Tukey pairwise connecting letters among treatments.

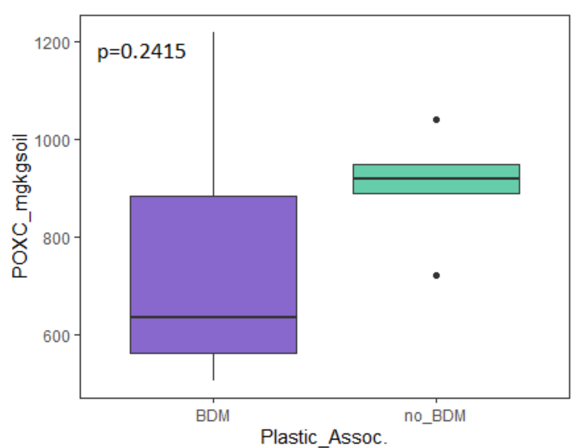

Figure 11: POXC (mg/kg dry soil) for Site 4. There was no significant difference between BDM and no BDM (P=0.2415).

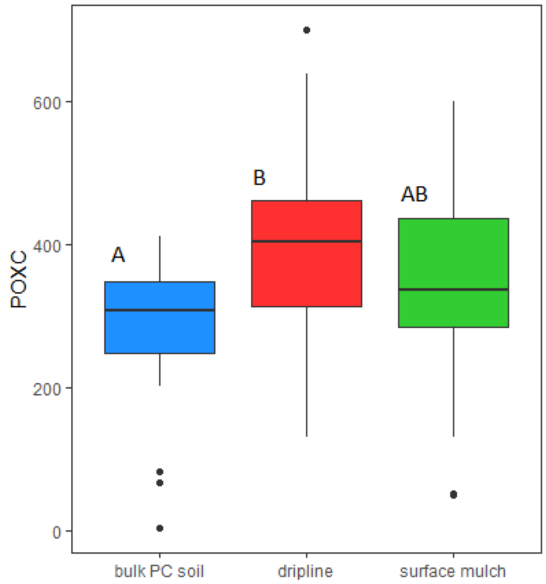

Figure 12: POXC for Sites 1-3 with a blocked design. The letters above each boxplot indicate the Tukey pairwise connecting letters among treatments.

Water holding capacity

The WHC was measured at each site. At Site 1 (Fig. 13A), WHC was significantly higher in the dripline soil than in the bulk PC soil (P=0.0261737). WHC in the surface mulch was not significantly different than the bulk PC soil and the dripline soil (P=3297862 and P=0.4634835 respectively). At Site 2 (Fig. 13B) there were no significant differences in WHC among the three plastic associated soils. At Site 3 (Fig. 13C), the WHC was only done for the bulk PC soil as there was not enough soil from the macroplastic fragment associated soils.

At Site 4, a t test was performed to determine whether the differences in WHC between the BDM field and the no BDM field were significant (Fig. 13D). There was no significant difference in WHC at Site 4 (P=0.4416).

