THESIS

THE EFFECTS OF SOIL STRUCTURE ON SOIL ORGANIC MATTER FORMATION AND PERSISTENCE: A MECHANISTIC APPROACH

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ABSTRACT

THE EFFECTS OF SOIL STRUCTURE ON SOIL ORGANIC MATTER FORMATION AND PERSISTENCE: A MECHANISTIC APPROACH

Two key factors theorized to affect soil organic carbon (SOC) dynamics are type of plant carbon (C) inputs and soil structure (i.e., soil aggregation), both are influenced by management practices and are considerably intertwined. Research surrounding these factors has increased in the last several decades as the threat of climate change has forced policy makers to find natural based solutions to rising CO_2 levels in Earth's atmosphere. Given that soil acts as the largest terrestrial C pool but has lost substantial amounts of C due to land use change and unsustainable agriculture, focus has shifted towards identifying better ways to manage arable lands that improve SOC storage. Among the conventional management practices tillage is likely the most studied, because of its damage to soil structure, leading to soil C losses. However, while research centered on tillage effects on soil aggregation and SOC cycling is vast, few studies explore how plant C input type (i.e., soluble *versus* structural) and disturbance (i.e., tilling) together affects SOC in soils with different degrees of aggregation.

We examined the effects of soil texture, disturbance, and plant input type on soil aggregation, C mineralization, and formation and persistence of plant input-derived SOC to better understand the mechanisms by which soil aggregates help form and protect SOC, specifically as particulate and mineral associated organic carbon (POC and MAOC). POC and MAOC are expected to be formed by distinct pathways, respectively from structural and soluble inputs. Because of their different mechanisms of protection, POC and MAOC are also expected to respond

differently to plant inputs and management practices, like tilling, that disturb soil aggregates. We aimed to parse the formation and persistence of POC and MAOC by adding ^{13}C labeled plant residue separated into soluble and structural plant constituents to determine how these physically distinct plant compounds contribute to either pool when soil is intact or disturbed. In an in-lab incubation using ^{13}C enrichment, we traced SOC over the course of one year in a factorial design with four factors: soil type*disturbance*plant input*harvest. Our results showed, as expected, that hot-water extractable (HWE) plant inputs contributed substantially to MAOC while structural plant components (SPC) inputs preferentially formed POC. Interestingly, we found that disturbance resulted in less HWE mineralized to CO_2 and more MAOC formation in the highly aggregated (HA) soil suggesting that increased mineral surface area caused more efficient dissolved OM sorption. Moreover, HWE-derived MAOC persisted in both the undisturbed (U) and disturbed (D) HA soils but not in low aggregation (LA) soils, indicating that persistence of MAOC is dependent on soil type and aggregation (i.e., soil physical structure). Although we did not observe significant differences in aggregate-occluded POC (oPOC) formation between HAD and LAD soils, we did see higher oPOC persistence in HAD soil compared to LAD soil. Greater accumulation oPOC in HAD from day 22 to the end of the incubation suggests, again, that soil type influences the persistence of POC through occlusion in aggregates. To corroborate this, we also found that LAD soil had the highest CO_2 mineralization of SPC plant inputs as SPC was left more unprotected in the soil with a low capacity to aggregate. Disturbance did not affect microbial biomass in either HA or LA soils. We saw more plant-derived microbial biomass C from HWE inputs compared to SPC inputs in the bulk soil, indicating that HWE inputs are assimilated into microbial biomass, thus incorporated into SOC with higher efficiency. Lastly, there was a significant drop in % plant-derived microbial biomass C in the bulk soil overtime, as expected.

However, because the % HWE-derived MAOC persisted in HA soils regardless of disturbance, we illustrated the importance of microbial necromass in addition to direct DOC sorption for SOC stabilization as MAOC. Overall, my study provides mechanistic understanding for the role of soil structure and aggregation on POC and MAOC formation and persistence which can help improve the representation of these processes in models, to provide better predictions of SOC changes with changes in management practices affecting disturbance.

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CHAPTER 1: INTRODUCTION

Globally, soil has an incredible capacity to store carbon (C) as soil organic matter (SOM), storing more carbon than the atmosphere and terrestrial vegetation combined (Jobbagy & Jackson, 2000). However, decades of land use change and unsustainable agricultural practices have depleted soil organic C (SOC) substantially, resulting in more CO_2 to the atmosphere. An estimated 133 Pg of SOC has been lost after conversion from native land to agricultural land alone (Sanderman et al., 2017). Moreover, mismanagement of agricultural systems promotes continued loss of SOC. Two key factors theorized to affect SOC dynamics are the type of C inputs and soil structure (i.e., soil aggregation), both influenced by management decisions and considerably intertwined.

Fresh plant residues improve soil structure by helping stabilize large (macro) aggregates (Blanco-Canqui & Lal., 2004) as coarser plant fragments are enmeshed with larger mineral particles and aggregate surfaces (Lutzow et al., 2006). Additionally, the amount and type of plant residues affects the properties of soil aggregates because distinct plant constituents interact with soil particles differently (Jastrow et al., 1998) but few studies relate C input physical properties to aggregate formation and stability (Chan and Heenan, 1999) even today. Amelung & Zech (1996) found that lignin, representative of structural inputs higher in C:N, was more decomposed on aggregate surfaces than inside aggregates, indicating persistence of lignin with physical protection from aggregate structures. In contrast, Poirier et al. (2005) concluded that lignin had no relative contribution to SOC inside of aggregates. And although lignin is more resistant to decomposition (Rasse et al., 2005) and represents a large proportion of inputs from plants to SOM (Kogel-Knabner, 2002), it is no longer believed to contribute to stable forms of SOM, which are rather believed to be derived from low molecular weight (LMW) compounds through bonding on silt and

clay sized minerals (Mikutta et al., 2006). Input of LMW soluble compounds, such as that from root exudates or plant input leachates, does in fact result in the most efficient formation of the mineral associated fraction of SOC, known as MAOC (Sokol et al., 2019; Cotrufo et al., 2022). However, we have limited knowledge of how MAOC formation from soluble inputs is affected by or affects soil aggregation.

Previous studies have shown mean weight diameter (MWD), a single quantitative indicator of soil structure (van Bavel, 1950), to be closely related to SOC concentrations, especially with particulate organic carbon (POC) (Samson et al., 2020). Despite this growing knowledge, there is still a lack of understanding regarding the mechanisms by which aggregation aids in long term SOC protection. Specifically, little attention has been given to how aggregates and soluble plant inputs interact. Since plant input physical properties (i.e., structural versus soluble) influences the partitioning of C inputs into SOC pools, such as POC and MAOC (Cotrufo et al., 2015; Cotrufo et al., 2022) that have varying functional attributes, turnover times, and stabilization mechanisms (Cotrufo and Lavallee, 2022), it's valuable to consider how soluble versus structural plant input components affect and are affected by soil aggregation separately. Certain SOC pools may have a higher dependency on aggregate stability than others. Moreover, soluble and structural plant inputs may promote aggregate formation differently but how does that affect their plant input derived C stabilization in POC and MAOC?

This Thesis explores how soil structure affects the formation and persistence of particulate organic carbon and mineral-associated organic carbon, by following the two-pathway model of POC and MAOC formation (Cotrufo et al., 2015). The first is formed through the physical transfer of structural plant inputs that defragment and decompose to form POC (Grandy & Neff, 2008; Haddix et al., 2016). In contrast, MAOC forms through direct sorption of dissolved organic carbon

(DOC) on to mineral surfaces, typically referred to as *ex-vivo*, or through the *in vivo* microbial assimilation of DOC, and subsequent sorption of microbial necromass on minerals (Kaiser and Guggenberger, 2000; Sokol and Bradford, 2019; Liang et al., 2017). Since soluble plant inputs, through exudation and plant input leachates, contribute to DOC in soil, they result in high MAOC formation (Sokol et al., 2019; Cotrufo et al., 2022). Additionally, microbial abundance and activity control both POC and MAOC formation and persistence by regulating plant structural input depolymerization, as well as the assimilation of DOC and its mineralization or necromass production.

Unlike MAOC, POC is not protected by chemical bonding to minerals (Lavallee et al., 2020), thus physical protection of POC is critical for managing systems that promote soil C storage and maintain crop productivity. Thus, in aerated mineral soils POC is less stable than MAOC (Cotrufo & Lavallee, 2022) and has been considered a sensitive indicator of land use change and disturbance (Chan, 2001; Kolbl and Kogel-Knabner, 2004). Persistence of POC differs depending on whether it is free (fPOC) or occluded (oPOC) within soil aggregates. If left undisturbed, oPOC can persist more similarly to MAOC (Haddix et al., 2020) and a higher mean residence time (MRT) has been observed in oPOC compared to fPOC (Puget et al., 2000). In one such study, oPOC showed a higher MRT by 30 years (Liao et al., 2006) determined by using natural abundance $\delta^{13}C$.

Disturbance increases aggregate turnover (Six et al., 1998) making oPOC once protected within an aggregate structure more vulnerable to microbial decomposition, thus more easily mineralized to CO_2 (Gupta & Germida, 1988; Elliot, 1986, Tebrugge & During, 1999; Six et al., 2002). For example, management practices like intensive tilling have been shown to disturb soil structure and destabilize soil aggregates, which can be detrimental to a soil's ability to store SOC long term (Pagliai et al 2004; Conant et al., 2007; Pires et al., 2017). Exposed oPOC via tillage

reduces SOC accumulation through increased SOC turnover (Blanco-Canqui & Lal., 2004). A recent meta-analysis showed that out of three structural soil indicators, namely wet aggregate stability, bulk density, and soil penetration resistance, wet aggregate stability was the most sensitive to tillage (Nunes et al., 2020).

Conversely, MAOC may not be as vulnerable to physical disturbance since the formation of MAOC often involves strong chemical bonds between the organic anions and clay particles, making MAOC more difficult to destabilize and for microbes to utilize and degrade (Blanco-Canqui and Lal, 2004). The microbial efficiency matrix stabilization (MEMS) framework introduced a paradigm shift in our understanding of MAOC formation where Cotrufo et al. (2013) hypothesized that MAOC was most prominently formed through labile, soluble plant components made accessible during early-stage decomposition and efficient microbial transformation. This theory has been supported in multiple studies (e.g., Kallenbech et al., 2016; Lavallee et al., 2018; Cotrufo et al., 2022) but none have explored how disturbance may affect this pathway. A conceptual model has been developed which incorporates physical protection of MAOC within microaggregates (< 250 µm) that are mediated by macroaggregates (> 250 µm) (Six et al., 2000b), corroborated by the findings of Fulton-Smith and Cotrufo (2019), but we lack understanding of how persistence of MAOC alone may change if physical protection is jeopardized.

Physical protection is built through plant inputs, microbes, and soil mineral interactions. Jastrow (1996) was one of the first soil scientists to describe this process, explaining that fresh residues promote the formation of macroaggregates as microbial, and plant derived mucilage binds soil mineral particles together. Plant residues broken down by microbial processes control the aggregation of soil particles (Watts et al., 2001) while soil structure regulates the biodegradation of organic residues by microbial decomposers at the micron scale (Juarez et al., 2013; Basile-

Doelsch et al., 2020), thus can affect both POC and MAOC. Because the size and activity of the microbial biomass C (MBC) can regulate SOC formation and mineralization (Li et al., 2018; Thiessen et al., 2013), I included the quantification of MBC in my study.

