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Microbial Response to Biodegradable Mulch: Can Degradation Rate Be Accelerated by Management?

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MICROBIAL RESPONSE TO BIODEGRADABLE MULCH: CAN DEGRADATION RATE BE ACCELERATED BY MANAGEMENT?

by

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Single-use, petroleum-based polyethylene mulch is ubiquitous in certified organic mulched vegetable systems, representing a broken nutrient cycle and a waste concern. Current organic-allowable biodegradable mulches cannot match the performance of polyethylene, in part because of the requirements that they contain 100% bio-based feedstock, and biodegrade within two years after soil incorporation. It is valuable to understand whether management can influence postharvest degradation rate of mulch films. Two biodegradable mulches: a potentially organic nonwoven polylactic acid and wood particle prototype (PLA), and a widely-adopted non-organic starch/copolymer blend, Bio360® (BLK), were used in field trials in two distinct ecoregions of Nebraska, at Lincoln (LNK) and Scottsbluff (SBF). We tested degradation rate, influence on soil microbial community, and microbial recruitment of buried mulch residue under five management treatments. Mulch mass loss, tensile strength, and qualitative presence by bulk recovery were not affected by management treatments which included cover cropping and high rates of compost. Likewise, management had little impact on microbial community structure present on mulch surfaces. Instead,

location and mulch type were strong drivers of degradation rate, while mulch type alone was the primary driver of mulch-associated microbial community. BLK mulch was nearly completely undetectable after 12 months of burial at LNK, but 67% of BLK mass remained at SBF. PLA mass loss was initially more rapid at SBF, but after 12 months this difference was not prominent with 33% and 37% remaining at SBF and LNK, respectively. Direct mass measurement is uncommon in field-based biodegradable mulch literature. We used a novel approach to direct mulch mass measurement: mesh bags and mass by combustion. While mesh bags are instrumental in detecting mass changes over time, we showed that they are a strong driver of microbial profiles present in soil and mulch sample fractions, so caution is warranted in interpreting mesh bag results as representative of field status of mulch.

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DEDICATION

This thesis is dedicated to every aspiring scientist who is disappointed to realize that the trickier work is turning their discovery into action.

This thesis is also dedicated to every public servant, advocate, activist, businessperson, worker, and homemaker who realizes that repairing the world is ultimately in their hands, and who also realizes that the fruits of science only shape this world through their hands.

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1. Introduction

1.1 State of the Art in Mulch Membranes

Mulch membranes are a common tool in annual horticulture production systems. Their primary functions are to manage weeds and soil moisture. Other benefits can include soil warming or cooling, nutrient leaching prevention, among others (Subrahmaniyan and Ngouajio, 2012). Use of mulch membranes is widespread because of the cost savings and yield increase associated with their use. In 2006, 162,000 ha of U. S. production used mulch membranes and global consumption was estimated at 2.6 million tons annually (Hayes *et* al., 2012; Briassoulis and Dejean, 2010). Demand in all regions has grown annually since, and mulch membranes are now the fourth most essential goods consumed by agriculture in China after chemical fertilizer, seed, and pesticide (Yang *et* al., 2015).

The current state of the art in mulch membrane is unequivocally low-density polyethylene (PE). Attributes of PE mulch film are durability, non-degradability, economy of scale, wide availability, and customizability – many colored or metallized products are available at various widths and thicknesses.

1.2 Ideal agronomic properties for mulch membranes

The properties of PE are ideal for mulch membrane as far as ability to provide immediate production gains, but disposal is its drawback. For producers, PE mulch allows ease of installation, cost effectiveness, customizability to purpose, and ready

access. It benefits consumers by reducing the cost of produce and ornamental plants. PE mulch films also have positive externalities to ecosystems, reducing irrigation requirements for production, decreasing required fertility input and nutrient leaching (Lippert *et* al., 1964; Bhella *et* al., 1988). Mulch films may also reduce the need for herbicides and insecticides in some cases. These benefits have become indispensable in modern production systems, especially in dry environments (Yang *et* al., 2015). Agronomically, the only short-term detriment of PE mulch use is the cost of disposal. An ideal mulch material would provide all benefits of PE but require no special management in addition to normal best practices after its working life.

1.3 The case for alternatives: drawbacks of PE mulch

Direct and indirect environmental contamination and dependence on non-renewable petroleum are also associated with PE mulch (Steinmetz *et* al., 2016). It is a single use product, so the amount disposed annually is roughly equal to the amount produced. The magnitude of global PE mulch film use underscores the gravity of its negative consequences and further justifies the pursuit of bio-based alternatives. This is especially true for certified organic production which is intended to "foster cycling of resources (and) promote ecological balance" (USDA). Along with external or long-term detriment, PE mulch has some immediate agronomic drawbacks that can be mitigated by other materials making the development of viable alternatives more promising.

The primary agronomic drawback of PE mulch film for producers is the requirement that it be removed from the field, usually every year. Costs associated with removal are labor, hauling, and disposal fees. Depending on these, postharvest cost for

using PE mulch ranges from 100-400 USD (Goldberger *et* al., 2015; Moore and Wszelaki, 2016), and these costs are projected to increase. Landfilling is the most common means of disposal, but sometimes burial or burning on site are used. Only about 1% of disposed PE mulch was recycled in 2006 (Kasirajan & Ngouajio, 2012). Agricultural polyethylene waste is a low-value feedstock for recycling due to photodegradation and contamination. PE mulch waste can carry 50% or even 80% of its total mass in adhered soil (Kasirajan & Ngouajio, 2012; Ghimire & Miles 2016). Hauling and disposal of such a waste represents an unusual form of soil erosion, an ecologically and agronomically detrimental process.

Another drawback to producers includes soil loading with plastic fragments due to incomplete removal, and possible negative impact on soil physical and biological properties (Zhang *et* al., 2015). For cost reasons, producers prefer to use mulch film of minimum thickness to realize the functional benefit during the working life of the mulch, and retain sufficient durability to remove the film completely and efficiently. The interaction of mulch film thickness with (unpredictable) soil conditions at time of removal may make complete removal impractical. Fragments of mulch left in soil have a negative impact on soil properties, persist for centuries, and overloading ultimately reduces the productive potential of a soil (Liu *et* al., 2014; Liu *et* al., 2017).

Overall, the balance of ecological drawbacks to benefits is unknown and probably variable depending on timescale and environment. The weight of research suggests that the short-term benefit of sustained PE mulch use generally comes at the

price of long-term harm to environment and soil productive potential (Steinmetz *et* al., 2016).

1.4 A general case for biodegradable mulches

Benefits of PE mulch films stem from their physical resilience during their working life, the period while the crop is in the field. Some drawbacks may manifest during the working life, including acceleration of SOC mineralization (Zhang *et* al., 2015). Further drawbacks stem from their resilience and resistance to degradation after their working life, when mulch material becomes a liability as a disposal burden or soil contaminant. This paradox has vexed the entire plastics paradigm since plastics became a dominant material for all types of single-use applications.

Among items contributing to total plastic waste, mulches are ideally suited for replacement with biodegradable material. End of life handling is to simply leave mulch films in place and soil incorporate by tillage, typically already a part of management programs.

Numerous degradable mulch formulations have been explored. Early photodegradable formulations were PE based with additives designed to weaken and fragment the polymer through light exposure (Kyrikou and Briassoulis, 2017). These have failed to gain wide adoption due to unreliable degradation, questionable fate of complete biodegradation, petroleum feedstock, and high cost (Kasirajan and Ngouajio, 2012). Various cellulosic and thermoplastic starch based biodegradable formulations have been developed and are currently available. These have been shown to have equivalent performance to PE in some scenarios, while in other settings they may

degrade prematurely allowing weeds to establish (Ngouajio *et* al., 2008). Cellulosic membranes are particularly susceptible to premature degradation and offer less soil temperature control (Miles *et* al., 2012). Formulations of thermoplastic starch/copolymer blends compare most closely with PE, and in cases of high labor and disposal cost they are favored over PE by producers.

In general, a viable alternative to PE must perform at least as well and the sum of all associated costs must be less than or equal to PE. However, one particular exception may be certified organic production.

1.5 Biodegradable mulch membranes for certified organic production

The philosophy and values underpinning certified organic agriculture conflict with the use of PE as a single use mulch. Nevertheless, PE film is highly effective and allowable for certified organic use. Thus, certified organic producers whose values eschew the use of PE mulch must compete with others who embrace PE mulch. Producers must either adopt PE mulch, or endeavor to market their products as "beyond organic" in order to get higher prices for their products to offset the increased cost of producing organically without PE mulch. This scenario is troubling because the same certified organic program created to act as a marketing signal of good stewardship may impede producers' ability to operate without dependence on non-renewable single-use petroleum products.

Consumers also have environmental and aesthetic concern regarding single use plastic, especially in certified organic agriculture. Use of plastics in other applications in certified organic production such as hoop structures, and groundcovers in outdoor

soilless certified organic production is a growing concern for advocates of ecologically integrated farming systems.

One possible avenue to reduce petroleum dependence and increase choice for certified organic producers is to develop practical bio-based alternatives to PE for mulching applications in certified organic systems.

Products must be allowable as soil amendments for certified organic production if they are intended to be incorporated into soil. This requires that any certified organic allowable biodegradable mulch comply with general certified organic input requirements, but currently allows the unlimited use of PE mulch film as it is intended to be removed completely from the system at the end of its use. The restriction that most challenges formulation of functional, certified organic allowable biodegradable mulch membranes is that they must not contain petroleum-derived substances. This restriction excludes all biodegradable thermoplastic starch films on the market because they contain petroleum derived plasticizers. Another requirement is that mulches are expected to biodegrade 90% within 2 years (ASTM D 5988/ISO 17556). This clearly defined rule for biodegradation rate is laboratory based, but the requirements for field degradation are less clear. A "reasonable level of degradation" must be achieved in the field application of these mulches.

1.6 Approaches for measuring mulch biodegradation

There are many barriers to reliable quantitative biodegradation estimates that are present in a field setting. Laboratory-based microcosm approaches to determining soil degradation of biodegradable mulches are attractive because they avoid these

challenges. Laboratory approaches are valuable to demonstrate whether a material is potentially fully soil biodegradable, and to determine potential biodegradation rate. Indirect methods such as respirometry are possible in the laboratory (ISO 17556, ASTM D5988). A wide variety of other indirect methods are possible in the laboratory based on polymer of interest, and some can be adapted to environmental samples (Eubeler *et* al., 2009; Lucas *et* al., 2008; Van der Zee, 2011; Spaccini *et* al., 2016). Results of such approaches tend to be less variable than field-based direct measurement of mass (Ghimire *et* al., 2017). However, field-based trials are necessary to corroborate results of controlled laboratory soil simulations in the actual environment where mulches will be used. Also, effect on biodegradation from environment, management practices, and their interaction can only be tested in the field.

A perfect measurement of in-field biodegradation requires the ability to determine the mass of mulch present regardless of size of particles that persist in soil, whether as original polymer or modified molecular form without having been metabolized to water, carbon dioxide or microbial biomass. A time-series of such a measurement would describe the progress of biodegradation. There are several barriers to achieving this measurement. Approaches based on recovering mulch from bulk soil are subject to error due to the difficulty of recovering small fragments; difficulty of separating soil, roots, and other debris from mulch; and the heterogenous distribution in soil after tillage (Ghimire *et* al., 2017; Miles *et* al., 2012). Indirect measurements of mulch mass loss in field soil are necessarily different from those possible in laboratory. Respiration, measured as released CO_2, is impractical due to the

small amount of mulch mass relative to all other sources of respiration in soil over the period of years expected for most biodegradation to occur. Unique chemical markers for mulch presence may be possible, but specific markers will differ depending on mulch type. It also would have to be validated that the absence of a chemical marker indicates full biodegradation of all chemical mulch components. Li *et* al. (2014) used percent mulch area remaining (PMAR) as an indirect measure of biodegradation.