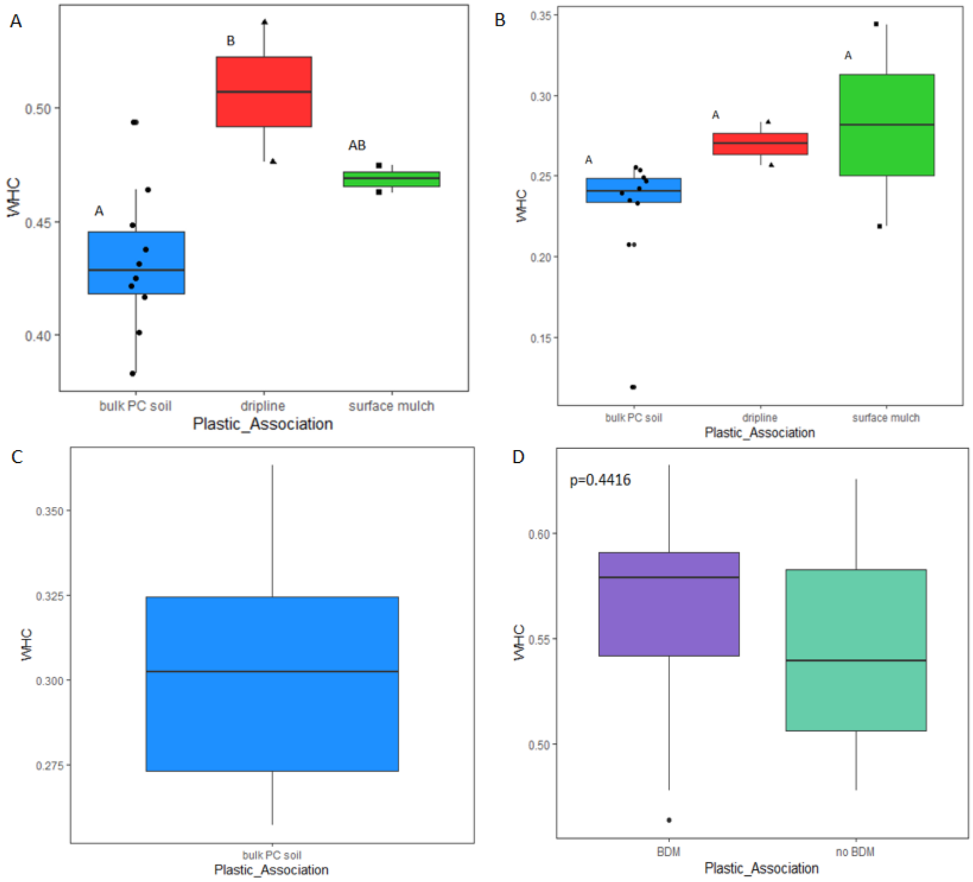

Figure 13: WHC for Site 1 (A), Site 2 (B), Site 3 (C) and Site 4 (D). The letters above each boxplot indicate the Tukey pairwise connecting letters among treatments. The WHC was only obtained for the bulk PC soil for Site 3. There was no significant difference for WHC for Site 4 (P=0.4416).

5. DISCUSSION

The presence of macroplastic fragments is omnipresent within the soil environment due to the vast capabilities of plastic in agricultural systems. Attempts have been made to quantify plastics within soils, yet the biological implications of plastic are still unknown (Fakour et al., 2021, Piehl et al., 2018). Studies mostly focus on microplastics, but it is essential that we first understand the implications of macroplastic fragments in the soil environment as macroplastics presumably break down to micro- and nanoplastics. These fragments will remain in soils indefinitely, leaching toxins into the environment (Steinmetz et al., 2020). For the protection of both the environment and public health, it is essential that we continue to study macroplastic fragments. Our study found that when macroplastic fragments are present in low carbon, fallowed soils, unique habitats form on the surface. This effect is muted when these sites are irrigated and fertilized, evident in Site 1.

At Sites 1-3, total inorganic nitrogen, POXC, and microbial biomass were highest in the surface macroplastic fragments in both the transect/quadrat and blocking design methods. Although significance varied, a clear pattern regarding higher microbial biomass was evident. The respiration data does not follow the same pattern, suggesting that the dripline and surface mulch communities are less efficient. This is the basis for our idea of a new biological hotspot within the plastisphere, which can be thought of like the rhizosphere due to shifts in biogeochemical signatures. We will continue to monitor both microbial biomass and soil respiration as we revisit these sites and expand to others.

Our results consistently showed that the presence of conventional macroplastic fragments in agricultural fields altered the behavior of soil decomposers and soil physical properties. The presence of biodegradable mulch also altered soil abiotic and biotic factors. It is important to understand whether the incorporation of plastic in soils overtime will change, as our results suggest. Our results provided evidence for the formation of a novel habitat on the surface of macroplastic fragments. Overall, it is apparent through these changed factors that the addition of plastic, regardless of its degradability, has an impact on overall soil health. The consequences of these impacts are unknown and will need to be further monitored. We will continue to monitor these fields, as it is important to understand how these systems change over time. Additionally, we will add a BDM incubation trial so that we can explore alternatives to conventional plastic.

6. CONCLUSION

Since farmers heavily rely on plastic in modern agriculture, it is important to study the implications of plastic within the soil environment. Although this paper aims to discuss the abiotic and biotic changes due to plastic, this paper also aims to spread awareness regarding the sheer amount of surface plastic that remains in soils after removal, as this number has not been officially quantified. We will continue to revisit Sites 1-4 and hope to expand this study by visiting more plastic contaminated sites.

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