Knowledge gaps in the mechanisms by which aggregation affects distinct SOM pools coupled with the role soluble *versus* structural plant input chemistry may play in these dynamics motivated my research. My overarching objective was to determine how soil structure affects the formation of DOC, fPOC oPOC, and MAOC from the addition of soluble or structural inputs, and their persistence, and in turn how these inputs contribute to regenerate structure in disturbed soils with inherently different aggregation. To achieve this objective, I set up a year long incubation experiment where I followed the fate of ^{13}C-enriched soluble (i.e., hot water extractable, HWE) and structural plant C (SPC) inputs into a fine textured highly aggregated (HA) soil and a coarse textured low aggregated (LA) soil, which were either disturbed (D) to break their structure or left undisturbed (U). I traced the input derived ^{13}C as it mineralized to CO_2 or formed DOC and free POC and stabilized into occluded POC and MAOC fractions.

With this experiment, I first asked, will the addition of plant inputs in soils stimulate aggregation? Is the degree of stimulation dependent on the inherent capacity of soils to aggregate (i.e., higher capacity in finer texture), their degree of disturbance and on the physical property of the input, i.e., soluble *versus* structural? I hypothesized that the fine textured disturbed soil would aggregate most after the addition of SPC inputs, because of efficient macroaggregate regeneration around SPC. Secondly, I asked does the formation and stabilization of POC and MAOC from SPC and HWE differ in soils with different aggregation? I hypothesized that HWE inputs would preferentially result in MAOC formation in highly aggregated, undisturbed (HAU) soil through diffusion and direct sorption to mineral surfaces and/or after incorporation into microbial biomass.

By contrast I hypothesized that SPC would preferentially result in fPOC and oPOC with higher oPOC found in the disturbed fine textured (HAD) soil, again, because of efficient physical protection from macroaggregate formation.

My third question was do soil aggregates protect soluble and structural plant components by way of different mechanisms and, if so, how will that affect different SOC pools? I hypothesized that HWE-derived MAOC persistence would not be modified by aggregation since organo-mineral bonding may withstand disturbance while SPC-derived POC persistence would be strongly increased by occlusion in aggregates. Lastly, I analyzed how disturbance affected plant input-derived microbial biomass C (pd-MBC) in the bulk soil. I predicted that pd-MBC would be higher from HWE inputs at each time point, regardless of disturbance. However, I thought that % pd-MBC would be less with SPC inputs in the disturbed soils because SPC is depolymerized *exvivo*, resulting in less efficient assimilation into MBC.

This type of novel research can enhance our understanding of how POC and MAOC benefit uniquely from aggregate protection, how plant input types influence distinct SOC pools, are affected by and influence aggregate stability, and which soil types have a higher capacity to regenerate SOM in degraded lands. Additionally, it could help inform SOM models that work to improve predictions of SOM-C dynamics in managed systems particularly. My hope is that this study will inform management decisions that encourage regenerative agriculture while also benefitting modeling predictions related to global changes of soil organic matter.

CHAPTER 2: MATERIALS AND METHODS

2.1 Soil collection and processing

In order to source two soils with contrasting levels of aggregation, physical, and chemical properties, I collected soil samples from the Long-Term Ecological Research site at the Konza Prairie Biological Station (KPBS) in eastern Kansas (39·0931 °N, 96·5586 °W) and at the State Forest State Park (SFSP) in northern Colorado (40·3041 °N, 106·0037 °W). Soils at KPBS are fine textured silty clay loams classified as Mollisols. The mean annual temperature (MAT) at KPBS is 12.9^{o} C and the mean annual precipitation (MAP) is 835 mm. Big bluestem (*Andropogon gerardii*) dominates the tallgrass prairie there with several other grass species, forbs, and woody plants (Knapp et al., 1998). The SFSP soil is classified as a Larand fine sandy loam (USDA, 1973). SFSP has a MAP of 597 mm and a MAT of 1.8^{o} C (State Forest State Park Management Plan, 2019). The dominant landscape is subalpine, lodgepole pine (*Pinus contorta*), and aspen (*Populus tremuloides*) forests. After particle distribution analysis, we confirmed both texture classifications.

In the fall of 2019, I collected topsoil (0-10cm) from both sites by spade from an area of 100 * 100 cm and promptly transported it in a cooler to the laboratory. There, soils were maintained at 4^{o}C until processed. Fresh soils were 8mm sieved, removing coarse rocks and plant material. A 30g 8mm sieved subsample was taken and oven-dried at 105^{o} C for 72 hours to determine soil moisture and porosity. The remaining soil was air-dried until used for the incubation experiment and further characterization analyses.

2.2 Incubation experiment design

The yearlong incubation experiment was set-up in a factorial design with four soil types (two soils * two disturbance levels), three plant input treatments and two harvest times, with each soil*plant input*harvest combination replicated four times, resulting in 96 total units. The two soils were renamed according to their aggregation level, see below how this was determined. The KBPS soil represented the highly aggregated soil while the SFSP soil was the low aggregated soil.

To generate disturbed soils, a large subsample of each 8mm sieved, air-dried soil was manually crushed in a large mortar and pestle to pass through a 250 µm sieve, ensuring disruption of all macroaggregates (Denef et al., 2002). This created two disturbance levels for each soil type, resulting in the four soil types of our experiment:

HAU: high aggregation undisturbed,

HAD: high aggregation disturbed,

LAU: low aggregation undisturbed,

LAD: low aggregation disturbed.

The three plant input treatments consisted of: a control with no addition, a structural plant C (SPC) addition, and an addition of plant hot water extractable (HWE) organic C. The two destructive harvests took place after 22 days (H1) from the beginning of the incubation to assess short-term input-derived SOC formation from the plant inputs and after 366 days (H2) to assess later SOC formation, and its persistence.

Each unit consisted of a soil sample placed in 110 mL specimen cups inside 1-gallon jars with fitted septa. An amount of 75.14g HAU, 87g LAU, 75g HAD, and 75g LAD was used for

each replicate (n=4). The mass difference was based on the initial water stable aggregate data reporting that 0.18% of HAU and 16.02% of LAU was made of gravel > 2000 µm, respectively. Mass was added so that all treatments had 75g of soil. Additionally, I made sure to remove rocks > 2000 µm while disturbing soils so that the rocks were not crushed into the 75g of soil for the D treatments.

2.3 Labeled plant input production and processing

The intermediate wheatgrass Kernza™ (*Thynopyrum intermedium*) was grown in a dual (^{13}C and ^{15}N) isotope labelling chamber as described in Soong et al. (2014). At maturity, the kernza was removed from the chamber. The aboveground biomass was harvested by clipping above the crown to separate roots from shoots and oven-dried at 60° C. Aboveground plant input was then cut into 2.5-3 cm pieces and separated into soluble (HWE) and structural (SPC) materials by boiling 50 g in 2L of DI water on a hot plate at 105° C for three hours. The HWE was then filtered through 20 µm mesh and freeze-dried. The remaining SPC was rinsed with DI and oven-dried at 60° C. A subsample of the bulk, SPC, and HWE plant input was finely ground with a ball mill and analyzed on an Elemental Analyzer – Isotopic Ratio Mass Spectrometer (EA-IRMS: Costech ECS 4010 elemental analyzer, Italy coupled to a Thermo-Fisher Delta V Advantage IRMS) to determine % C, % N, ^{13}C atom%, and ^{15}N atom%. The initial plant input data is presented in Table 1. For this work, I only report and discuss C data and will revisit N data at a later time.

The SPC and HWE were mixed into the air-dried soil to avoid disturbing soil aggregates. The SPC was mixed in at a rate of 3.5 mg C/g soil, similar to Gentile et al. (2011) and the HWE was added at 0.63 mg C/g soil. Amount of HWE added was calculated to be consistent with the plant input C proportion between SPC and HWE, since in our initial plant input the HWE only had

18% of the C found in the SPC. After plant input addition, all soils were brought up to 61-67% water-filled pore space by the addition of DI water

Table 1: Carbon (C) and nitrogen (N) concentrations, and their isotopic composition for the initial bulk soils and all plant input types used in the experiments. Data are averaged over 3 laboratory replicates. Standard error is reported in parentheses for each where HAU is the high aggregation undisturbed soil, HAD is the high aggregation disturbed soil, LAU is the low aggregation undisturbed soil, LAD is the low aggregation disturbed soil, SPC is the structural plant component, and HWE is the hot water extractable (water soluble) plant component.

	C	**N**	**^{13}C**	**^{15}N**
Sample	**%**	**%**	**atom %**	**atom %**
HAU bulk soil	4.29 (0.03)	0.35 (<0.01)	1.0937 (<0.01)	0.3676 (<0.01)
HAD bulk soil	3.91 (0.05)	0.32 (<0.01)	1.0943 (<0.01)	0.3668 (<0.01)
LAU bulk soil	1.65 (0.03)	0.12 (<0.01)	1.0831 (<0.01)	0.3677 (<0.01)
LAD bulk soil	1.40 (<0.01)	0.10 (<0.01)	1.0832 (<0.01)	0.3666 (<0.01)
Bulk plant input	42.76 (0.24)	1.68 (0.07)	4.4390 (<0.01)	6.2201 (0.05)
SPC	44.71 (0.18)	1.01 (0.02)	4.3242 (0.01)	6.1676 (0.06)
HWE	30.58 (0.09)	3.95 (0.04)	4.4372 (<0.01)	6.7831 (0.02)

2.4 Respiration measurements

Soil and plant input respiration were quantified for the duration of the incubation on the H2 units, by measuring CO_2 efflux and atom % ^{13}C-CO_2. CO_2 concentrations were measured on an infrared gas analyzer (LI-COR biosciences Lincoln, NE, USA) every 1-4 days for the first three weeks, once a week for a month, twice a month, and then monthly resulting in 27 total measurements. Measurements were performed by injecting a known volume of gas from the jar head space, collected by syringe from a sealed, rubber septa on the lid. To avoid excessive CO_2 build up in the jars, units were flushed with CO_2 free air every or every other measurement for the first 3 months and then after every measurement for the remaining time points. Initial soil moisture was maintained throughout the incubation by performing routine checks and adding DI if

necessary. Gas samples for atom% ^{13}C-CO_2 analysis were collected in evacuated glass vials with fitted septa every other CO_2 measurement until measuring occurred once a month. Then ^{13}C gas samples were collected every measurement (once a month). All CO_2 gas samples were run on an IRMS (Europa 20-20, Sercon Ltd., Crewe, UK) for the determination of ^{13}C atom%.

2.5 Harvest

At each destructive harvest, a final gas measurement was taken for CO_2 efflux and ^{13}C atom%. Samples were then extracted from jars and 8mm sieved to remove the remaining SPC. The remaining SPC was oven-dried at 60^{o}C and weighed. A 12-15g soil subsample was 2mm sieved fresh and placed in a -80^{o}C freezer for microbial biomass. The remaining soil was air-dried for at least 72 hours. Once air-dried, a 20g subsample was set aside for WSA analysis. All soil left was 2mm sieved for fractionation and storage.