1.7 Introduction to the current study

Currently the most widely used biodegradable mulch films are formulated from thermoplastic starch/copolymer blends. Several formulations are available commercially which are mostly based on the proprietary polymers under the trade names of MaterBi® and BioTelo®. These mulches have been thoroughly vetted for complete soil degradation, they are understood to biodegrade completely (Bastioli, 1998). They have also become economically competitive with conventional PE mulch in areas where labor and disposal costs are high. As such their adoption is growing rapidly. However, all such formulations rely on petroleum for some fraction of their copolymer component which excludes them from use in certified organic systems.

Polylactic acid is a promising feedstock for mulch membrane because it is known to biodegrade, and formulations derived from 100% bio-based feedstock can have the strength, flexibility, and resilience required of a mulch membrane (Masaki *et* al., 2005; Hakkarainen *et* al., 2000). However, it has been challenging to achieve sufficiently rapid biodegradation rates in polylactic acid mulch formulations (Siwek *et* al., 2015; Miles *et* al., 2012; Wortman *et* al., 2016; Martín-Closas *et* al., 2016). As a result, no polylactic

acid mulch membrane has yet been commercialized. There is particular interest in understanding what kinds of management practices may accelerate biodegradation of biodegradable mulches in general, and polylactic acid mulches in particular. Degradation rate of xenobiotic polymers found in mulch membranes is controlled by numerous factors that broadly fall into the categories of mulch composition or soil environment. Organic mulch additives such as alfalfa and soy meal have been shown to affect the rate of molecular weight decrease of the polylactic acid polymer (Thompson *et* al., 2019).

Ultimately any difference in mulch biodegradation is due to differences in behavior of soil microbial communities in contact with the mulch substrate. Manipulation of microbial communities has been shown to modulate degradation rate in polylactic acid (Karamanlioglu & Robson, 2013; Hakkarainen & Albertsson, 2000). Some common management practices are known to shift soil microbial communities. Cover cropping and compost amendment have been shown to influence soil microbial community composition and function (Barel *et* al., 2019; Finney *et* al., 2017; Butcher and Lanyon, 2005; Toyota and Kuninaga, 2006). Compost tea has garnered grower enthusiasm as a microbe-enhancing input. It is a preparation of small amounts of compost in aqueous suspension used as biological inoculum, though little evidence suggests that commonly applied rates can reliably shift microbial community (Scharenbroch, 2013). Often certified organic management plans utilize multiple strategies intended to benefit soil life, with hopes of achieving synergy between them.

The current study investigates post-harvest soil biodegradation of a novel prototype polylactic acid mulch loaded with wood particles (PLA), and a commercially available thermoplastic starch/copolymer blend mulch, Bio360® (BLK), under five management strategies in a vegetable production system typical of certified organic production field at two contrasting ecoregions of Nebraska – Western High Plains and the Western Corn Belt (Chapman *et* al., 2001). It is hypothesized that 1) direct recovery of mulch from bulk soil can estimate mulch mass in soil and 2) mulch type and management can affect rate of mulch mass loss and tensile strength. We also used climate and soil data in multivariate models to determine their potential impact on mulch mass loss.

2. Materials and Methods

2.1 Site descriptions and climate

Field trials were established in 2017 in two ecoregions of Nebraska – Western Corn Belt, Lincoln, NE (LNK), and Western High Plains, Scottsbluff, NE (SBF) to determine the effects of various management practices and location-driven climate and soil conditions (Table 1) on the degradation rate of two potentially biodegradable mulches in soil.

The current study focuses on mulch mass loss during the period after soil incorporation only, but during the working life of the mulch while it is on the soil surface, i.e. cropping season, weathering is also sustained (Sec. 2.5). We report precipitation and temperature information from the period of mulch installation until the second sampling event (Figure 1). At LNK, temperature and precipitation were near

the 10-year normal for the area. Temperature during the study period at the SBF site was near normal precipitation was ~25% increased from the normal.

2.2 Experimental design

The study was established at two locations using split-split-plot, randomized complete block design with three replications, and four observation timepoints for mulch mass loss. Each block was a pair of main plot rows. Main plots were 65.84m long x 1.83m wide extending the length of the experiment, separated by 0.30 m buffers. Main plots were split into two 1.83 x 32.92 m subplots. Subplots were split into six 1.83 x 5.49 m sub-subplots (Figure 2). Main plot treatments were two mulch types: black Bio360® (BLK) biodegradable plastic mulch formulated from the Mater-Bi® polymer (Novamont S.P.A.; Shelton, CT, USA) and a prototype bio-based polylactic acid and wood particle mulch (3M Company, St. Paul, MN; abbreviated hereafter as PLA). Mulches were applied to main plots and used for the 2017 growing season. Other treatments were established in fall post-harvest. Subplot treatments were removal status of mulch: removal (CTL) and incorporation (INC). Sub-subplot treatments were five management strategies: compost (COM), cover crop (COV), compost extract (CEX), a "kitchen sink" management (SNK) comprised of all three management practices, and a no amendment control (NA). These treatments were established Fall 2017 and maintained throughout the experiment.

2.3 Mulch descriptions

Mulch properties, including thickness and weight, are outlined in Table 2. The BLK mulch is Bio360® a leading biodegradable mulch formulated from thermoplastic

starch and poly-ε-caprolactone and formed by a film blowing process (Bastioli, 1998). BLK mulch was supplied by Johnny's Selected Seeds (Winslow, ME, USA). The PLA mulch is a prototype product of 3M Company (Minneapolis, MN, USA). It is a 100% bio-based product. The source materials used in its production are wood and polylactide resin lactic acid produced by fermenting sugar, typically from either corn or sugar cane. PLA was comprised of three distinct layers. The inner layer was black blown micro fiber polylactic acid (21% mulch mass) impregnated with wood particles approximately 0.5mm in diameter (62% mulch mass). This inner layer was somewhat fragile. Both outer layers were spun bond poly lactic acid (17% mulch mass; Lim, 2010). The outer layers were stronger, white in color, and translucent (Figure 3). PLA thickness was 1.14 mm. Roll width was 1.07 m and roll length was approximately 74 m. Weight was 298.1 g/m^2.

2.4 Sub-subplot treatment descriptions

Compost for the COM and SNK treatments was applied and incorporated by tillage with the mulches fall 2017. Compost was topdressed after harvest in fall 2018. Compost rate was adjusted to supply a target of 504 kg/ha total N. Compost applied at LNK was a municipal yardwaste compost applied at a rate of 57 Mg/ha and 60 Mg/ha (dry weight) in 2017 and 2018, respectively. Compost applied at SBF was a beef feedlot manure compost applied at 42 Mg/ha and 51 Mg/ha dry weight in 2017 and 2018, respectively.

Compost extract typically understood to be a suspension of compost in water including fine particulate and soluble fractions of compost. It is not clearly defined by

USDA organic regulations, but it is allowable for certified organic production as long as it is included in a producer's organic system plan and approved by their certifier (Samuelson *et* al., 2019). It is usually intended as a microbial inoculant, applied at such low rates as to supply negligible amounts of nutrient fertility. Compost extract for this study was prepared by vigorously kneading compost inside of a nylon filter bag with 400 µm openings while submerged in water. 60 g fresh (20 g dry equivalent) compost was used per liter of water. Any compost remaining in the filter bag was discarded. We selected a kitchen and yardwaste vermicompost on criteria recognized by popular sources to be desirable, including presence of darkly colored fungal hyphae, protozoa, and nematodes of diverse feeding groups visible at 400x magnification (Lowenfels and Lewis 2010, Soil Food Web School, 2019). Compost extract was applied to CEX and SNK plots by a coarse spray at a rate of 3742 L/ha every spring and fall. It was applied within 48 hours of tillage in fall 2017, at time of cover crop planting in spring 2018, and after mowing crop residues in fall 2018.

Cover crop was adapted to the available fallow period between cropping in COV and SNK plots. A mustard cover crop (var. Mighty Mustard® Pacific Gold) was sown at a rate of 22.4 kg/ha by broadcasting and lightly incorporated by hand raking. Mustard was sown 23 Mar. 2019 and re-sown 20 Apr. 2019 at LNK, and sown 23 Apr 2018 at SBF. It was terminated at a height of approximately 0.3 m by flail mowing and hand hoeing on 23 May 2018 and 30 May 2018 at LNK and SBF, respectively. After 2018 harvest, a cover crop of cereal rye (*Secale cereale*) and hairy vetch (*Vicia villosa*) was sown by

broadcasting and raking at a rate of 112 kg/ha and 44.8 kg/ha, respectively. Rye and vetch were sown 28 Sept. 2018 and 24 Sept. 2018 at LNK and SBF, respectively.

The SNK "kitchen sink" treatment was established to assess whether any synergistic or additive effects of the four active management treatments were present. COV, COM, and CEX were applied as described above to SNK plots.

NA plots received no management other than uniformly applied management ie. fertility, irrigation, and cultivation. The NA treatment was repeated twice within each subplot because an intended fallow irrigation treatment became impractical to maintain. We learned this after two events in which one inch of irrigation was applied to the SNK prior fallow irrigation plots at LNK only. This intervention is assumed to be negligible.

2.5 Pre-experiment field management

In order to expose mulches to a season of weathering by normal use during vegetable production prior to soil incorporation, a mulched crop of sweet pepper (*Capsicum annuum* var. Carmen) was produced. In the spring of 2017, the experimental fields were rototilled to a depth of 20 cm. Mulch treatments and drip tape were applied to main plots using a mulch layer/bed shaper implement. Sweet pepper was transplanted into the mulch in a single row at 0.6 m spacing. After harvest in the fall of 2017, crop residue was mowed, and the incorporation status subplot treatment was implemented. Mulches were removed or left the field on 28 Sept. 2017 and 5 Oct. 2017 at LNK and SBF, respectively. Removed mulch was gently cleaned and stored temporarily for use in mesh bags (described later). Within 48 hours of removal sub-

subplot treatments of COM and CEX, as well as these components of the SNK treatment were applied, and experimental fields were tilled with a spading machine implement (Celli Y70 spading machine, Celli SpA, Forlì, Italy <double check this>) on 29 Sept. 2017 and 5 Oct. 2017, at LNK and SBF respectively.

2.6 Mulch and soil sampling from mesh bags

Two approaches were used to track degradation of mulch: direct recovery using a wide-diameter soil probe, and a mesh bag approach.

2.6.1 Mesh bag preparation

To prepare mesh bags, mulch squares measuring 10 cm^2 were cut from the gently washed reserved mulch that was removed from the CTL subplot treatment. Each square was weighed then paired with a methanol-washed aluminum label embossed with a unique ID. Mesh bags were 26 x 15 cm nylon mesh bag with 200 µm openings and hook and loop closure.

Soil for filling mesh bags was collected from sub-subplots under the CTL treatment after mulch removal, application of COM and TEA treatments, and tillage. This soil was sieved to 1 cm and stored at 4^o C for less than one week before use in mesh bags to be buried in corresponding sub-subplots within INC subplots. 250g soil was added to each bag, then a mulch square was set on this soil, then another 250g soil was added followed by the label. Eight mesh bags were prepared in this way for burial in each sub-subplot within INC subplots. Mesh bags were buried to occupy a depth of 5-10 cm within each INC sub-subplot in a grid pattern of two rows with four bags each, spaced 0.61 m between rows and 0.91 m within rows (figure 4). Two mesh bags were

recovered at approximately six-month intervals, and a total of four recovery events over two years.

2.6.2 Mesh bag sampling

At the time of this thesis, two recovery events have been completed. Mesh bag burial position within plot was assigned a number (1-8) and two numbers were randomly selected for recovery for each plot and recovery event. At recovery, bags were placed in one-gallon plastic bags, randomly assigned to biological testing (B bags) or physical testing (P bags), and placed in coolers in the field, then held at 4° C for maximally one week before processing.