2.6 Water stable aggregate and mean weight diameter determination

Water stable aggregate (WSA) analysis was performed on initial soils from field collection and on incubated soils collected at both harvest times. A 50g subsample of 8mm sieved air-dried field soil (n=4) was used to determine baseline by wet sieving (Six et al., 2000b). To retain enough soil for multiple analyses, a 20g subsample of incubated soil was used. Specifically, we separated large macroaggregates (> 2000 µm), small macroaggregates (2000 µm - 250 µm), free microaggregates (250 µm – 53 µm) and free silt & clay (< 53 µm) particles. Soil was placed in a humidifying chamber for 30 minutes and then distributed over a 2000 µm sieve, submerged in DI water and left to slake for 5 minutes. The sieve was gently moved up and down fifty times over a two-minute period to allow the < 2000 µm fractions to pass through. Large macroaggregates left on the sieve were collected in a pre-weighed loaf pan. The supernatant was then poured over the

250 µm sieve and the process was repeated with each sieve size for the remaining soil. All fractions were oven-dried at 60ºC and weighed. The large macroaggregate, small macroaggregate and microaggregate size fractions were corrected for rocks and sand by dispersing a subsample of the oven-dried fraction with 0.5% Sodium hexametaphosphate (NaHMP). Rock and sand weights were scaled up and subtracted from each size fraction before calculating mean weight diameter (MWD). MWD was determined using Equation 1 (Van Baval, 1950) where X_i is the mean diameter of any particular size range of aggregates separated by sieving (i.e., large macroaggregates = 5) and w_i is the weight of aggregates in that size range as a fraction of the total dry weight of soil used.

$$(1)\quad \text{MWD} = \sum_{i=1}^{n} \left(w_i \overline{X}_i\right)$$

2.7 Soil fractionation

Soil organic matter fractions were separated from air-dried, 2mm sieved soil using physical fractionation, as described in Haddix et al., 2020. An aliquot of 5.75-6.25g for each sample was shaken with DI water and centrifuged at 1855 *g*. The dissolved organic matter was decanted off, weighed, and placed in the freezer. Once decanted, sodium polytungstate (SPT) at density 1.85 g/cm^3 was added to the soil before being set in a vacuum chamber to remove any air trapped in the aggregates. Pellets were spun down on the centrifuge for 30 minutes. Free light particulate organic matter was aspirated off, SPT rinsed out, and the remaining heavy fraction was dispersed using 0.5% NaHMP with 12 glass beads for 18 hours. Occluded POM (oPOM) + heavy coarse OM (hcOM) was separated from mineral associated organic matter by wet sieving over a 53 µm sieve. I define the pool as oPOM+ hcOM because I did not do an additional density separation to float off the oPOM and the hcOM is sand-sized. However, hcOM has been shown to contribute

minimally to overall OM in the sand-sized fraction (Cambardella and Elliot 1994; Soong et al., 2016; Haddix et al., 2020). Each fraction was oven-dried and run on the EA-IRMS to determine total %C, %N, ^{13}C atom%, and ^{15}N atom%, as described above for bulk soils. Dissolved organic matter was run on the Shimadzu – Total Organic Carbon Analyzer (TOC) for nonpurgeable organic carbon (NPOC) and total nitrogen (TN) concentration and then freeze-dried to obtain ^{13}C atom%, and ^{15}N atom% on the EA-IRMS. In this study we report and discuss the C values only, and thus refer to the fractions as fPOC, oPOC+hcOC, MAOC and DOC.

2.8 Microbial biomass

Microbial biomass was determined using a chloroform-fumigation extraction method (Vance et al., 1987) with 20 mL of 0.5 M K_2SO_4 and 4 grams of 2mm sieved, fresh soil that was kept frozen at -80°C until 2 days before the extraction, when it was thawed at 4° C. All extractions were frozen at -20°C until being run on the Shimadzu TOC for NPOC and TN concentration and then freeze-dried and run on the EA-IRMS for ^{13}C atom%, and ^{15}N atom%, as described above.

2.9 Data analyses

All stable isotope abundance data was retrieved as a δ-value ($^0/_{00}$) relative to the standard and converted to atom% according to the following equation (2):

$$(2)\ \text{atom\%} = 100 * (\delta\text{-value } (^0/_{00}) + 1000) / [(\delta\text{-value } (^0/_{00}) + 1000 + (1000/R_{standard})]$$

where $R_{standard}$ is the VPD-B $^{13}C/^{12}C$ 0.0112372 (Fleisher et al., 2021).

Contribution of plant input-C to the CO_2 efflux, the SPC residue remaining, bulk soil, SOC fractions, and microbial biomass were determined using a two-end member isotopic mixing model

where the control treatment (no plant input added) and the enriched plant input atom% ^{13}C values were used as two end members. The isotopic mixing model is as follows (equation 3):

$$(3)\ f_{\text{Plant input}} = \frac{\text{atom\%}^{13}C_m - \text{atom\%}^{13}C_s}{\text{atom\%}^{13}C_{\text{Plant input}} - \text{atom\%}^{13}C_s}$$

where $f_{\text{Plant input}}$ is the plant input derived C in the CO_2, SPC residue remaining, bulk soil, SOC fraction, or microbial biomass, atom%$^{13}C_m$ is the ^{13}C atom% of the mixture, atom%$^{13}C_s$ is the ^{13}C atom% of the natural abundance back-ground (control treatments averaged over soil type n=4 at each harvest), and atom%$^{13}C_{\text{Plant input}}$ is the ^{13}C atom% of the initial SPC or HWE inputs.

To determine the atom% ^{13}C-CO_2 in between samplings, when sampling only occurred every other efflux measurement, we assumed a linear change between the two-time measurements and used the time-weighted average using the prior and latter atom% ^{13}C-CO_2 values (Stewart et al., 2013). To determine the plant input derived C (pd-C) in each of these pools, $f_{\text{Plant input}}$ values were multiplied by the total mg C in the pool of interest and then divided by the mg C of plant input added. The pd-C was determined on each individual unit for all analyses. However, CO_2 efflux and pd-C-CO_2 were only determined on H2 units. We assumed that H1 units would have responded similarly, thus calculated any H1 results for CO_2 and pd-C-CO_2 using H2 units. To directly compare HWE and SPC pd-C, we reported the results using percentage pd-C since the two plant input types have different % C and were not added at the same rate.

Formation efficiency was determined at both harvests by dividing the amount of labeled pd-C in each soil fraction by the total amount of plant input processed (residue C loss + pd-C in bulk soil) (Lavallee et al., 2018). The SPC C lost was calculated by subtracting the remaining pd-

C in plant residues at the end of the incubation from the initial C added. We assumed that all HWE was processed by the first harvest at 22 days.

2.10 Statistical analyses

All statistical analyses were performed in R version 4.2.0 (R Core Team, 2018) using the *emmeans* package (Length, 2022). Separate full linear models were created for aggregate fractions, total SOM, SOM fractions, residue remaining, CO_2 mineralization, formation efficiency, and microbial biomass C. The response variables were MWD and % plant input-derived C. We fit separate models for each fraction (DOC, fPOC, oPOC + hcOC, MAOC) since the response variables differ drastically across the fractions. A log-transformation was utilized for all models to fulfill the assumptions of normality and equal variance when using a four-way ANOVA. Pairwise comparisons were made across treatments using a Tukey adjustment. Differences with a p-value < 0.05 were considered significant.

Three units were determined to be leaking throughout the incubation experiment, thus were removed as outliers for all comparisons made involving CO_2 respiration data. Observations removed included unit 41 (HAU SPC), 42 (HAD SPC), and 44 (LAD SPC). Additionally, interpolations were made for the CO_2 respiration data on day 2 due to a clogged needle. The µg C/g soil for units 10 (HAD HWE), 30 (HAD SPC), 33 (HA HWE), and 34 (HAD HWE) were determined by using the average slope and intercept from the other associated treatment units at day 2. Other interpolations were made at day 22 (unit 40; LAD control), day 25 (units 35 and 40; LA HWE and LAD control), day 39 (units 36,46,47; LAD HWE, HAD HWE, LA HWE) and day 53 (unit 1; HA control). The measurement was determined bad because it produced a negative incremental value, indicating a leak or clogged needle. For these data, I was able to use the

respiration rate from the time points before and after the bad measurement to calculate slope and intercept. I then applied those values to get µg C/g soil.

CHAPTER 3: RESULTS

3.1 Aggregate mean weight diameter

Mean weight diameter was significantly modified by all the experimental treatments (Table 2; Figure 1). Overall, HA soils had a significantly larger MWD (1.92 mm) than LA soils (0.22 mm). The disturbance treatment significantly decreased MWD by 1.24 mm.

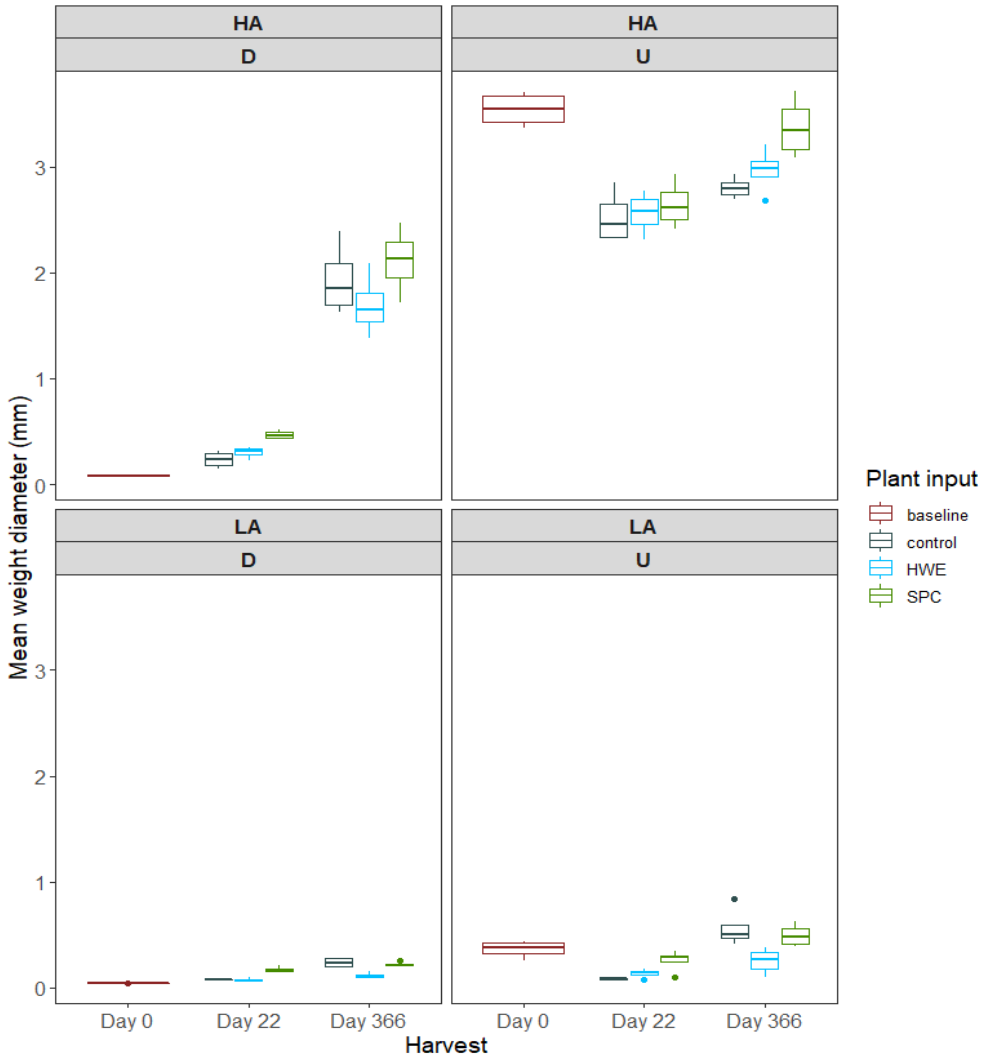

Figure 1: Mean weight diameter (MWD) for all treatments at both harvests (n=4) where high aggregation (HA) soil is on the top panels and the low aggregation (LA) soil is displayed on the bottom panels. The disturbed (D) treatment is on left and undisturbed (U) treatment is on the right. The hot water extractable (HWE) in blue represents the soluble plant input and SPC in green represents the structural plant input treatment. The baseline MWD for each soil is displayed in brown at day 0. Error bars represent the standard error of treatment averages.

Plant inputs also affected soils MWD, with the SPC input resulting in significantly higher MWD when compared to the no input control (9%) and the HWE inputs (12%). Although we observed an initial drop in MWD from the baseline (average loss of .21 mm) at day 22, soils aggregation increased by 0.6 mm between day 22 and day 366.