B Bags were cut or carefully ripped open on a 4mm sieve. Mulch fragments were recovered, loosely adhering soil brushed off with gloved hands, and stored at -20° C. Remaining soil was homogenized, 100g was stored at -20° C, and 100g was air dried and stored at room temperature. P bags were handled identically, except that all mulch fragments were air dried, and in the extreme treatments (SNK & NA) 3-5 g soil adhering or closely associated with mulch was air dried separately for future enzyme analysis.

2.7 Mulch and soil sampling from bulk soil

2.7.1 Soil chemical sampling

Soil samples used for soil chemistry (SOM, pH, and NO_3) were taken from each sub-subplot by eight 20 cm cores at time of mulch incorporation (fall 2017), and at the spring and fall 2018 sampling events. Soil chemical analysis was performed by Ward Laboratories (Kearny, NE, USA).

2.7.2 Mulch sampling from bulk soil

Mulch presence in bulk soil was estimated using a large soil probe (a golf hole cutter), 20 cm long with 10.2 cm internal diameter. Five 3 m diagonal transects were assigned to intervals within each sub-subplot. At sampling, we removed 8 cores at 0.3 m intervals along the transect and recovered mulch fragments from each (Figure 4). This approach resulted in sampling 653 cm^2 of soil surface area (0.65% of sub-subplot area) to a depth that ensured all mulch buried in this area was recovered. Each of the five transects were randomly assigned to a sample date. The first sample event was fall 2017, 20-22 days after initial tillage. This was to establish a baseline of mean and variability of recovered mulch mass among sub-subplots. Baseline sampling was 19 Oct. 2017 and 26 Oct. 2017 at LNK and SBF, respectively. From this sampling event, all mulch fragments were recovered, washed to remove soil mass, air dried and weighed. Total recovered mulch mass will be recorded again at the final sampling event in fall 2019. For the intervening sampling events we recorded a binary response for each core, presence of a mulch fragment of diameter greater than 24mm was recorded as a "yes", absence was a "no". A maximum of three small fragments were collected and tested for tensile strength at each sampling event (details to follow). Then all remaining mulch and soil was returned to its respective core hole.

2.8 Mulch tensile strength

Tensile strength decline is a measure of degradation that can detect changes in polymer integrity that may be occurring even if mass change is absent. Our method was designed to quickly and easily determine whether the mulch was becoming weaker,

independent of it apparent integrity, area recovered, or mass. It is cheap, simple and repeatable, but not the kind of thing for materials physicists. We hypothesized differences in mulch resistance to piercing between sampling events (time), in response to management treatments, and in response to recovery source (bulk soil vs mesh bag). Mulch tensile strength was reported as Newtons required to pierce a layer of mulch. Measurements were taken using a force gauge (FDX 100, Wagner Instruments, Greenwich, CT, USA) with 45° conical 8 mm diameter tip. Mulches measured were recovered from mesh bags (P bags), and from all INC sub-subplots recovered by large core. A single layer of mulch was fitted over the mouth of a plastic 15 mL conical tube and secured by a cap with a 1 cm drilled hole (Figure 5). The probe of the force meter was pushed through the hole in the cap to pierce the mulch. PLA mulch collected spring 2018 held together in its original 3-layer form, so this material was pierced (Figure 3). However, outer scrim layers of PLA had separated when collected fall 2018 from bulk soil, and in mesh bags both layers were free from one-another so tensile strength of a single PLA layer was tested at fall 2018 and future events, corresponding to just one white fragment in Figure 3a. When measuring bulk soil recovered fragments from the large cores, up to three measures were taken from three separate fragments and averaged. The mulch fragments from mesh bags were pierced in two places and averaged.

2.9 Mulch mass determination

Two approaches for determining mulch mass from recovered mulch fragments were used: washing and combustion. Mulch fragments recovered by large core in fall of

2017 and recovered from mesh bags in spring of 2018 were weighed after washing. Mulch was discerned by appearance. Visible mulch of any particle size, that was cohesive enough to be separated from soil was recovered. Mulch washing methods were adjusted for each mulch type in an effort to maximize recovery. For BLK mulch, we held mulch fragments in 0.25 mm sieves and gently polished surfaces under running water until visibly clean. After allowing to air dry at room temperature, any adhering soil particles that did not pass through the sieve were removed. The cleaned air dry mulch fragments were weighed. Washing PLA mulch was more challenging because soil was embedded in the matrix of spunbond fibers (Figure 3). Washing and flotation was used to separate mulch fibers and soil particles. Mulch was polished in a basin of water until visibly clean. This resulted in particles of the wood and dark middle layer of PLA to detach from the stronger white PLA layers. These particles were recovered by mixing the basin with soil and small mulch particles, allowing to settle for ten seconds, then pouring the basin of water over 0.5 mm sieves, leaving some of the heavier soil particles behind. The mulch particles were mingled with plant debris. This mixture was again immersed in water, after a brief settling, plant debris was removed and particles of mulch suspended in the water were recaptured with the sieve. Recovered mulch was air dried and weighed.

Samples collected in fall 2018 were too fragile to separate from soil by washing, therefore a combustion method was used for samples collected fall 2018 and later. Ash content of soil, fresh mulch, and soil-covered mulch samples were determined then mulch mass ratio in whole sample was calculated. First, recovered mulch samples were

air dried with associated soil particles. Root material was removed as much as possible. Samples were dried at 60°C in aluminum tins and weighed. Samples were combusted in a muffle furnace and weighed. The furnace was programmed to ramp to 550° C over a two hour period, hold at 550°C for four hours, then cool for 8-10 hours before samples were removed. Furnace temperature was approximately 130°C when samples were removed.

Ash content of soil and fresh mulch was used in calculation. Loss on ignition of soil ranged from 4.5 - 7.9%, and 2.5 - 2.9% at LNK and SBF, respectively. Ash content of fresh mulches was 0.45% (0.02% standard deviation) and 0.17% (0.08% standard deviation) for PLA and Bio360, respectively (M, Formula 1).

Soil ash content was found to vary significantly between plots within each location. To account for this, ash content was determined for mulch-free soil from each mesh bag (Formula 1, S). Change in mesh bag mulch mass was reported as percent mulch mass loss by dividing the mass of each recovered mulch fragment by its original mass.

Formula 1: Mass by loss on ignition

Used to determine grams of mulch in the dry sample of recovered mulch fragments.

$$Mulch\ Mass = G \times \frac{P - S}{M - S}$$

G – mass in grams of 60°C oven dried sample of soil and recovered mulch

P – fraction of sample mass remaining after combustion

S – fraction of soil mass remaining after combustion

M – fraction of mulch mass remaining after combustion

2.10 Soil temperature

Soil temperature was monitored in all sub-subplots with temperature sensors (Hobo; Onset, Pocasset, MA, USA). Sensors buried at a depth of 5 cm automatically logged temperature every four hours through the duration of the study. All sensors were programmed to record at the same times. Monthly mean soil temperature was used in further analysis.

2.11 Soil moisture

Soil moisture was measured approximately weekly starting at the beginning of the 2018 production season. Soil moisture was recorded as soil water potential in kPa, approximately weekly in extreme management sub-subplots (SNK and NA) using Watermark 200SS soil moisture sensors (Irrometer Co., Riverside, CA USA) buried to 30 cm at SBF and 20 cm at LNK. Sensors read soil water potential from 0 kPa (saturated) to -200 kPa (completely dry). Values were averaged to yield earl, mid, and late season soil moisture for further analysis.

2.12 Fatty acid methyl ester analysis (FAME)

Saprophytic fungal, bacterial, and total microbial biomass associated with mulch and soil were estimated by fatty acid methyl ester (FAME) analysis. In the current study soil and mulch ester-linked FAMEs were extracted from B bag mulch fragments and soil

by alkaline methanolysis (Grigera *et* al., 2007; Jeske *et* al., 2018). Briefly, approximately 10 g field-moist soil, and 1-5 g of field-moist mulch plus closely associated soil were incubated in 0.2 M methanolic KOH. pH was neutralized with acetic acid and FAMEs were separated into hexane, filtered through an Arcodisc® CR 13 mm syringe filter with 0.2 µm PTFE membrane, evaporated to dryness, and resuspended in hexane containing 0.05 mg ml^{-1} methyl-nonadecanoate as an internal standard. FAMEs were quantified by gas chromatography on an Agilent 7890 GC fitted with an HP-Ultra 2 (Agilent) capillary column (50 m, 0.2 mm I.D., 0.33 µm film thickness). Identity of FAMEs was confirmed on an Agilent 7890 GC fitted with an Agilent 5975 mass selective detector. FAMEs were quantified on the basis of nmol FAME per gram organic matter lost on ignition (nmol FAME/g LOI) for the sake of making comparisons between soil and mulch microbial biomass on a per carbon. Fatty acids are named by the IUPAC system described in Drijber et al., 2000). The biomarker C18:2*cis*9,12 was used for saprophytic fungi. Bacteria was the sum of 16 FAMES: iC14:0; iC15:0; aC15:0; C15:0; iC16:0; i10MeC17, iC17:0; aC17:0, cyC17(9,10), C17:0, i10MeC18; a10MeC18; 10MeC18:0; 10MeC19:0, cyC19(9,10), and cyC19(11,12).

2.13 Statistical analysis

2.13.1 Analysis of variance

Analysis of variance was conducted with the GLIMMIX procedure using SAS 9.4 (Cary, NC, USA) to test for effects of treatments on mulch mass loss, tensile strength, and fragments recovered by large core. Analysis of variance was performed treating locations as separate experiments because locations were considered fixed effects, the

model used was more stable when locations were separated because we had no evidence to suggest that variability was constant between locations, and because statistically analyzing main effect of location was not possible because there would be no replication if we considered location as a whole plot. A split-split-plot in time model was used in which mulch type was the whole plot, management was the subplot, and time was the sub-subplot. Block and block x mulch, and block x mulch x management were random variables. Mulch, management, time, and all possible interactions among these were fixed variables. All possible interactions were evaluated. When an effect was significant, mean separation was performed using Tukey's HSD with the LSMEANS function. Normality was assumed for all response variables except fragments recovered by large core which was analyzed as the binary response of number of cores with mulch/number of cores searched.

In the case of percent mulch mass loss, residual plots suggested adequate normality, so we assumed a normal distribution. BLK mulch at LNK was practically unrecoverable and fully degraded at the Fall 2018 sampling. To avoid violating assumptions of equal variance, we tested the effect of management, mulch type, and their interaction within LNK at the Spring 2018 sampling only. Then, the effect of time, management, and their interaction at LNK was tested within PLA mulch. Analysis for SBF followed the full split-split-plot in time model

2.13.2 Power analysis

Power analysis for baseline mulch mass recovery by large core was performed with SAS using the POWER procedure. Mulch mass in soil at the time of baseline

sampling shortly after incorporation was assumed to be equal across locations and sub-subplots, which had been subject to management treatments for a maximum of two weeks.

2.13.3 Principal component analysis (PCA)

Variability in temperature, moisture, and soil properties unrelated to experimental treatments existed between and within sites. We used multivariate techniques to infer whether these may be drivers of mulch degradation.

Presented are four PCA biplots, each representing both locations. Groups "all managements" and "extreme managements" (SNK & NA) are plotted separately, and spring 2018 is plotted separately from fall 2018, yielding four analyses (Figures 6-9). We arrived at this presentation after PCA was performed within location, within mulch type, and within location by mulch type. This approach yielded few trustworthy insights because we suspected spurious correlations due to the many comparisons and relative scarcity of data points, so data from both locations were considered together.

Analysis was performed in R (version 3.6.0, R Foundation for Statistical Computing, Vienna, Austria) using packages "ggplot2", "HSAUR", "ggfortify", "maptools", and "Hmisc". We used the prcomp function to perform principal component analysis (PCA) as an iterative data reduction step. First, the most granular data was plotted: nitrate, soil OM, and pH at three sample dates (baseline fall 2017, spring, 2018, and fall 2018); monthly temperature; early, late, and mid-season moisture; 2018 corn yield; spring and fall large core recovered mulch tensile strength; spring and fall mesh bag recovered tensile strength; and spring and fall mass loss percent.

Microbial and soil moisture data was only available for extreme treatments; soil moisture as well as spring and fall FAMEs (bacterial, saprophytic fungal, and total) from mulch and soil was only available for extreme (SNK and NA) management treatments. These data were added to the above and PCA was performed within these extreme management treatments. Analysis was separated into spring and fall periods such that included variables were biologically relevant to the period of degradation. Related variables (ie. monthly temperature) whose eigenvectors were similar in magnitude and direction were consolidated iteratively by averaging. Presented biplots were generated using response variables averaged across periods relevant to their respective mulch degradation observation. For example, data collected during summer 2018 was not included in PCA that included the spring 2018 mulch mass loss.

In the case of the extreme managements at fall 2018, no FAME data was available from BLK mulch because it was unrecoverable. In order to perform PCA, microbes colonizing mulch in this case was considered to be identical to microbes colonizing soil. Regressions comparing principal components to variables were performed with the cor.test function. The rcorr function was used to perform regressions comparing mulch mass loss at both periods with their respective consolidated variables: soil organic matter, pH, nitrate, soil temperature, bacterial biomass and fungal biomass (Table 3).

3. Results and Discussion

3.1 Bulk recovery – mulch mass baseline

Mulch mass recovered by large core was highly variable (Table 4). Management had no significant effect on mass recovered from the baseline sampling at LNK ($p = 0.43$) or at SBF ($p = 0.15$). This was expected as this sampling event was completed within several days of treatment application. Despite known difference in mulch mass incorporated between BLK and PLA, variability of this recovery method was such that there was no significant main effect of mulch at LNK ($p = 0.10$) or SBF ($p = 0.06$). Given this variability, sample sizes to achieve acceptable power for detecting anything but very large differences was not possible within our design. Given the mean and standard deviation from mulches pooled across location, a simple two-sample t-test for difference of means with power of 0.8 would be achieved with n per group = 68 in order to detect a 50% difference from our measured PLA mean (difference of 10.96 g/654 cm^2), and n per group = 64 to detect the same relative difference from the BLK mean (0.449 g/654 cm^2). These results informed the decision to record a binary response from each of 8 cores at future sampling events, until the final sampling when recovered mass will be weighed again.

Based on the mass per area of fresh mulches, and assuming no in-season degradation during the working life of the mulch before soil incorporation, the expected amount of mulch to recover by this method was 11.4 g and 0.9g for PLA and BLK, respectively (Table 4). Our average recovered amount matched the estimated amount for BLK when location was pooled (n=32; Table 4). But our average recovered PLA was

21.9 g, twice the expected amount and significantly different from 11.4 g ($p = 0.01$ by one-sample t-test). The probable primary cause of this is that PLA had a high tensile strength and the cutting edge of the core failed to cleanly cut the mulch. As a result, mulch was recovered that laid outside actual cylinder of the core volume. Another factor was probable imperfect washing of the recovered mulch fabric, fine adhered soil and biofilm increased mulch mass as measured. These results underscore the problematic nature of direct quantitative recovery of mulch from bulk field soil.

3.2 Bulk recovery – binary response for mulch presence in large cores

Bulk soil recovery provided qualitative information on the persistence of mulch in soil revealing overall trends in mulch macro-fragment decay. Figure 10 shows the ratio of cores containing mulch at SBF and LNK at both sample periods. Neither management, mulch type, nor time resulted in differences between number of cores containing visible mulch. However, at LNK the effect of mulch type and the interaction of mulch type with time approached significance at $p=0.061$ and $p=0.055$, respectively. The counterintuitive result of non-significant differences in Figure 10 arises from groups containing only zeroes. In fact, out of all 144 cores searched within BLK mulch at LNK from Fall 2018, only one contained a mulch fragment (in a TEA sub-subplot). In this scenario, it may be inappropriate to statistically analyze this grouping of data (BLK at LNK, fall 2018) due to a violation of the assumptions of variance in the binary distribution. Instead we can observe that it stands out as divergent from the other groups, but we cannot have confidence in the probability statement about the likelihood of a true difference.

Consistent with others' results, the bulk sampling approach failed to detect significance even among evidently large differences, as well as detecting any trend in small mulch degradation change. Wortman *et* al. (2016) and Ghimire *et* al. (2017) attempted a similar sampling method but with more labor intensive mulch area or mass measuring, they found the approach to be unreliable and highly variable requiring excessive sampling to achieve useful estimates of mulch quantity in soil. This is consistent with our baseline sampling described above. The binomial approach was intended to efficiently detect dramatic changes in mulch presence, similar to a grower searching several shovels of soil. Despite difficulty in applying statistical analysis to the binomial response, our data shows that a quick practical search for remaining mulch can detect large differences which were consistent with the difference in mulch loss from mesh bags, described below.

3.3 Mesh bag recovery - mulch mass remaining

Evident sources of error are important to report from the Spring 2018 sampling when washing and weighing was used for recovered mulch mass determination. First, several BLK mulch fragments gained weight, apparently due to soil and/or microbial biomass too tightly bonded to mulch surfaces to be removed without fragmenting the mulch. Second, the middle layer of the PLA mulch had completely lost any cohesion and appeared very similar to soil particles in color and size (Figure 3). As a result, the process of recovering and washing these micro-fragments was imperfect. Similar to BLK, tightly adhered soil was apparent after washing and drying PLA which introduced error in the opposite direction.

At Scottsbluff, NE (SBF), no significant management effect was present for mesh bag mass loss (p=0.95). Lincoln, NE (LNK) was analyzed in two parts to exclude the predominantly zero mass recovered of BLK mulch in fall 2018. No significant management effect was present at LNK within PLA (p=0.28), or within Spring 2018 (p=0.90). Significant effects of mulch type and time were present at both locations (Figure 11). The interaction of mulch and time approached significance at SBF (p=0.07), while this interaction could not be formally tested at LNK due to violation of statistical assumptions by the near zero amount of mulch recovered from BLK treatment at Fall 2018. Spring 2018 BLK mulch mass loss after winter burial was less than 5% at both locations. Mean remaining BLK mulch mass was 2.1% at the LNK Fall 2018 sampling, only 3 out of 18 bags had recoverable fragments. Mean remaining mulch mass was 67% at SBF, with all mesh bags containing recoverable BLK mulch. At LNK within PLA, effect of time was significant (p=0.01) with 74% remaining in Spring 2018, and 37% in Fall 2018. At SBF, PLA mulch mass remaining was not significantly changed between Spring 2018 and Fall 2018, with 48% and 33% remaining, respectively (Figure 11).

These results demonstrate that the compounded differences between locations outweighed any imposed differences due to management within location. This interpretation is in agreement with results of Li *et* al. (2014) in which high tunnel vs field environment also had minimal effect on degradation while location effects were present. Compost and cover crop use input organic matter and often shift soil microbial communities (Finney *et* al., 2017; Butcher and Lanyon, 2005; Toyota and Kuninaga, 2006). Differences in the soil environment due to location have a distinct impact on

degradation rate of biodegradable mulches, but these relevant differences are not easily manipulated by management. Even our relatively high N-based rates of compost (42-60 Mg/ha) failed to shift soil environment to any extent relevant to mulch biodegradation. Management decisions in systems that include biodegradable mulch should be informed by general best management, rather than expectations of accelerated biodegradation of mulch mass. Still unexplored in true field environments are specific microbial inoculants shown to accelerate mulch or mulch polymers in laboratory setting (Thompson *et* al., 2019; Motoo *et* al., 2012).

3.4 Mulch tensile strength

The force required to pierce a layer of fresh BLK and a single outer layer of PLA was 4.23 and 17.47 N, respectively. Management had no significant effect on mulch tensile strength at any location/mulch combination ($p \geq 0.62$). Both mulches at both locations became significantly less resistant to piercing over time. The effect of source was significant at LNK for PLA mulch, and it approached significance at SBF for BLK mulch ($p=0.01$ and $p=0.056$, respectively). Mesh bag recovered PLA mulch was consistently more resistant to piercing (Table 5). This result of tensile strength disparity between recovery methods suggests a habitat difference between bagged and bulk soil. The mesh bag mulches were neither subjected to the mechanical wear of the spading implement during soil incorporation nor to disturbance by surface cultivation by hand hoeing. Also, the influence of arthropod shredders and the priming effects of other soil fauna would have been largely excluded by the 200 µm opening size of mesh bags (Karberg *et* al., 2008).

BLK mulch strength had the opposite trend with respect to recovery source, it was less resistant when recovered from mesh bags (Table 5). This result is probably due to the way mulch was sampled from bulk soil. No fragments were recovered from bulk soil that were too small to be pierced, so the sampling was biased towards larger, more in-tact fragments. Fragments that were too deteriorated to pierce in bags were recorded to break at 0 newtons. Fall 2018 BLK was not recoverable at LNK, but it was recovered at SBF and required less force to pierce than the 0.5 N threshold for detection by the instrument.

Considered together, these tensile strength results offer insight to the limitations of a "percent mulch area remaining" (PMAR) approach to measuring biodegradation of mulch (Li *et* al., 2014). We found that deterioration of strength is occurring over time even while fragments large enough to be pierced appeared similar over time. Mulches with more than one ply like PLA can separate into fragments of lesser strength and more area than their original form. Also, fibrous mulches like PLA can become stretched without completely losing integrity, increasing their area and decreasing their tensile strength, an obstacle also noted by Li *et* al. (2014).

3.5 Principal component analysis of potential biodegradation factors

Principal component analysis was used with a focus toward discerning soil environment differences that may be associated with mulch mass loss at the two sampling events. Figures 6-9 are biplots of this analysis, and table 3 shows regressions between mulch mass loss and all variables used in PCA. Location differences were key drivers of the first principal component (PC1). PC1 explained 62% of spring and 69% of

fall variation during 2018 when all management treatments were considered (Figures 6 and 7). When microbial and moisture data was added and analysis was constrained within the extreme management treatments, the strength of PC1 decreased to 52% in spring and 46% in fall. In all four cases, clusters representing location appear to segregate along PC1. Thus, the differences most strongly attributable to location such as pH and SOM can be generally illustrated by the most strongly horizontal arrows on figures 6-9. The second principal component (PC2) tended to predict mulch type, being most characterized by mulch mass loss, microbial biomass extracted from mulch, and sometimes soil nitrate. No trends resulting from management treatment were apparent from this analysis in the presented figures or in the iterations during data consolidation.

3.5.1 PCA of Spring 2018, all managements

Figure 6 shows segregation by mulch type on PC2. PC2 correlated significantly with fall 2017 nitrate ($r = 0.34$, $p < 0.003$), average soil temperature from burial to first sampling ($r = 0.33$, $p < 0.003$), and mulch mass loss ($r = -0.84$, $p < 0.0001$). At this sampling event, BLK mulch mass loss was less than 5% at both locations, and PLA mulch mass loss was 33% at LNK and 55% at SBF. This is reflected by the mulch mass loss arrow pointing towards the lower right quadrat of the plot, occupied by points BLK in SBF. Fall 2017 nitrate and soil temperature were increased towards the upper side of PC2. There is a negative association between mulch mass loss vs fall nitrate and temperature along PC2. This association is weak but may be explained by increased N immobilization by the wood particles present in the PLA mulch and by the heat

absorbing properties of the black mulch material. Even after soil incorporation some mulch fragments remained on the soil surface.