Table 2: Results from the linear model of the effect of soil type, disturbance level, plant input type, and harvest and their interactions on mean weight diameter.

Effect	**Mean weight diameter (p value)**
Soil	<0.001 ***
Disturbance	<0.001 ***
Plant input type	<0.001 ***
Harvest	<0.001 ***
Soil:Disturbance	<0.001 ***
Soil:Plant Input	0.001 ***
Disturbance:Plant Input	0.492
Soil:Harvest	<0.001 ***
Disturbance:Harvest	<0.001 ***
Plant Input:Harvest	<0.001 ***
Soil:Disturbance:Plant input	0.317
Soil:Disturbance:Harvest	<0.001 ***
Soil:Plant Input:Harvest	<0.001 ***
Disturbance:Plant Input:Harvest	0.460
Soil:Disturbance:Plant Input:Harvest	0.062

Significant interactions occurred across the experimental treatments affecting MWD (Table 2). A three-way interaction occurred between soil, disturbance, and harvest time. We observed significant increases in MWD for disturbed treatments (all $p < 0.001$). However, there was a much higher MWD gain for HAD (0.25 mm) than for LAD (0.07mm) between day 0 and day 22. Moreover, between day 22 and 366, HAD gained another 1.58 mm in MWD while LAD only gained an additional 0.09 mm. Interestingly, both HAU and LAU lost MWD at day 22 compared to the baseline MWD, so had no significant increases in MWD from day 0 to day 366.

LAU had 64 % higher MWD at day 366 compared to day 22 ($p < 0.001$). The increase in HAU from day 22 to day 366 was not significant.

The effects of plant input type also interacted with harvest time and disturbance. From HWE inputs, HAD soil increased in MWD by 0.3 mm (<0.001) from day 0 to day 22 and another 1.4 mm ($p<0.001$) from day 22 to day 366. LAD increased in the first 22 days ($p=0.010$) by nearly 0.05 mm, but changes were not significant ($p=0.2316$) from day 22 to day 366. HAU, however, had no significant change in MWD over time unlike LAU ($p=0.040$), which increased by 0.12 mm from HWE inputs from day 22 to day 366. This same pattern held true with SPC plant input addition. HAD showed a 78 % higher MWD ($p<0.001$) from day 22 to day 366. In fact, HAD regained enough MWD to make differences between time 0 HAU and HAD insignificant ($p=0.0687$) at day 366 with SPC inputs. There was only evident gain for LAD between day 0 and 22 ($p < 0.001$) and not between day 22 and day 366 but, surprisingly, LAD also reaggregated enough by day 366 to be insignificantly different from time 0 LAU with SPC inputs ($p=0.1201$). LAU had a significantly higher MWD by day 366 ($p < 0.001$), nearly doubling from day 22 from 0.26 to 0.49. Additionally, LAU surpassed baseline LAU by 0.13 mm by day 366 ($p=0.8197$)

Finally, we observed that even in the control treatment HAD had clear differences when compared to HAU at day 22 ($p<0.001$) but not by day 366 ($p=0.513$). The MWD of HAD was 2.29 mm smaller than HAU on day 22. By day 366, there was only a difference of 0.87 mm. However, we still observe evident differences in HAD at day 366 from HAU day 0 ($p=0.010$) where HAD day 366 was still 1.6mm less than HAU time 0. In the LA soils, both LAU and LAD showed insignificant differences from baseline time 0 by day 366 ($p=0.3273$, $p=0.4250$).

3.2 CO_2 mineralization and plant input decay

When averaging over disturbance, plant input type, and harvest, HA soils showed a higher cumulative CO_2 respiration (+ 493 µg C/g soil) than LA soil (Figure 2). Both the HWE and SPC treatments showed higher respiration than control treatments (Table 3; Figure 2). SPC addition resulted in more than double the amount of C mineralized compared to the control and HWE treatments while HWE inputs only resulted in a 7% higher CO_2 efflux than the control. In the first 22 days, CO_2 flux rate increased rapidly but then dropped drastically from day 22 to the end of the incubation. Disturbance had no main effect on C mineralization (Table 3, Figure 2). A few interactions were also observed across treatments and harvest time (Table 3). Particularly, disturbance interacted with plant input type yielding some interesting results. We only observed evidence of disturbance effects in the no-input control treatment (p=0.002) and not in either of the two plant input treatments. The D control treatment had 14 % higher CO_2 respiration than the U control treatment if averaging over day 22 and 366.

Table 3: Results from the linear model of the effect of soil type, disturbance level, plant input type, and harvest and their interactions on the cumulative CO_2, percent plant input-derived (% pd) C-CO_2, SOC (total soil organic C pool) and residue. Residue is the structural (SPC) plant input remaining after harvest.

Effect	Total CO_2 (p value)	% pd-C-CO_2 (p value)	% pd-SOC (p value)	% pd-residue (p value)
Soil	<0.001 ***	<0.001 ***	0.509	<0.001 ***
Disturbance	0.116	0.25	<0.001 ***	0.001 ***
Plant Input	<0.001 ***	0.022 *	<0.001 ***	NA
Harvest	<0.001 ***	<0.001 ***	0.254	<0.001 ***
Soil:Disturbance	0.003 **	0.031 *	<0.001 ***	0.084
Soil:Plant Input	0.027 *	0.178	<0.001 ***	NA
Disturbance:Plant Input	0.050	0.003 **	<0.001 ***	NA
Soil:Harvest	<0.001 ***	0.010 **	<0.001 ***	<0.001 ***
Disturbance:Harvest	0.531	0.997	0.555	0.008 **
Plant Input:Harvest	<0.001 ***	<0.001 ***	<0.001 ***	NA
Soil:Disturbance:Plant Input	0.710	0.933	0.003 **	NA
Soil:Disturbance:Harvest	0.297	0.367	0.140	0.199
Soil:Plant Input:Harvest	0.177	0.264	0.043 *	NA
Disturbance:Plant Input:Harvest	0.959	0.99	0.469	NA
Soil:Disturbance:Plant Input:Harvest	0.556	0.359	0.123	NA

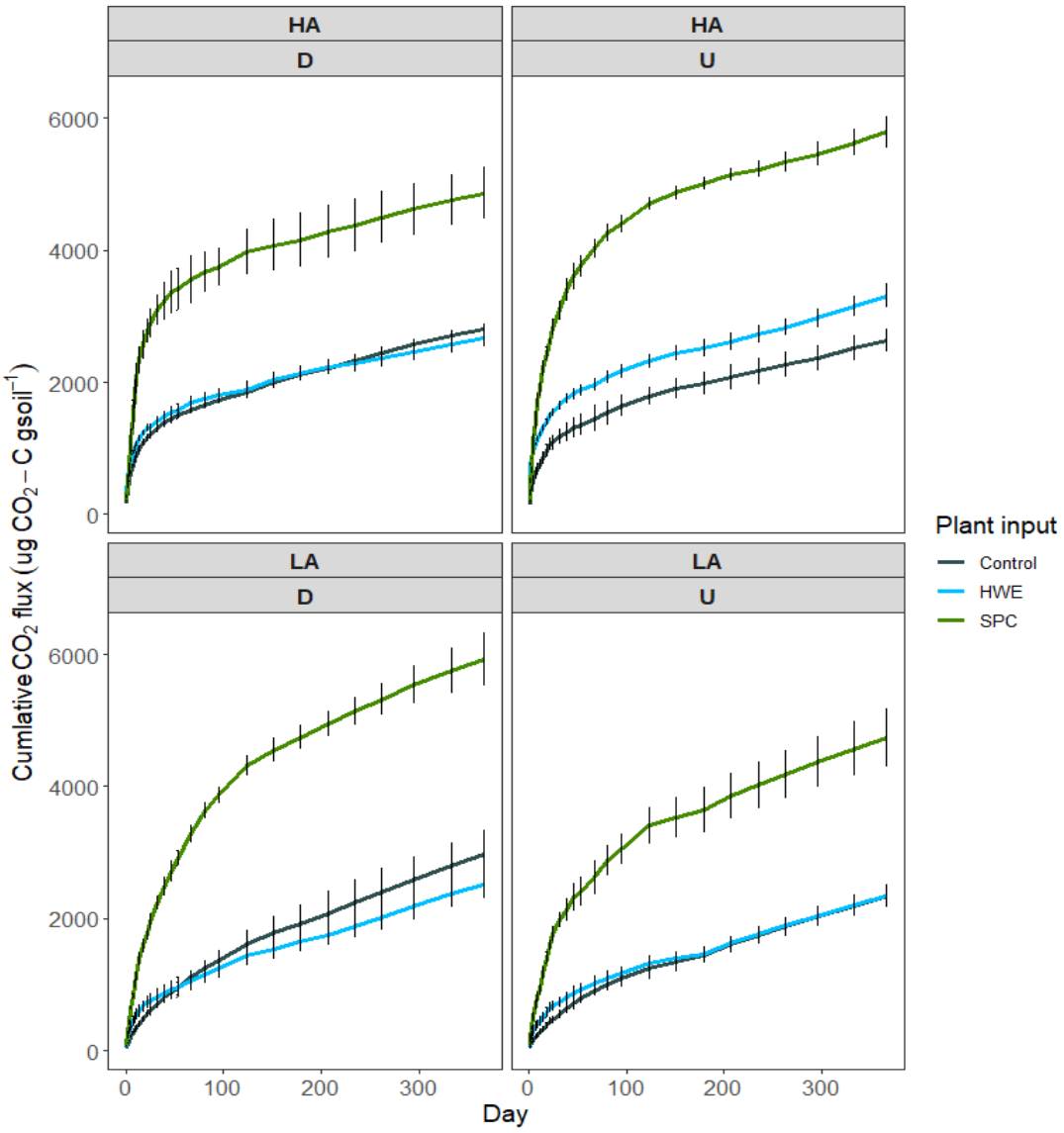

Figure 2: Cumulative CO_2 flux for all treatments over the full incubation where high aggregation (HA) soil is on the top panels and the low aggregation (LA) soil is displayed on the bottom panels. The disturbed (D) treatment is on the left and undisturbed (U) treatment is on the right. The hot water extractable (HWE) in blue represents the soluble litter addition and SPC in green represents the structural plant litter addition. SPC was mixed in at 3.5 mg C/g soil and HWE was added at 0.63 mg C/g soil. Error bars represent the standard error of treatment averages.

Although disturbance had no main effect on percent plant input-derived (pd-) C-CO_2, all other factors significantly affected % pd-C-CO_2 (Table 3, Figure 3). HA soil had a 15% higher %

pd-C-CO_2 than LA soil. By the end of the experiment, the HWE inputs had higher % pd-C-CO_2 by 58% compared to the SPC input. Overall, % pd-C-CO_2 was 30% higher on day 366 compared to day 22.

An interaction occurred with plant input and disturbance (Table 3). HWE % pd-C-CO_2 was higher in undisturbed soils (63%) than in disturbed soils (53%) (Figure 3). Additionally, there was 18% higher pd-C-CO_2 with HWE inputs ($p=0.002$) compared to SPC in undisturbed soils. Factoring in time with the different plant inputs, we observed a significant difference in % pd-C-CO_2 between HWE and SPC at day 22 ($p<0.001$) but not by day 366 ($p=0.331$). HWE resulted in 27% more pd-C-CO_2 on day 22.