3.5.2 PCA of Fall 2018, all managements

Among all managements at the fall 2018 sampling, mulch type nearly disappears as a driver of either principal component, while location still strongly affects PC1 (Figure 7). Percent mulch mass loss is roughly the same in BLK vs PLA mulch when pooled across location. But differences are clear when location is considered separately, BLK persisted more than PLA at SBF, while it was nearly entirely unrecoverable at LNK (Figure 11). This interaction with location results in the strong correlation between all factors and mulch mass loss shown in table 3. Because BLK was lost so completely at LNK, all location driven soil differences also associate with mass loss. All factors modeled by Figure 7 are strongly correlated with PC1 ($p < 0.001$), while PC2 was only correlated with fall 2018 nitrate ($r = 0.85$, $p < 0.0001$) and mulch mass loss ($r = -0.025$, $p = 0.04$). These results revealed a prominent location-driven difference in soil properties which was not strongly influenced by management and resulted in divergent summer degradation rate of BLK mulch, but similar degradation rate of PLA mulch. PLA is known to degrade more quickly at higher temperatures, Satti et. al. (2008) found only 10% mineralization of PLA after 180 days of incubation at 30° C. The wood component of PLA mulch is expected to be readily degradable in most soils. It appears that most PLA mulch mass loss in fall 2018 was due to wood mass loss, which was roughly equal between locations. As for difference in BLK mulch degradation between locations, it is not possible to definitively parse the relative impact of the various differences in soil

properties between location, but clearly the fine-textured, high organic matter, warmer summer soil conditions of LNK resulted in more rapid BLK mulch degradation. Such a marked degradation difference in BLK degradation between locations, and the above edaphic properties suggests that warmer moister conditions influenced LNK mulch, assuming that the drier air, coarser soil, and periodic drip irrigation at SBF caused cyclical moisture in the top few inches that was not detected by the watermark sensors buried at 30 cm. This explanation fits with optimal conditions for Mater-Bi® degradation described by Basioli *et* al. (1998). Given the function of mulch film to regulate moisture in the most shallow horizons of soil, mulch itself may accelerate mulch residue degradation, especially in drier climates.

3.5.3 PCA of Spring 2018, extreme managements

Constraining analysis to extreme management treatments allowed inclusion of microbial biomarker abundance (Figure 8). Significant correlations existed between PC2 and the fungal biomarker from mulch ($r = -0.84$, $p < 0.0001$), bacterial biomarker from mulch ($r = -0.52$, $p = 0.01$), and mulch mass loss ($r = -086$, $p < 0.0001$). PC2 explained 19% of variation and was mainly driven by increased degradation and microbial biomass present on PLA relative to BLK mulch. The inclusion of wood particle in PLA is intended to recruit and stimulate soil microbes, specifically fungi. Li. *et* al. (2014) suggested fungal biomass in soil to be stimulatory of PLA biodegradation. PLA in this study was effective at recruiting and increasing fungal biomass on mulch surfaces, supporting that the mass loss measured in PLA was due to biodegradation.

When analysis was constrained to PLA (biplot not shown), mulch mass loss and mulch-associated fungal biomass were not correlated ($r = -0.03$, $p = 0.93$) and did not share a correlation with a principal component. The increase in fungal biomass on mulch was not correlated with PC1 ($r = 0.15$, $p = 0.49$), which was dominated by location-associated differences in this analysis. This demonstrates that PLA mulch recruited and expanded fungal biomass to a similar extent at either location. The apparent association between mulch-associated fungal and bacterial biomass and mulch mass loss shown by figure 8 is driven by mulch type at the spring 2018 sampling event, whereby PLA lost more weight and hosted greater fungal and total biomass. Within PLA mulch, fungal biomass on mulch surfaces was not predictive of mulch mass loss, or strongly influenced by location, so soil fungal community may not be a useful target for management to increase degradation rate of PLA mulch.

Soil bacterial biomass and soil fungal biomass were associated only with location, suggesting that mulch had little influence on biomass of surrounding soil, and that mulch microbial colonization was not limited by the recruitment pool of either location's soil. Rather, location differences in degradation rate are probably due to various compounded factors possibly including, but evidently not limited to pH, SOM, nitrate, bacterial and fungal biomass in soil.

3.5.4 PCA of Fall 2018, extreme managements

PCA of fall 2018 extreme managements included the greatest number of possible predictors of mulch mass loss. New predictors in this analysis were fall microbial biomass variables and soil moisture averaged across the cropping season (Figure 9). Key

insights are highlighted by comparison of the fall 2018 PCA with and without microbial factors. These new factors and exclusion of non-extreme managements reduced the explanatory power of the PCA. Fall 2018 analysis including all managements and excluding microbial and moisture factors explained a cumulative 82% of variation in the first two principal components (Figure 7), while this analysis among extreme treatments only, including the microbial and moisture factors accounted for less variation in the first two principal components, 59% (Figure 9). Most of this loss of explanatory power was from PC1. Factors most strongly influenced by location (pH, SOM, temperature, spring NO_3) still characterized this axis. PC2 became most strongly characterized by soil moisture and mulch mass loss. It also correlated significantly with spring mulch bacterial FAMEs ($r = 0.41$, $p = 0.04$), fall mulch bacterial FAMEs ($r = 0.45$, $p = 0.02$), and fall mulch fungal FAMEs ($r = 0.47$, $p = 0.02$), but no other variables (Figure 9). These new variables caused mulch types to segregate along PC2, as they had not in the "all management" analysis of fall 2018. This shows that mulch type is a stronger driver of microbial colonization than either location or management treatment. The mulch types segregate along PC2 in a fashion that interacts with location. This may be partly an artifact due to the required assumption that BLK mulch microbial colonization was identical to its mesh bag soil colonization. Nevertheless, this PCA reveals that PLA mulch recovered from SBF now contained more fungal biomass per g OM than PLA from LNK. To explain this shift from spring, wood particle of PLA mulch at LNK may have been consumed more completely by fall than the wood in PLA at SBF, which may have become colonized and consumed more slowly. This is consistent with the cooler, drier

climate and sandier soil with less water holding capacity at SBF (Table 1, Figure1). This explanation is inconsistent however with the greater degree of mulch mass loss at spring 2018 in SBF. Though every effort was made to recover and wash mulch recovered from mesh bags in spring 2018, we suspect that the inner layer of the PLA mulch was so friable that it escaped the sandwiching effect of the stronger outer layers both during loading and mulch recovery from mesh bags. Particles of this inner layer may have been unrecoverable due to its physical properties rather than biodegradation.

The association between soil moisture and mulch mass loss is notable because moisture between locations was not dramatically different. Drip irrigation was provided as required through the growing season according to our soil moisture measurements. Also, within location variation was fairly high (Figure 12). Thus, spurious correlation based on location difference is less concerning with the relationship between moisture and mulch mass loss. Moisture availability significantly correlates with fall 2018 mulch mass loss and may have been a limiting factor for mulch biodegradation. However, the similarity in measured moisture between locations may not have existed where mulch was degrading. Mulch mainly occupied a depth of 0-10 cm. Sensors were placed at agronomically relevant depths according to soil type, 20 cm at LNK and 30 cm at SBF. SBF received less precipitation and has coarser soil (Table 1, Figure 1), as well as drier air. So it is reasonable to suspect that mulch fragments experienced a greater disparity of available soil moisture than measured. If this is true, then the interesting insight is that PLA degraded to an equal extent between locations by the fall 2018 sampling

(Figures 11 & 12). This is explained by increased ability of fungi to utilize substrates in reduced water conditions.

Conclusion

This study investigated degradation of two biodegradable mulch membranes under a typical certified organic field vegetable production system across two distinct locations and five management practices. Mulch mass loss, change in tensile strength, and qualitative presence by bulk recovery was not different between management treatments despite large differences in organic matter input. Degradation was instead influenced by location, mulch type, and recovery method (ie. mesh bag or bulk soil). Specific factors influencing mass loss seem to differ between mulches, but only broad site-related differences can be described. BLK mulch was nearly completely undetectable after 12 months of burial at LNK, but 67% of BLK mass remained at SBF. BLK degraded more rapidly in the LNK environment of fine textured soil, low pH, higher SOM, higher precipitation, higher temperature, and more soil moisture. PLA mulch loss was initially more rapid at SBF, but after 12 months of burial, location difference was not prominent with 33% and 37% remaining at SBF and LNK, respectively. Because PLA mulch contains 38% polylactic acid, this indicates that some portion of the polymer degraded in field conditions after 12 months of burial, at least beyond detection by direct recovery. It remains unclear to what extent these materials will biodegrade, and the challenge of in-situ field study of biodegradation is the most important barrier to assessment of mulch biodegradation across many environments. Tensile strength measurement showed that deterioration progressed over time even when area of

individual fragments of material appeared in-tact. PLA weakened by separating into its component layers, as well as by the possible influence of fauna and cultivation which was more present outside the mesh bags. Both mulch types lost strength over time indicating that deterioration may not be directly related to mass or area loss. To demonstrate field biodegradation it will be necessary to improve methods for confirming complete biological transformation of mulch materials. The mass by combustion method used here is a promising method for determining mulch mass after burial, but improvements should be made on the mesh bag method for tracking buried mulch. The approaches in this study are suited to show agronomically relevant levels of degradation, but we are not able to answer questions regarding persistent microfragments of potentially biodegradable mulch.

Tables

Table 1: General geographic information and soil properties of experimental sites.

	Lincoln, NE (LNK)	Scottsbluff, NE (SBF)
Latitude	40°50'13.2"N	41°53'33.4"N
Longitude	96°39'50.4"W	103°40'54.0"W
Elevation	351 m	1198 m
Mean annual precipitation[a]	78.7 cm	38.1 cm
Soil series[a]	Zook	Tripp
Texture class[a]	Silty clay loam	Very fine sandy loam
Soil subgroup classification[a]	Fine, smectitic, mesic Cumulic Vertic Endoaquolls	Coarse-silty, mixed, mesic Aridic Haplustolls
Soil pH[b] range	6.2-8.2	7.9-8.3
Soil pH[b] mean	7.1	8.1

[a]Soil series, texture, and subgroup classification were obtained from the Web Soil Survey online resource (Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Web Soil Survey. Available online at the following link: https://websoilsurvey.sc.egov.usda.gov/. Accessed [5/1/2019])

[b] Soil pH was measured in a 1:2 soil:water ratio by Ward Laboratories, Kearney NE, USA

Table 2: Mulch background and specifications.

Abbreviation	PLA	BLK
Trade Name	-	Bio360®
Manufacturer	3M Corporation	Novamont
Composition	polylactic acid (38%) and wood (62%)	thermoplastic starch and poly-ε-caprolactone
Roll Length	~74 m	1220 m
Roll Width	1.07 m	1.22 m
Thickness	1.14 mm	0.015 mm
Weight	298.1 g/m^2	20.2 g/m^2

PLA – polylactic acid and wood particle mulch; BLK – Bio360® mulch.

Table 3: Correlations between mulch mass loss and each predictor variable included in PCA across both mulches and locations.

Predictor of mulch mass loss		Correlation of predictor with mulch mass loss			
		All Management		Extreme Management (SNK & NA)	
		Spring 2018	Fall 2018	Spring 2018	Fall 2018
fall 2017 NO_3	Pearson r	-0.29	-	-0.31	-
	P-value	*	-	*0.14*	-
spring NO_3	Pearson r	-0.22	0.45	-0.11	0.55
	P-value	*0.07*	***	*0.61*	**
pH	Pearson r	0.23	-0.56	0.13	-0.64
	P-value	*0.06*	***	*0.54*	**
SOM	Pearson r	-0.25	0.55	-0.17	0.55
	P-value	*	***	*0.41*	**
spring Temp	Pearson r	0.13	-	0.01	-
	P-value	*0.27*	-	*0.95*	-
fall Temp	Pearson r	-	0.53	-	0.47
	P-value	-	***	-	*
fall NO_3	Pearson r	-	0.23	-	0.26
	P-value	-	*	-	*0.22*
sM bacterial FAME	Pearson r	-	-	0.23	0.71
	P-value	-	-	*0.27*	**
sM fungal FAME	Pearson r	-	-	0.59	0.17
	P-value	-	-	**	*0.41*
sS bacterial FAME	Pearson r	-	-	-0.09	0.31
	P-value	-	-	*0.68*	*0.14*
sS fungal FAME	Pearson r	-	-	0.27	-0.23
	P-value	-	-	*0.20*	*0.28*
fS bacterial FAME	Pearson r	-	-	-	0.41
	P-value	-	-	-	*
fS fungal FAME	Pearson r	-	-	-	-0.52
	P-value	-	-	-	**
fS bacterial FAME	Pearson r	-	-	-	0.28
	P-value	-	-	-	*0.19*
fM fungal FAME	Pearson r	-	-	-	-0.13
	P-value	-	-	-	*0.55*
soil moisture	Pearson r	-	-	-	0.54
	P-value	-	-	-	**

*FAME source abbreviations: s- spring 2018, f – fall 2018, M – extracted from mesh bag mulch, S – extracted from mesh bag soil. Significance codes: * - p<0.05, ** - p<0.01, *** - p<0.0001.*

Table 4: Baseline bulk recovery estimation of mulch mass in soil.

Mulch	Location	Mass Recovered (g/654 cm2)	Coefficient of Variation
PLA	LNK	19.6	136%
	SBF	24.3	74%
	both	21.9	103%
BLK	LNK	0.7	92%
	SBF	1.1	98%
	both	0.9	100%

PLA – polylactic acid and wood particle mulch; BLK – Bio360® mulch; LNK – Lincoln, NE; SBF – Scottsbluff, NE. Mass recovered is reported in g/645 cm^2 because this is the area sampled by eight large cores. Separate locations per group N = 18; pooled locations per group N = 32.

Table 5: Tensile strength across time, mulch type, and recovery source. Fall 2018 BLK was not recoverable at LNK from mesh bags and rarely recoverable from bulk soil, it was recovered from both sources at SBF, but it often required less force to pierce than the 0.5 N threshold for detection by the instrument.

Location	Time	Source	Force to Pierce (N) Mulch	
			PLA	BLK
LNK	Spring 2018	Mesh Bag	25.0 A	0.50 A
		Bulk	17.3 B	0.51 A
	Fall 2018	Mesh Bag	9.1 C	-
		Bulk	8.1 C	0.03 B
SBF	Spring 2018	Mesh Bag	11.1 a	0.53 ab
		Bulk	10.9 a	0.89 a
	Fall 2018	Mesh Bag	9.5 ab	0.00 b
		Bulk	7.5 b	0.31 ab

PLA – polylactic acid and wood particle mulch; BLK – Bio360® mulch; LNK – Lincoln, NE; SBF – Scottsbluff, NE.

Figures

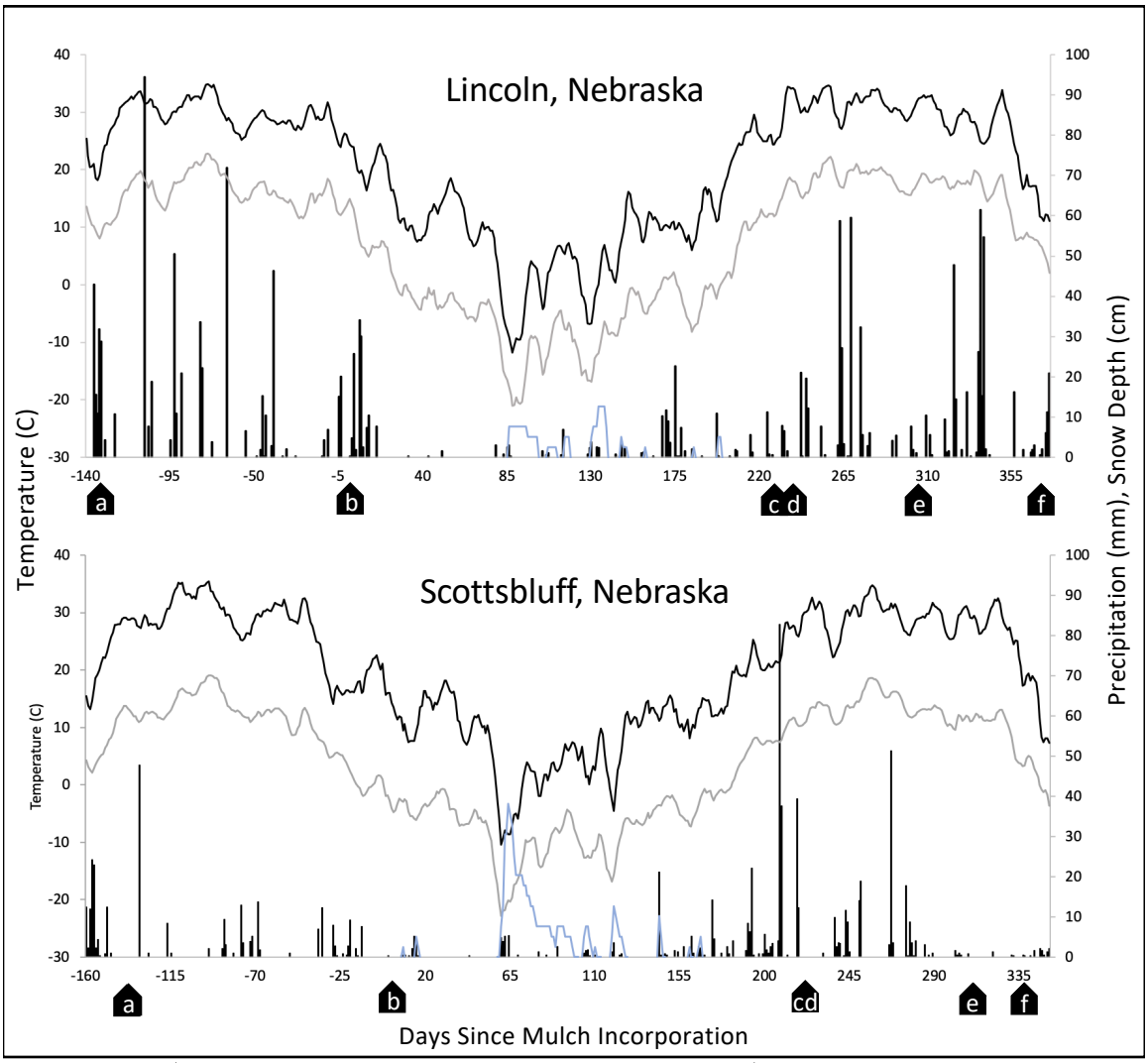


Figure 1: Climate from 16 May 2017 to 9 Oct 2018 at LNK and SBF locations. Black and gray lines are weekly rolling averages of daily high and low temperature. Bars are precipitation. Blue line is depth of snow accumulation. Data courtesy of the High Plains Regional Climate Center stations within 12 m elevation of sites, located 10.5 and 4.7 km from LNK and SBF sites, respectively. Cumulative precipitation before soil incorporation at LNK was 61.6 cm, and 90.2 cm after incorporation. Cumulative precipitation before soil incorporation at SBF was 26.6 cm, and 53.5 after incorporation (irrigation was also applied during the growing seasons).

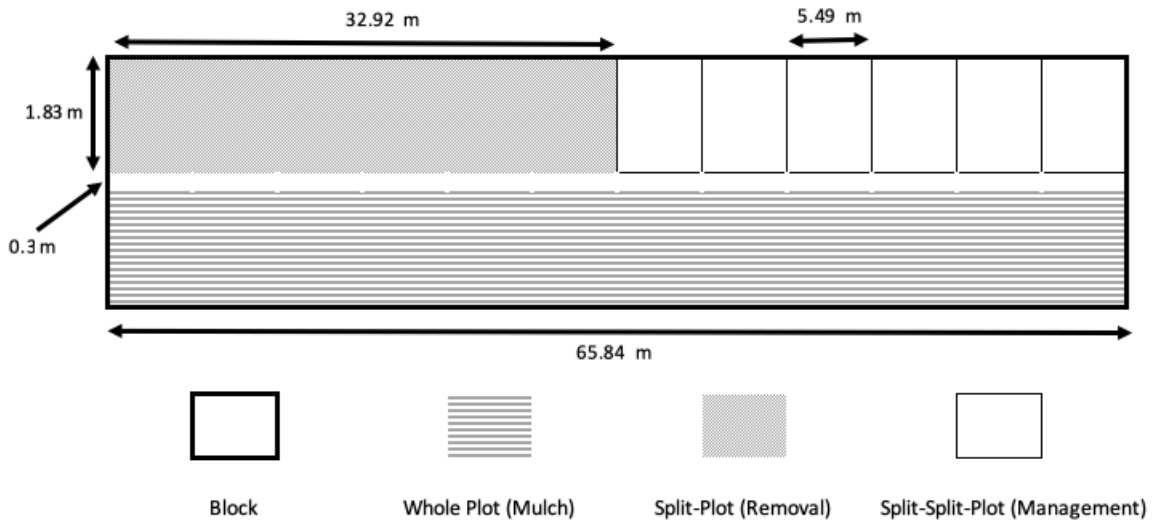


Figure 2: Field layout of each block.