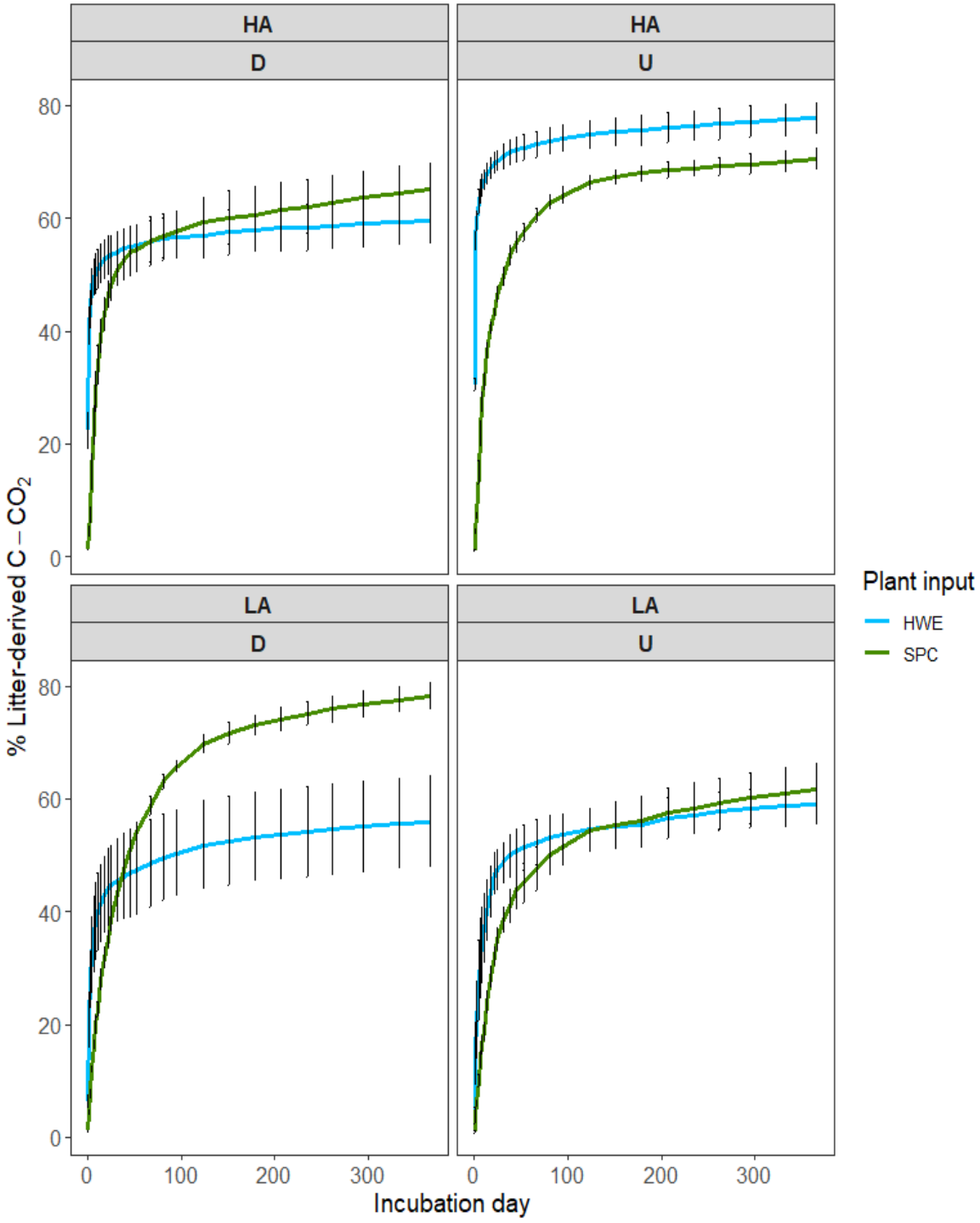

Figure 3: Percent plant input-derived C-CO_2 for all treatments over the full incubation where high aggregation (HA) soil is on the top panels and the low aggregation (LA) soil is displayed on the bottom panels. The disturbed (D) treatment is on the left and undisturbed (U) treatment is on the right. The hot water extractable (HWE) in blue represents the soluble plant input and SPC in green represents the structural plant input. Error bars represent the standard error of treatment averages.

The amount of SPC residue remaining, which can be interpreted as the dynamic to litter mass loss, was affected by soil type, disturbance, and harvest (Table 3). HA soils had slightly, but significantly more, SPC residue remaining overall by 0.5% than LA. Disturbed soils had roughly 25% residue remaining compared to 30% in undisturbed soils. Not surprisingly, we observed much less residue remaining on day 366. Averaged over soil and disturbance, SPC lost 79% of its C from day 22 to day 366. Lastly, we had evidence that soil type did have a significant effect on SPC remaining at day 366 but not at day 22 ($p<0.001$ and $p=0.7465$). HA soils had 14% SPC remaining while LA soils only had 5% left by the end of the incubation (Figure 4).

When looking at % pd-SOC in the bulk soil, we observed a main effect of disturbance and plant input type but not of soil type or harvest (Table 3). More SOC formation occurred in disturbed soils by 15%. Additionally, we observed higher % pd-SOC from HWE inputs. HWE inputs resulted in 27% pd-SOC while only 19% pd-SOC formed from SPC (Figure 4).

Notably, disturbance only influenced SOC formation in the HA soil and not LA with both the HWE and SPC inputs (Figure 4). HAD had higher % pd-SOC from HWE by 17% compared to HAU ($p=0.028$) and 48% higher with SPC inputs ($p<0.001$).

A three-way interaction with soil, disturbance, and plant input was evident (Table 3). HAD HWE had significantly (20%) higher % pd-SOC than LAD HWE ($p=0.003$). There was no significant difference between HAD and LAD with SPC inputs ($p=0.478$). Interestingly but consistently with SPC residue remaining, we observed the opposite relationship when soils were undisturbed. HAU had lower % pd-SOC than LAU (12% and 20% respectively, $p<0.001$) with SPC addition. No significant difference was evident with HWE inputs ($p=.999$).

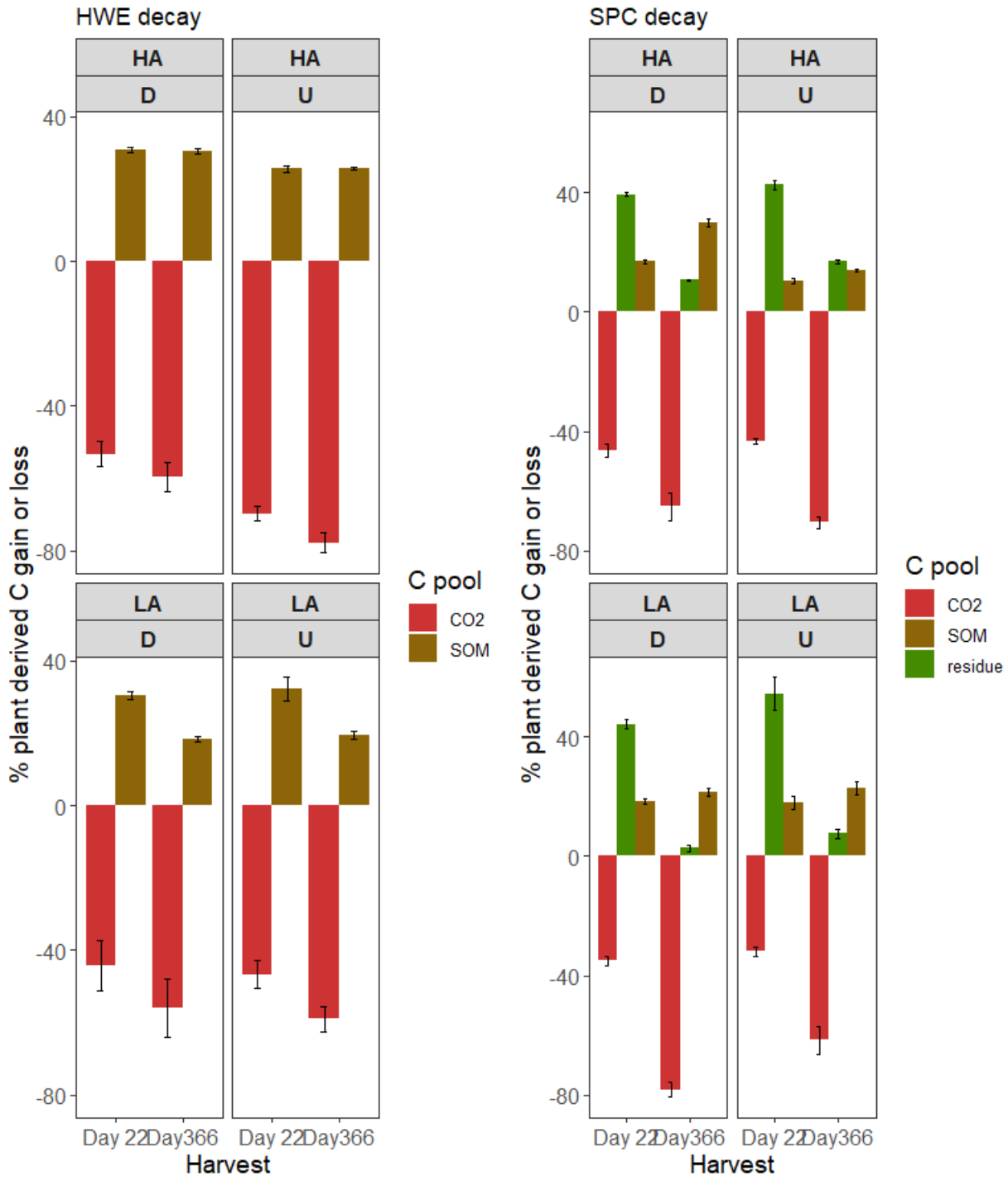

Figure 4: Distribution of percent plant input-derived C lost (thus presented as negative values) as CO_2, incorporated into the bulk soil organic matter (SOM) or remaining as structural plant (SPC) residues at day 22 and day 366 harvest. High aggregation (HA) soil is on the top panels and the low aggregation (LA) soil is displayed on the bottom panels. Soluble (HWE) plant input type is displayed separately (left) from the SPC treatment (right) since no residue remained from HWE inputs. The disturbed (D) treatment appears first and undisturbed (U) treatment second. Added plant input mineralized to CO_2 is displayed in red, total SOM formation is shown in olive brown, and SPC remaining is in green. Error bars represent the standard error of treatment averages.

3.3 SOC fractions formation and persistence

We observed significant differences in % pd-DOC and % pd-fPOC from the main effects of soil, plant input type, and harvest, but there was no main effect of disturbance (Table 4). Higher % pd-DOC was present in HA soil than LA soil by 16%. HWE plant input contributed 65% more pd-DOC than SPC. By day 366, % pd-DOC decreased from 0.9% to only 0.4% (Figure 5).