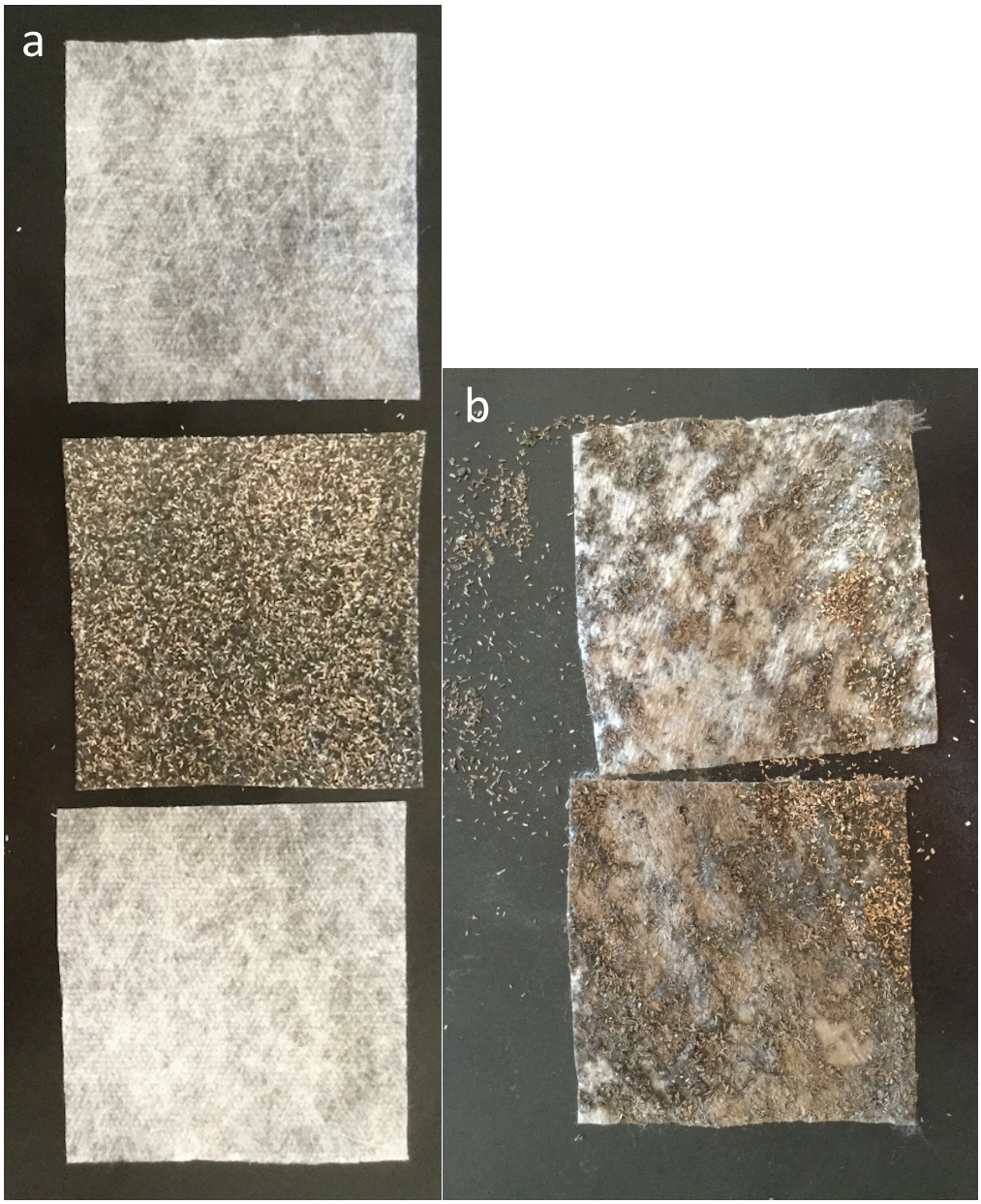


Figure 3: (a) Non-weathered PLA mulch peeled into its component layers. Lighter colored squares are the stronger outer "scrim" layers of spun bond PLA fiber, darker speckled layer is spun bond PLA with additives to block light from the soil surface, and impregnated with wood particles. Mass breakdown of the total mulch is 21%, 17%, and 62% white PLA scrim, dark PLA filler, and wood particle, respectively. (b) PLA mulch after weathering during working life, fall 2017 SBF.

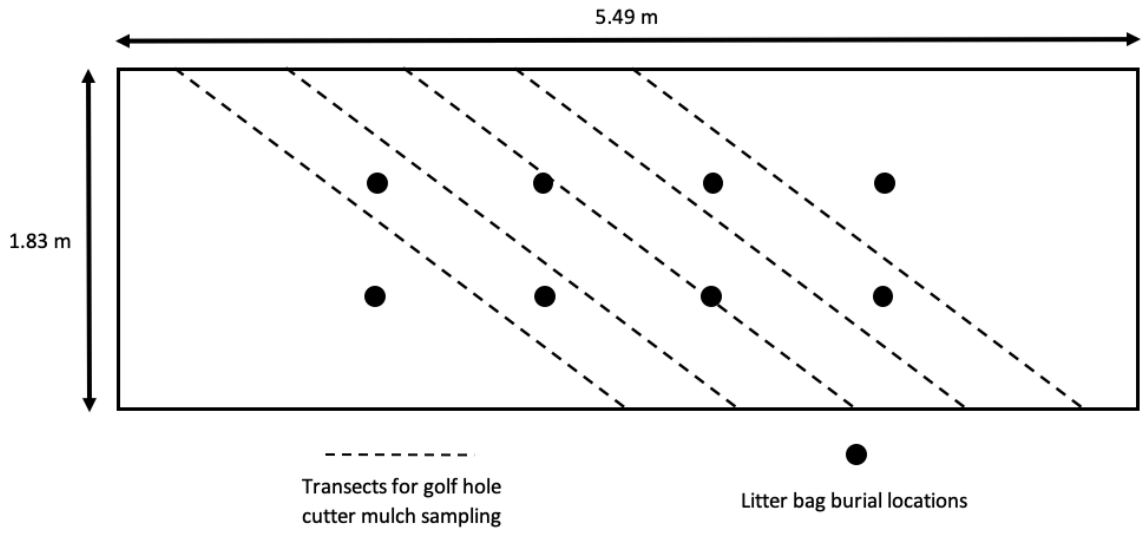


Figure 4: Scale diagram of each INC sub-subplot showing mesh bag burial pattern and transects for bulk soil sampling for mulch presence by large core (golf hole cutter). Cores were taken from eight points, spaced 0.3 m, along each transect. If a core location overlapped with a mesh bag location, the core was taken at a safe distance from the mesh bag and any other transect to prevent damaging buried mesh bags or overlapping with any past or future core sample.

Figure 5: Conical tube and drilled cap apparatus to secure mulch fragment for piercing with force meter.

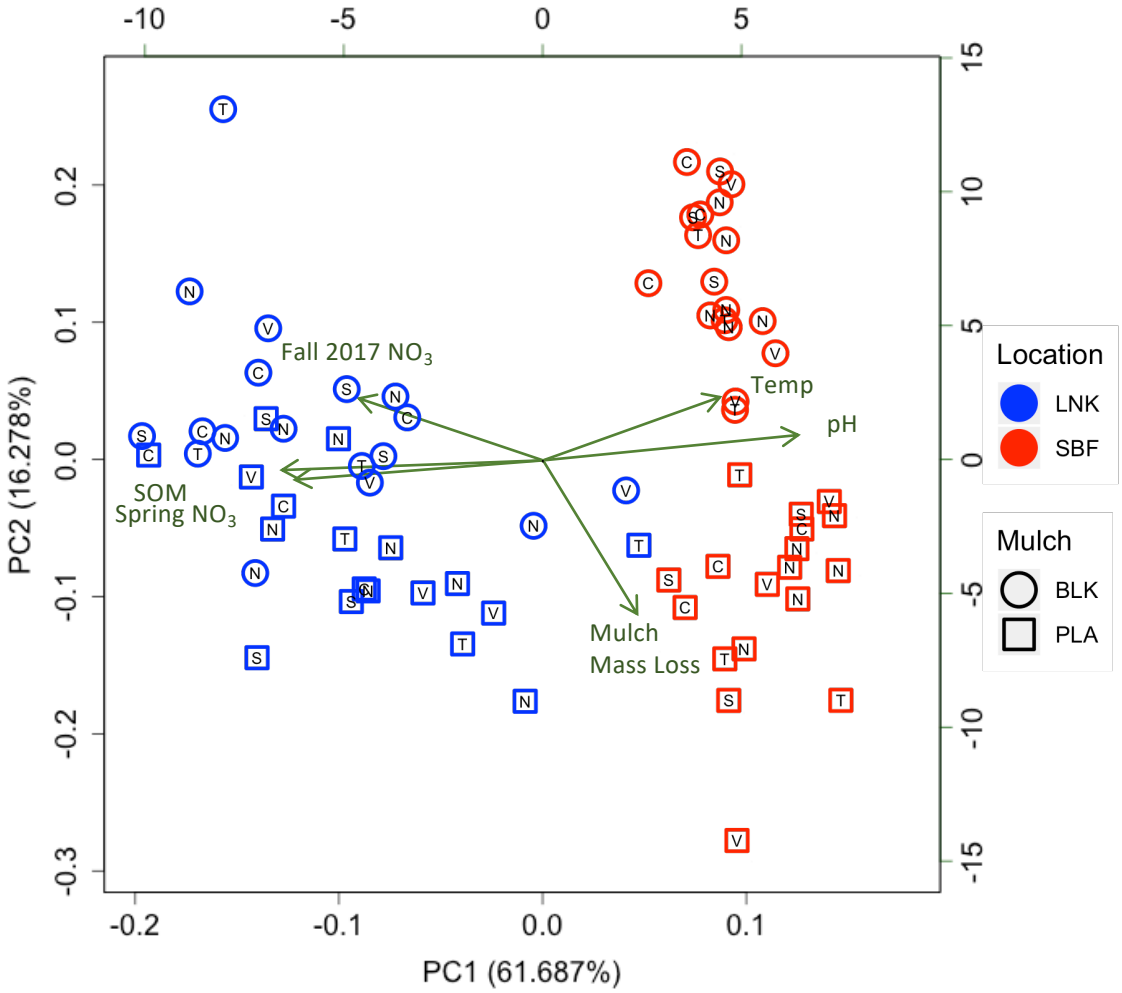


Figure 6: PCA of spring 2018 mulch mass loss and soil properties during period of potential influence among all management treatments. Management abbreviated further for point labels: C – COM (compost), V – COV (cover crop), T – TEA (compost extract), S – SNK (kitchen sink)

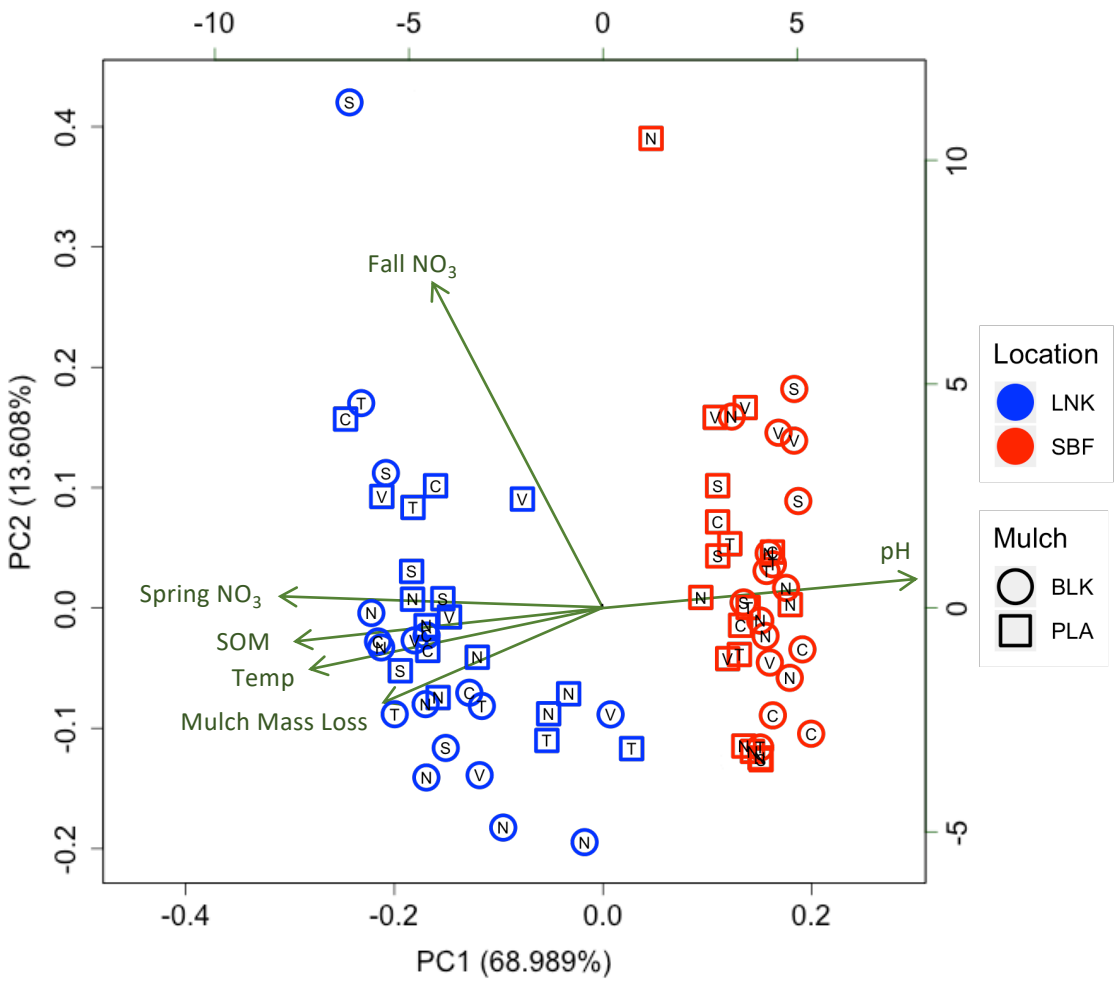


Figure 7: PCA of fall 2018 mulch mass loss and soil properties during period of potential influence among all management treatments.