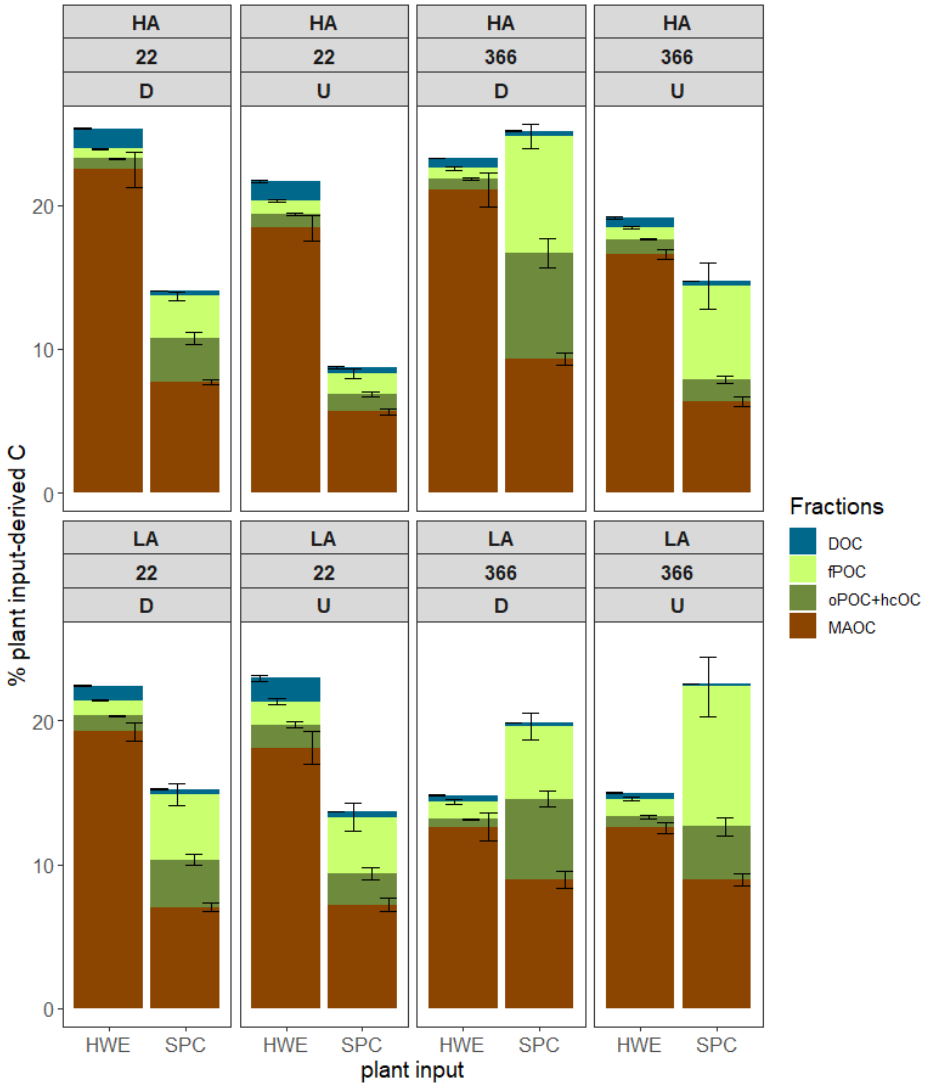

Figure 5: Distribution of percent plant input-derived C for all soil fractions across each treatment combination at each harvest (n=4) where high aggregation (HA) soil is on the top panels and the low aggregation (LA) soil is displayed on the bottom panels. Day 22 data is displayed on the left and day 366 data is on the right. The disturbed (D) treatment appears first and undisturbed (U) treatment second. DOC in blue represents the dissolved OC, fPOC is the free particulate OC in light green, oPOC+hcOC is the occluded particulate + heavy coarse OC in dark green and MAOC is the mineral associated OC in brown. Error bars represent the standard error of treatment averages.

Looking at % pd-fPOC, LA soil had 22% higher pd-fPOC than HA soil. We observed higher fPOC from SPC than HWE plant input. SPC resulted in 5% pd-fPOC compared to only 1% HWE pd-fPOC. Total % pd-fPOC was around 2% at day 22 and nearly doubled by day 366 (Figure 5).

The interaction between soil type, plant input, and harvest showed that HA soils had half as much SPC % pd-fPOC as LA soils ($p=0.012$) at day 22 but was nearly identical at day 366 (7% for both). The treatment of disturbance only had a significant effect when interacting with plant input and harvest. In disturbed soils, % pd-fPOC from SPC was greater than in undisturbed soils at day 22 (4% and 3% respectively, $p=0.0352$). However, this difference was not significant by day 366 ($p=0.855$). When looking at just the disturbed soils, differences in % pd-fPOC from SPC remained significant overtime. We observed that the % SPC pd-fPOC increases by 43% from day 22 to day 366 ($p=0.022$).

Unlike % pd-DOC and % pd-fPOC, disturbance did have a main effect on % pd-oPOC+hcOC and % pd-MAOC in addition to soil and plant input type. Harvest did not have a main effect (Figure 5, Table 3). Surprisingly, we observed that LA soil had 11% more pd-oPOC+hcOC than HA soil. Undisturbed soils had 43% less % pd-oPOC+hcOC than disturbed soils. However, this was only true for SPC inputs. Both LAD and HAD had a higher % pd-oPOC+hcOC compared to the undisturbed soils (34%, $p=0.031$ and 74%, $p<0.001$ respectively). As predicted, we observed that SPC promoted more oPOC+hcOC than HWE by a difference of 73%. There was a significant interaction between soil and disturbance showing that undisturbed LA soils had 2% pd-oPOC+hcOC while HA soils only had 1% ($p<0.001$). There was no significant difference in disturbed soils ($p=0.885$).

Table 4: Results from the linear model of the effect of soil type, disturbance level, plant input type, and harvest and their interactions on each soil fraction where % pd-is percent plant input-derived, DOC is the dissolved organic carbon (OC), fPOC is the free particulate OC, oPOC+hcOC is the occluded + heavy coarse OC, and MAOC is the mineral associated OC.

Effect	% pd-DOC (p value)	%pd-fPOC (p value)	% pd-ohcOC (p value)	%pd-MAOC (p value)
Soil	0.018 *	<0.001 ***	0.018 *	0.013 *
Disturbance	0.798	0.898	<0.001 ***	<0.001 ***
Plant input	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***
Harvest	<0.001 ***	<0.001 ***	0.186	0.335
Soil:Disturbance	0.013 *	0.070	0.001 **	<0.001 ***
Soil:Plant input	0.187	0.348	0.044 *	<0.001 ***
Disturbance:Plant input	0.538	0.038 *	<0.001 ***	0.403
Soil:Harvest	0.419	0.060	0.006 **	0.023 *
Disturbance:Harvest	0.995	0.253	0.254	0.773
Plant input:Harvest	0.003 **	<0.001 ***	<0.001 ***	<0.001 ***
Soil:Disturbance:Plant input	0.071	0.042 *	0.023 *	0.094
Soil:Disturbance:Harvest	0.060	0.961	0.169	0.454
Soil:Plant input:Harvest	0.771	0.047 *	0.004 **	<0.001 ***
Disturbance:Plant input:Harvest	0.574	0.018 *	0.249	0.532
Soil:Disturbance:Plant input:Harvest	0.368	0.439	0.241	0.811

For the MAOC fraction, we observed that HA soil had 12 % more pd-MAOC than LA soils. With all other factors averaged, disturbance resulted in a significant increase in MAOC by 13%. We also have clear evidence that HWE plant input contributed more to MAOC than SPC (Figure 5). HWE resulted in 18% pd-MAOC compared to 8% pd-MAOC from SPC. An interaction between soil type and harvest demonstrated that HA and LA soils had differences in % pd-MAOC persistence (Figure 5; Table 4). At day 22, there was no significant difference in % pd-MAOC between HA and LA soils (p=0.9984). However, by day 366, we observed a vast difference in % pd-MAOC as HA had 19% more pd-MAOC than LA (p=0.005). Furthermore, this is evident when looking at the three-way interaction between soil, plant input, and harvest (Table 4). On day 22, we observed no significant difference in HWE % pd-MAOC in HA and LA soils (p=0.6827). By day 366, there was a clear difference (p<0.001) as HA retained HWE % pd-MAOC. By the end of

the incubation, there was 33% more HWE pd-MAOC in the HA soil. Lastly, an interaction between soil type and disturbance, although not technically significant (Table 4) reveals that there were significant differences between HAU and HAD % pd-MAOC ($p=0.002$) but not between LAD and LAU with HWE inputs. HAD had higher % pd-MAOC by 20 %.

3.4 SOC fraction formation efficiency

The formation efficiency (FE) of DOC was affected by all main effects (Table 5, Figure 6). HA DOC FE was greater than LA by 16%. Although barely significant, disturbed soils showed lower DOC FE than undisturbed soils by 14%. HWE formed DOC more efficiently than SPC (0.007, 0.004 respectively) and the FE of DOC decreased by more than half overtime. An interaction between disturbance and harvest showed that FE DOC is significantly different at day 22 ($p=0.003$, 21% higher in U soils) but not by day 366 ($p=0.8815$) where undisturbed soils had higher FE initially by 21%.

For fPOC FE, we observed no main effect from disturbance (Table 5, Figure 6). However, LA had 21% higher FE of fPOC than HA soil. SPC forms fPOC more efficiently than HWE with an FE value of 0.054 compared to 0.008. fPOC FE increases overtime as more is incorporated into SOC. We observed a 26% higher FE of fPOC between day 22 and day 366. An interaction occurs between soil, plant input, and harvest. HA soil more than doubles fPOC FE from day 22 to day 366 ($p=0.0002$) from SPC inputs while LA soil has no significant change.

FE of oPOC+hcOC differs significantly from main effects of soil, disturbance, plant input and not harvest (Table 5, Figure 5). LA had 16% faster oPOC+hcOC formation compared to HA. Disturbed soils had higher FE than undisturbed (0.027 and 0.017, respectively). Plant input type had the most drastic effect on FE for oPOC+hcOC. SPC inputs had higher FE by a factor of 5.

With SPC inputs, HAD and LAD had no significant difference in FE of oPOC+hcOC (p=0.984) nor did LAD from LAU (0.071). However, HAD showed significant differences in FE, forming oPOC+hcOC 67% more efficiently than HAU (p<0.001).

For the MAOC fraction FE, we observed a main effect of all factors (Table 5). Higher MAOC FE occurred in HA than LA (0.119 and 0.109) soils. A 7% higher FE of MAOC was observed in disturbed soils compared to undisturbed. The HWE plant input had higher FE than SPC by 40%. MAOC formed more efficiently at the beginning of the incubation: FE of MAOC at day 22 was 0.112 but down to 0.101 day 366. Soil and disturbance interacted (Table 4), where HAD had higher FE than LAD (p<0.001) and HAU (p<0.001), but there was no significant difference between LAD and LAU (p=0.7489) or HAU and LAU (0.5920). The MAOC FE of HAD was 0.129, LAD was 0.107, and HAU was 0.108.

Table 5: Results from the linear model of the effect of soil type, disturbance level, plant input type, and harvest and their interactions on the formation efficiency (FE) of each soil organic C fraction where DOC is the dissolved OC, fPOC is the free particulate OC, oPOC+hcOC is the occluded + heavy coarse OC, and MAOC is the mineral associated OC.

Effect	**DOC FE (p value)**	**fPOC FE (p value)**	**oPOC+hcOC FE (p value)**	**MAOC FE (p value)**
Soil	<0.001 ***	<0.001 ***	0.005 **	0.029 *
Disturbance	0.043 *	0.39	0.005 **	0.012 *
Plant input	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***
Harvest	<0.001 ***	0.044 *	0.086 .	<0.001 ***
Soil:Disturbance	0.999	0.155	0.002 **	<0.001 ***
Soil:Plant input	0.941	0.187	0.034 *	<0.001 ***
Disturbance:Plant input	0.927	0.085	<0.001 ***	0.138
Soil:Harvest	<0.001 ***	0.018 *	0.002 **	0.006 **
Disturbance:Harvest	0.003 **	0.373	0.177	0.465
Plant input:Harvest	0.804	0.013 *	0.002 **	0.117
Soil:Disturbance:Plant input	0.048 *	0.056 .	0.016 *	0.167
Soil:Disturbance:Harvest	0.010 *	0.684	0.214	0.722
Soil:Plant input:Harvest	0.304	0.003 **	0.023 *	0.191
Disturbance:Plant input:Harvest	0.812	0.026 *	0.182	0.324
Soil:Disturbance:Plant input:Harvest	0.877	0.703	0.304	0.199

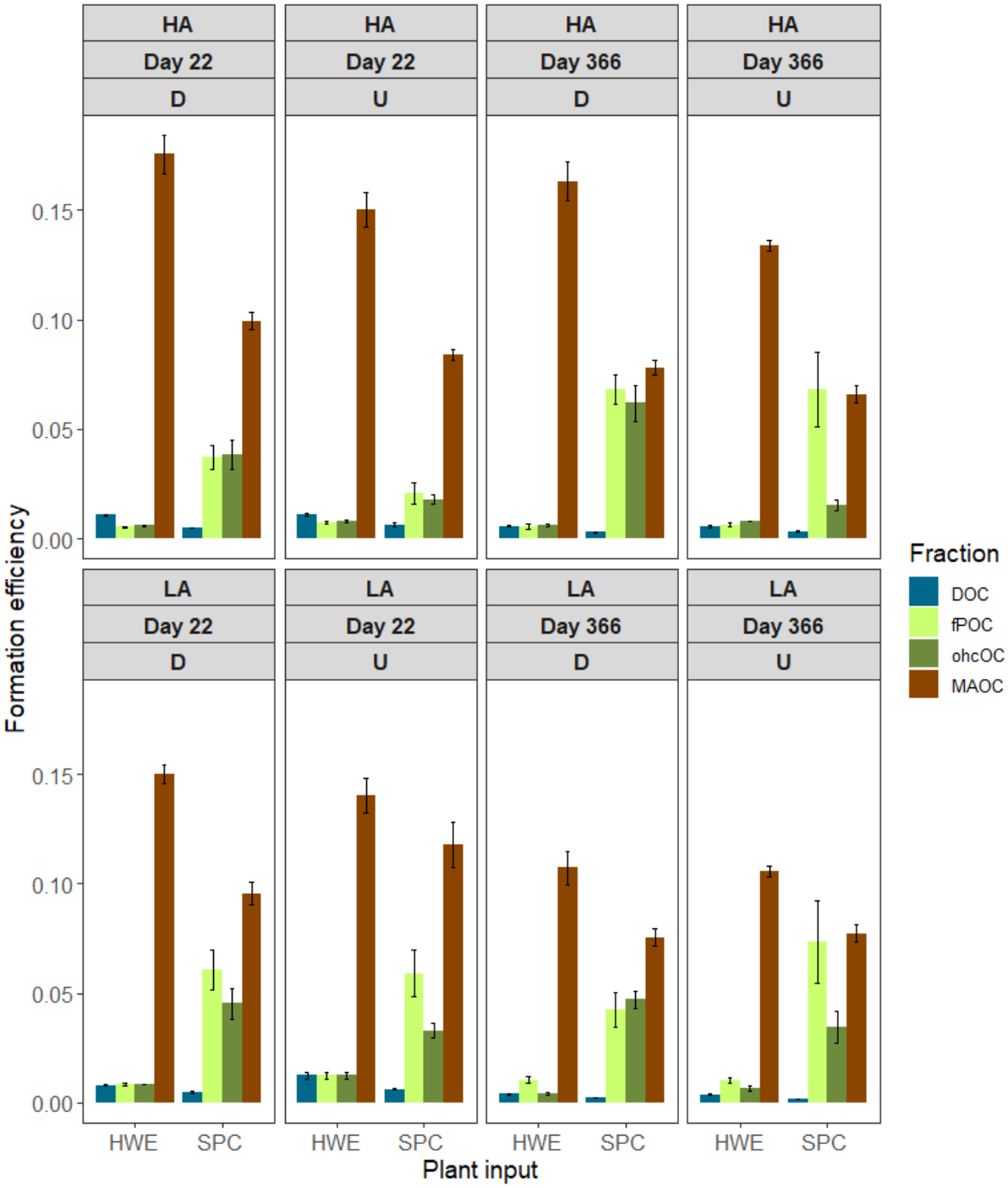

Figure 6: Formation efficiency for all soil organic carbon fractions across each treatment combination at each harvest (n=4) where high aggregation (HA) soil is on the top panels and the low aggregation (LA) soil is displayed on the bottom panels. Day 22 data is displayed on the left and day 366 data is on the right. The disturbed (D) treatment appears first and undisturbed (U) treatment second. Plant input type is on the x-axis. DOC in blue represents the dissolved organic carbon (OC), fPOC is the free particulate OC in light green, oPOC+hcOC is the occluded particulate + heavy coarse OC in dark green and MAOC is the mineral associated OC in brown. Error bars represent the standard error of treatment averages.

3.5 Microbial biomass C

The percent plant input-derived MBC was dependent on soil type ($p < 0.001$), plant input ($p = 0.012$), and harvest ($p < 0.001$). Disturbance was not a significant main effect. HA soils had 12% pd-MBC while LA soils only had 7%. We observed a similar difference between plant input types with 11% pd-MBC from HWE and 7% from SPC. Overtime, the % pd-MBC decreased drastically by 86% from day 22 to day 366. The only significant interaction occurred with soil and harvest ($p < 0.001$) but as shown in the main effect of harvest, we observed that all differences for both HA and LA soils from day 22 to day 366 had a p-value less than 0.001. However, % pd-MBC decreased in HA soil by 82% while we observed a difference of 92% in LA soils by the end.

Table 6: The microbial biomass carbon (MBC) concentration measured in mg MBC/g soil. HWE is the soluble plant input treatment and SPC is the structural plant input treatment. Standard error (se) is displayed in the far-right column

Soil	Disturbance	Harvest	Plant input	mg MBC/ g soil	se
HA	D	Day 22	control	0.043238	0.005235
HA	D	Day 366	control	0.03395	0.00177
HA	U	Day 22	control	0.04505	0.006012
HA	U	Day 366	control	0.034963	0.002492
LA	D	Day 22	control	0.030146	0.003819
LA	D	Day 366	control	0.029034	0.001034
LA	U	Day 22	control	0.028916	0.003678
LA	U	Day 366	control	0.023329	0.003102
HA	D	Day 22	HWE	0.097038	0.001951
HA	D	Day 366	HWE	0.044775	0.00109
HA	U	Day 22	HWE	0.088575	0.004123
HA	U	Day 366	HWE	0.039713	0.002368
LA	D	Day 22	HWE	0.046165	0.004239
LA	D	Day 366	HWE	0.021058	0.002978
LA	U	Day 22	HWE	0.050325	0.005859
LA	U	Day 366	HWE	0.020733	0.00243
HA	D	Day 22	SPC	0.1064	0.011786
HA	D	Day 366	SPC	0.0423	0.004525
HA	U	Day 22	SPC	0.100888	0.003771
HA	U	Day 366	SPC	0.046738	0.002549
LA	D	Day 22	SPC	0.054506	0.002836
LA	D	Day 366	SPC	0.016725	0.000746
LA	U	Day 22	SPC	0.050003	0.000918
LA	U	Day 366	SPC	0.023865	0.001481

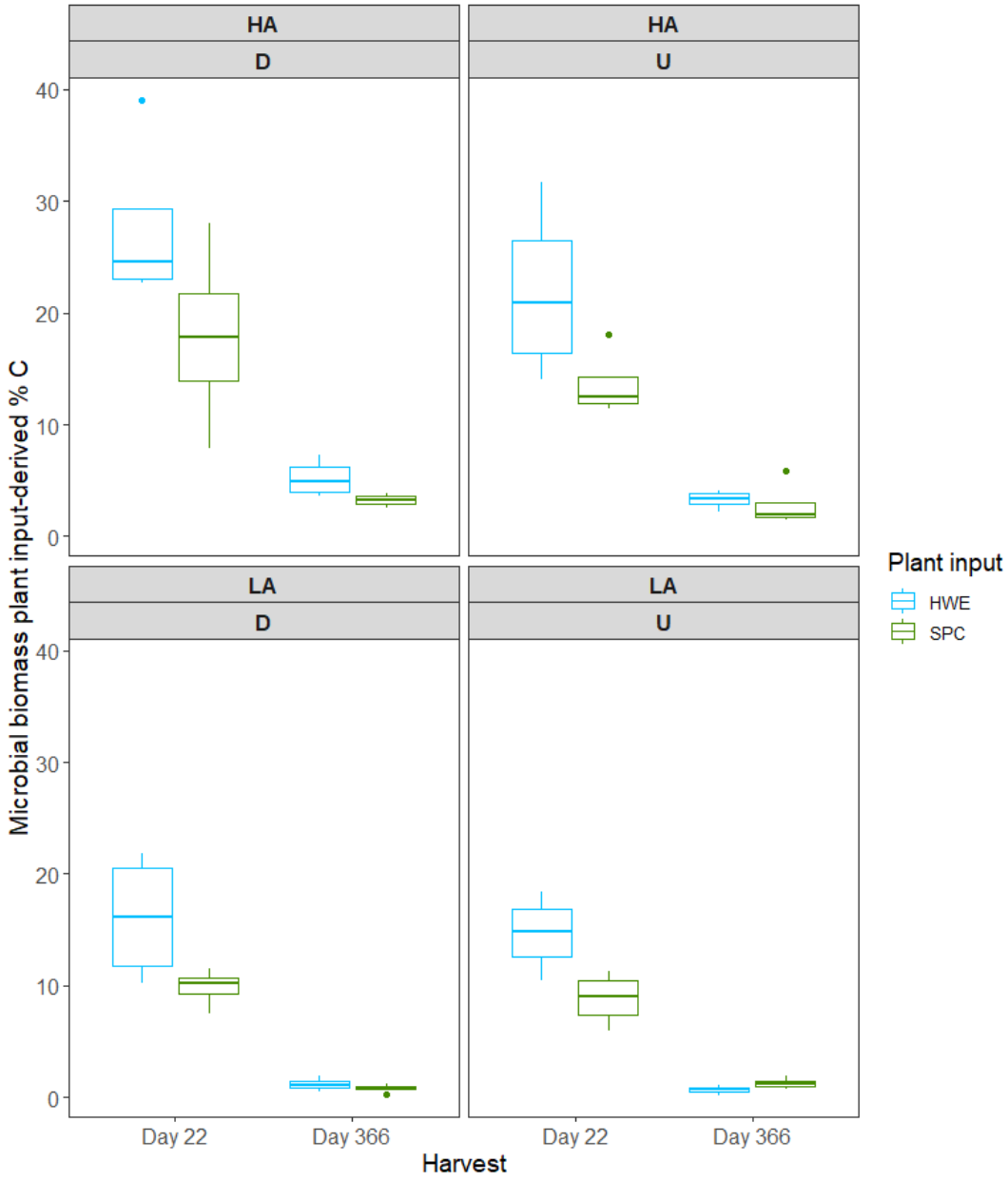

Figure 7: The percent plant input-derived microbial biomass C for all treatments at both harvests where high aggregation (HA) soil is on the top panels and the low aggregation (LA) soil is displayed on the bottom panels. The disturbed (D) treatment is on left and undisturbed (U) treatment is on the right. The hot water extractable (HWE) in blue represents the soluble plant input and SPC in green represents the structural plant input. Error bars represent the standard error of treatment averages.

CHAPTER 4: DISCUSSION

4.1 Aggregate mean weight diameter

We found clear differences in aggregation between the two soil types, confirming that we succeeded in comparing a highly aggregated soil to a soil with poor aggregation. Both the HA and LA soils were able to reconstitute soil aggregates after disturbance with both plant input types, but more so with SPC. This is evidenced by HAD and LAD soils no longer showing a significant difference in MWD from the undisturbed baselines after just one year from SPC inputs. This supports our hypothesis that plant inputs would stimulate reaggregation with structural inputs, specifically, resulting in the highest MWD gains. Both soil types regained aggregation relative to their initial baselines despite vastly different structure.

This finding is consistent with De Gryze et al. (2005) who found that aggregate formation increased similarly in three soils with different textures, all showing a positive linear relationship between amount of residue added and aggregate formation. In contrast, Bach et al. (2010) performed a field experiment and found that soil texture affected aggregate formation in restored grasslands that were all seeded with similar native grasses. They found no significant changes in MWD in the loamy fine sand soil but reported an "exponential rise" in MWD in the silty clay loam. Contrasting results point to the difficulties of defining one or two factor as the most influential on MWD. Despite ample studies aimed at identifying the controls of MWD, there doesn't seem to be a consistent question and experimental design that yields support for a limited number of factors. For example, Mbagwu et al. (1994) focused on the effects of soil property on MWD and found that chemical properties were most influential in comparison to physical and mineralogical properties. Chivenge et al. (2011) studied litter quality and determined that low

quality inputs resulted in higher MWD. A PCA performed by Ciric et al. (2012) identified water retention, bulk density, and pH as the most significant factors in MWD prediction, but Kamamia et al. (2021) determined that organic C had the highest influence on MWD. Although uncertainty is what drives research efforts aiming to include soil aggregation in predictive SOM-C models, it may be beneficial to simplify predictions of MWD to the interaction of litter quality (i.e., plant input type) and soil structure so that soil aggregation can be incorporated into models, like the MEMS (Zhang et al., 2021).

Important to note is that despite the HAD soil regaining enough aggregation to be deemed insignificantly different from HAU baseline MWD by day 366, we still observed a difference between their MWD of 1.6 mm. Thus, I suggest that the disturbance response would have been even more insignificant if the incubation had continued beyond a year or if the incubation was *in-situ*. Interestingly, LAU surpassed LAU baseline MWD with SPC and the control plant input treatment. This points to the benefits of coupling no-till or conservative till management with increased residue addition and/or retention, especially in agroecosystems with soil that has relatively poor aggregation. If arable soil is afforded time to rest and regenerate aggregates, then perhaps occasional tillage events may not be as destructive as has been portrayed in conversations surrounding agricultural management. In fact, a recent meta-analysis highlighted that occasional tillage occurring every 5-10 years actually benefits soil physical properties like aggregation and even crop yields (Peixoto et al., 2020)

4.2 CO_2 mineralization and SOC formation

Soil texture and microbial biomass have both been shown to regulate mineralization of OC to CO_2 from soils (Luo et al., 2016; Spohn & Chodak, 2015; Numa et al., 2021). Additionally, plant input type affects CO_2 mineralization given that soluble and structural plant constituents have

vastly different availability for microbial processing and soluble plant components can enter the mineral matrix *ex vivo* without microbial processing (Liang et al., 2017). Results from my study substantiate each of these factors believed to significantly influence CO_2 mineralization. Higher cumulative CO_2 respiration in the HA compared to the LA soil with plant inputs was surprising at first. However, we confirmed that HA soils had higher microbial biomass than the LA soils (Table 6). It's well documented that soils with higher microbial biomass (MB) respire more CO_2 and that MB abundance and respiration respond similarly to environmental changes (Hofman et al., 2004; Zhang et al., 2018; Chen et al., 2019, Holden & Treseder, 2013) but higher microbial biomass can also lead to more microbial processing of fresh residues (Van Groenigan et al., 2010), thus conversion into stabilized SOM. Although I did not find higher % pd-SOC in HA soils compared to LA soils even though HA has higher MB and a finer texture, I did see a soil type effect on % pd-SOC when looking at the SOC fractions separately (Figure 5; Table 4) which I'll discuss later.

As expected, higher initial plant C input mineralization (% pd-C-CO_2) occurred from HWE inputs due to higher availability to microbes but the rate quickly reached plateau, unlike respiration of SPC plant inputs, which continued throughout the incubation. Other studies have found rapid reduction in microbial processing of soluble plant inputs (Haddix et al., 2016) and we found further evidence that the HWE remaining was contributing almost entirely to MAOC. However, contrary to our hypothesis that disturbance would stimulate soluble plant input C mineralization, we observed that HWE inputs in the HAD soil had lower % pd-C-CO_2 than HAU. Initially, we thought this may be a result of microbial biomass being negatively impacted by disturbance, but our % pd-MBC data does not support this mechanism (Figure 7).

We propose that disturbance increased mineral surface area of the HA soil, exposing more direct OM sorption sites for DOM, thus decreasing HWE mineralization. This interpretation is

supported by recent evidence of fast stabilization of DOM (i.e., glucose) on soil minerals without previous microbial processing (Ismal et al., 2023). Moreover, we observed 20 % higher HWE pd-MAOC in HAD soil compared to HAU soil when averaging over time. This indicates that MAOC formation was not dependent on aggregation and, instead, was more dependent on mineral surface availability. The above theory is supported by the findings of Singh et al. (2016) where DOC adsorption was found to be positively correlated to the specific surface area of soil mineral fractions. In fact, efforts to quantify the capacity of minerals to adsorb DOC signifies the importance of mineral surface availability for DOC sorption, thus potential long-term SOC storage from the formation of MAOC. It was estimated that 107 Pg DOC could be adsorbed across six soil orders globally (Abramoff et al., 2021), a number similar to the amount of total SOC predicted to have been lost from land conversion to agricultural land suggested by Sanderman et al. (2017).

It was overwhelmingly clear that MAOC formed primarily and most efficiently from HWE inputs, adding to the growing support (Kallenbech et al., 2016; Haddix et al., 2016; Lavallee et al., 2018; Cotrufo et al., 2022) of the MEMS theory (Cotrufo et al., 2013) which posited that soluble higher quality residues are more efficiently stabilized on mineral surfaces than lower quality residues, due to their higher efficiency of microbial necromass production, but also direct sorption (Ismal et al., 2023). Moreover, our data suggests that a soil with higher aggregation potential will have higher persistence of MAOC, regardless of disturbance. The % pd-MAOC in both the HAU and HAD soil was nearly the same at both harvests, a similarity not observed in either the LAU or LAD soil, indicating that MAOC did not persist in the soil with inherently poor structure. We speculate that MAOC persistence in HA is a result of efficient macroaggregate generation and, thus, more occluded microaggregates within the macroaggregates that entrap MAOC. Although we did not quantify proportion of occluded microaggregates or % plant input derived C in each

aggregate size class, several studies corroborate this speculation. For example, in a field incubation using ^{13}C labeled plant inputs, Fulton-Smith & Cotrufo (2019) found that physical protection of microaggregates within macroaggregates resulted in higher % pd-MAOC. Additionally, Plaza et al. (2013) concluded that physical protection of MAOC in occluded microaggregates was likely the most important mechanism for C stabilization in no till agriculture directly linking slow macroaggregate turnover to increased microaggregate formation and C protection as proposed by Six et al. (2000b).

We also found support for our hypothesis that SPC contributes primarily to POC pools, consistent with the two-pathway model proposed by Cotrufo et al. (2015), and that more aggregation results in less SPC mineralized to CO_2. Regeneration of aggregates was happening consistently in the HAD soil resulting in less % pd-C-CO_2 than in the LAD soil. In fact, LAD soil had the highest % pd-C-CO_2 from SPC inputs by the end of the incubation despite having lower microbial biomass. This illustrates that SPC was more exposed to microbial decomposition in LAD than in HAD soil over time, which is corroborated by our observation of persistently low MWD in the LAD soil.

The increase in fPOC overtime supports the theory that more complex, structural plant constituents enter the SOC pool as POC later in the decomposition process as they fragment (Cotrufo et al., 2015, Soong et al., 2015). Higher formation efficiency of fPOC occurred on day 366 than on day 22 as more SPC was transformed into either fPOC or oPOC+hcOC and less mineralized to CO_2. Both LAD and HAD soils formed more oPOC+hcOC than the undisturbed treatment with SPC inputs. Consistent with the MWD data, the formation of oPOC+hcOC in D soils illustrates that plant inputs stimulate macroaggregate formation, which in turn results in

higher formation efficiency of oPOC+hcOC compared to soils with lower aggregate formation rates (in this experiment, the U soils).

Despite there being no significant differences in % pd-oPOC+hcOC between HAD and LAD soils by day 366, we still observed a 4% increase in HAD soil from day 22 to day 366 and only a 2% increase in LAD soil. Thus, I can assume the HAD soil continually formed oPOC+hcOC as significant gains in aggregation occurred. The observed higher oPOC+hcOC in LA soils may be a result of higher hcOC formation than oPOC as it is a sandier soil with a much higher proportion of sand-sized fraction. However, the formation and function of hcOC is still being debated and some studies have shown that it contributes trivial amounts to total POM (Cambardella and Elliot 1994; Soong et al., 2016). Additionally, a second density fractionation to separate occluded POC from heavy-coarse OC has been reported to decrease total C recovery (Poeplau et al., 2018). To build on this experiment, one might consider performing the second density separation and quantifying % pd-C in the oPOC and hcOC separately, but we chose to forego the possibility of increased C loss and follow the same fractionation performed by Haddix et al. (2020).

4.3 Microbial Biomass C

I lack any evidence that disturbance affected microbial biomass in my study when looking both at % plant input-derived MBC and MBC abundance in the bulk soil. Laub et al. (2021) observed a strong connection between MB, aggregate C, and aggregate formation, suggesting that higher MB from higher quality plant inputs would result in higher aggregate formation and, ultimately, MAOC. Our findings are consistent with Laub et al. given that we detected higher % pd-MBC (and higher % pd-MAOC) from HWE inputs, in support of our hypothesis. We observed a clear drop in % pd-MBC from day 22 to day 366, as expected, but % pd-MAOC persisted in both

the HAU and HAD soil from HWE inputs. This result points to the importance of microbial necromass for MAOC stabilization and adds to the growing evidence that a significant amount of the OM stabilized in soils is composed of microbial-derived compounds (Lutzow et al., 2006).

4.4 Future suggestions

Although we created a robust experimental design to test our hypotheses, no experiment is without limitations. Ideally, I would have used multiple soils along a textural gradient. A limiting factor was the feasibility of conducting gas measurements on enough units to capture different soil types with each disturbance*litter*harvest treatment. Additionally, the forest soil I collected had a lot more rocks > 2mm than anticipated. I did adjust for this (described in Chapter 2), but there was no guarantee that each LAU unit had exactly the same soil mass as all the other units. Lastly, it may be beneficial for future experimental designs using these same treatments to test not only more soils with different textures but to also use agricultural soils. Since this was a mechanistic study, we decided that using soils outside of agroecosystems was justifiable. However, if focusing on management and tilling effects, it would be prudent to use agricultural soils on a textural gradient

CHAPTER 5: CONCLUSION

The findings of my study have important implications directly linked to SOC formation and stabilization, adding to evidence that supports the two-pathway model and advancing our understanding of the mechanisms by which soil aggregation affects SOC dynamics. As discussed, my results show clear differences in SOC persistence between the HA and LA soils. Demonstrating that a disturbed soil with high mineral content and a known capacity to aggregate can regenerate SOC effectively over such a small timescale can inform future management decisions. Focusing efforts on degraded lands that meet these criteria in agroecosystems and beyond could expedite soil sequestration, especially with higher quality plant inputs that have more soluble compounds. MAOC persistence from HWE inputs in both the disturbed and undisturbed highly aggregated soil substantiates the role of low molecular weight compounds and efficient microbial assimilation of these compounds to OC stabilization.

Additionally, the evidence we put forth confirming that soil aggregation can be restored with plant inputs alone should inspire management practices in agriculture that aim to increase inputs and retain residues for enhanced soil function. It's likely that adopting rotations that intensify (i.e., cover cropping) and diversify crops, and leave crop residues would result in more efficient SOC formation via increased macroaggregate formation that leads to oPOC. However, this may not result in SOC persistence if the disturbance occurred in soil with poor structure. Soils with a low capacity to aggregate would benefit the most from higher plant inputs in tandem with no or little disturbance (i.e., no tillage) to reduce the likelihood of increased mineralization of the more structural plant constituents.

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