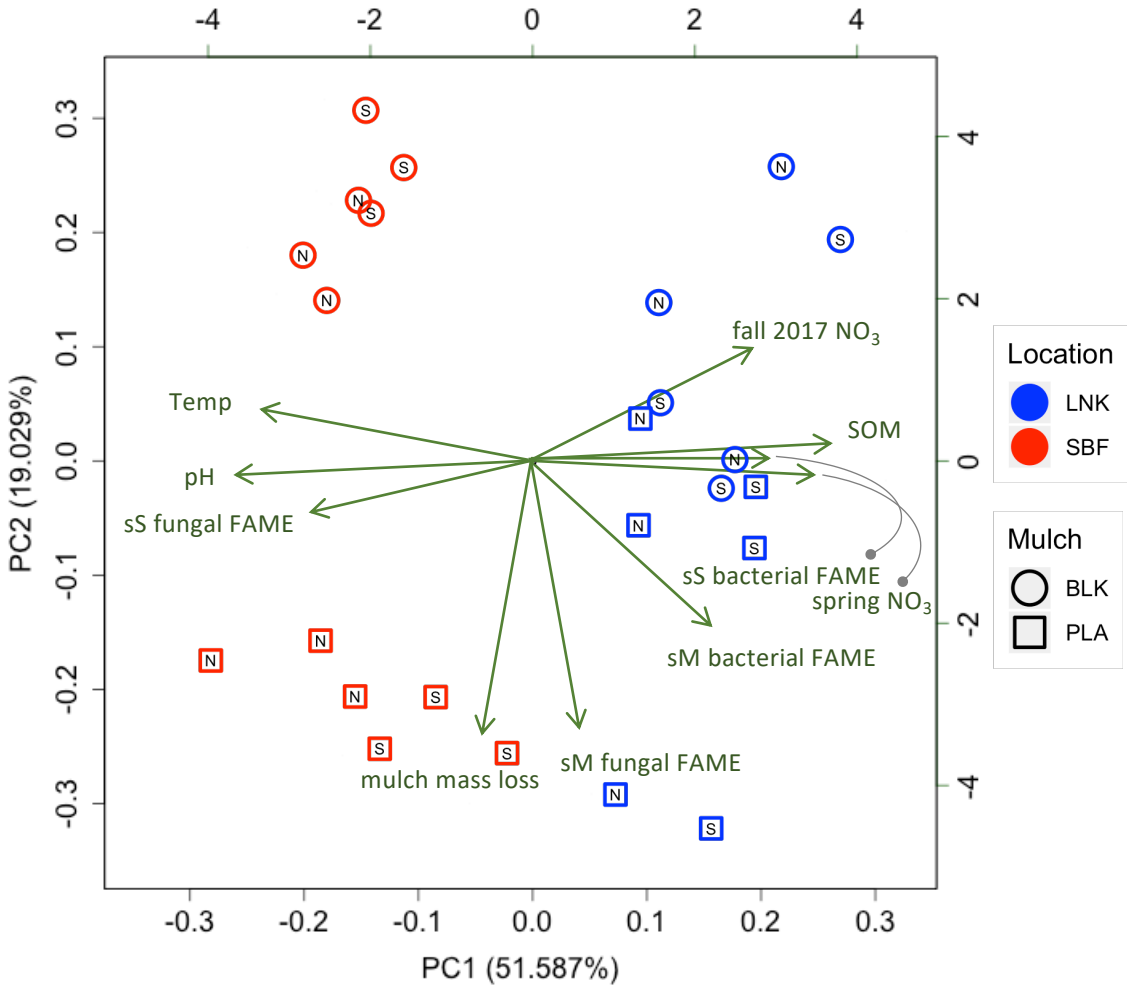


Figure 8: PCA of Spring 2018 mulch mass loss, soil properties and microbial biomass on mesh bag mulch and soil during period of influence among extreme (SNK & NA) management treatments. FAME abbreviations: s - spring 2018, S – soil recovered, M – mulch recovered. “fungal” refers to saprophytic fungal marker FAME.

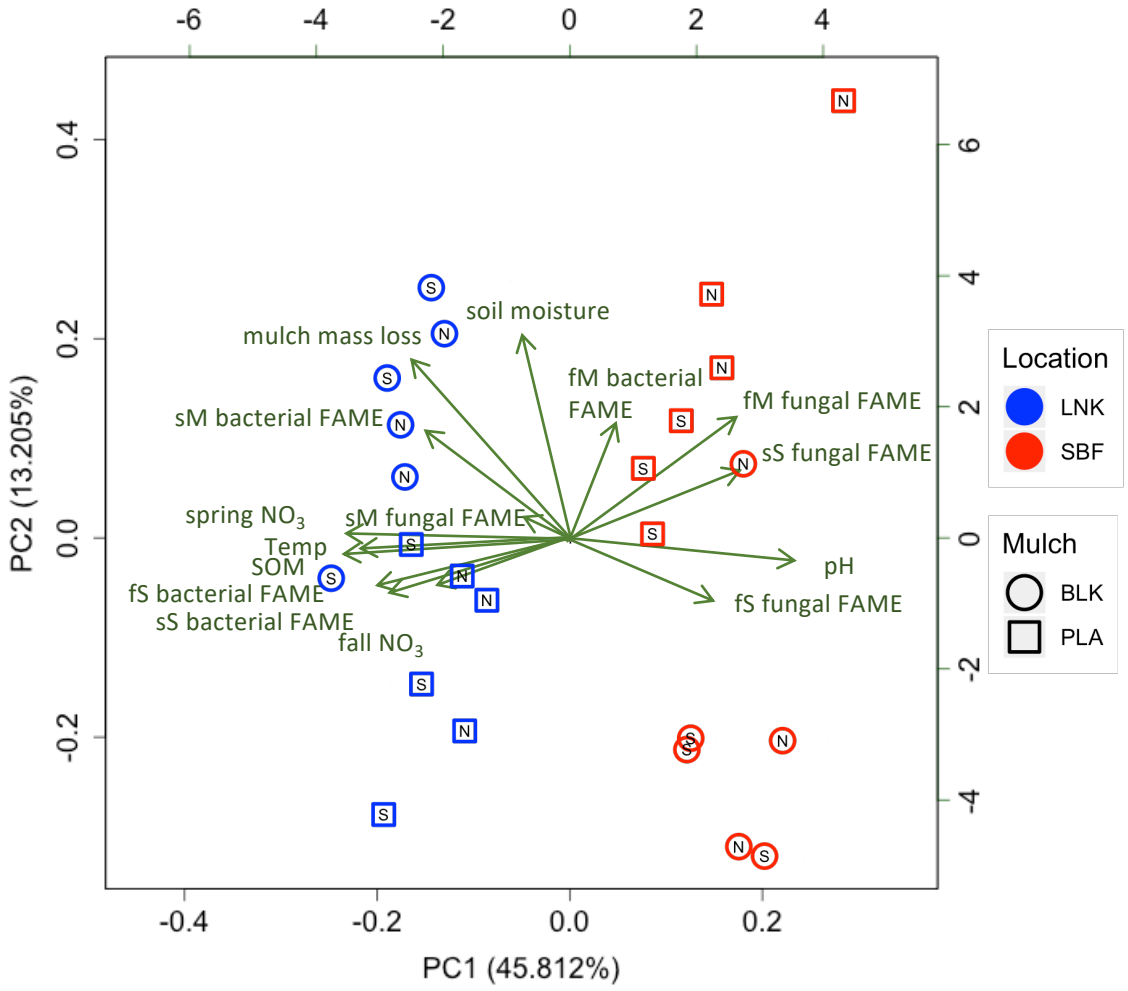


Figure 9: PCA of Fall 2018 mulch mass loss, soil properties and microbial biomass on mesh bag mulch and soil during period of potential influence among extreme (SNK & NA) management treatments. FAME abbreviations: s - spring 2018, f – fall 2018, S – soil recovered, M – mulch recovered. “fungal” refers to saprophytic fungal marker FAME.

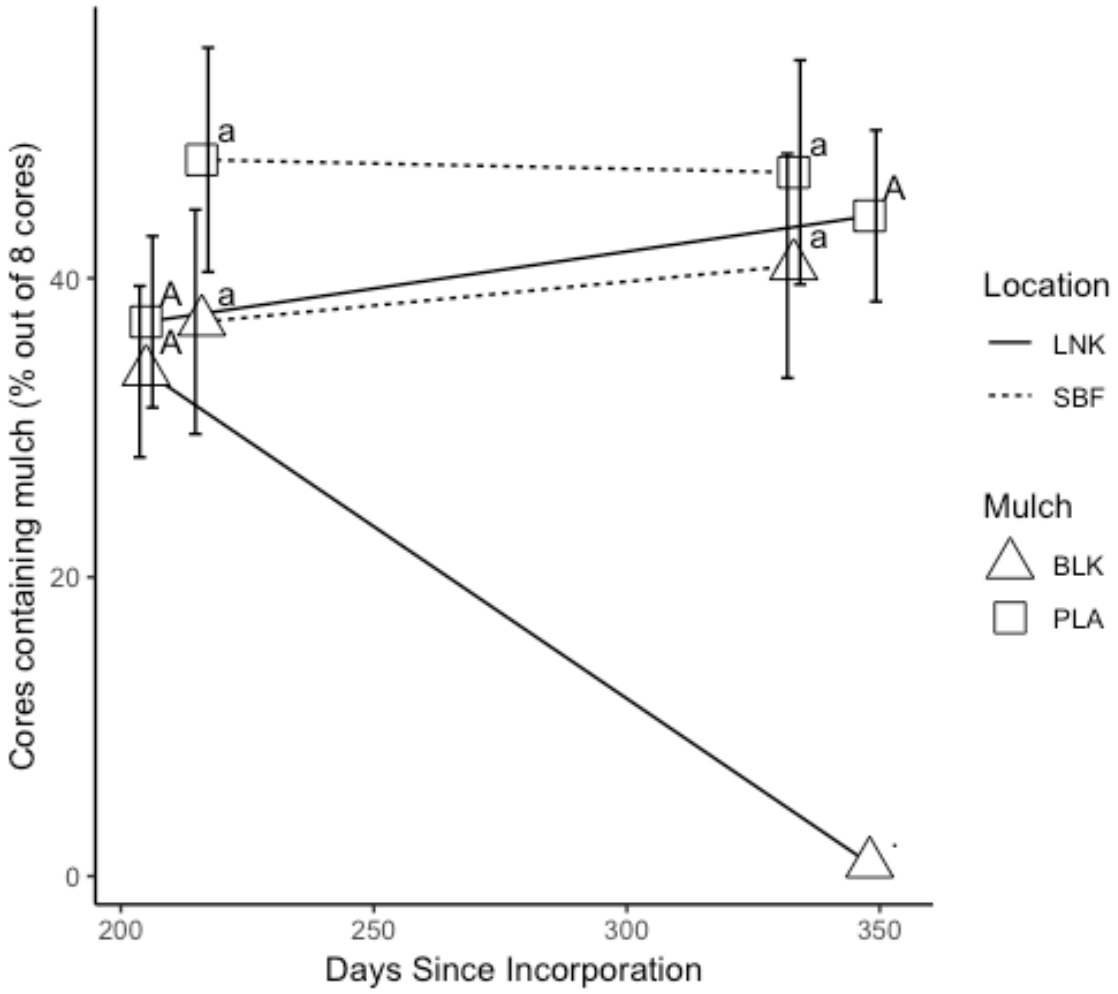


Figure 10: Qualitative mulch presence at two sample events. Values are mean "successes" out of eight attempts to recover a 2.5 cm diameter fragment of mulch from a 10.2 cm diameter by 20 cm depth core of soil. Error bars are standard error. BLK mulch at LNK on the later sampling date contained too many zero responses to analyze with the others, its error bar and significance code are removed.

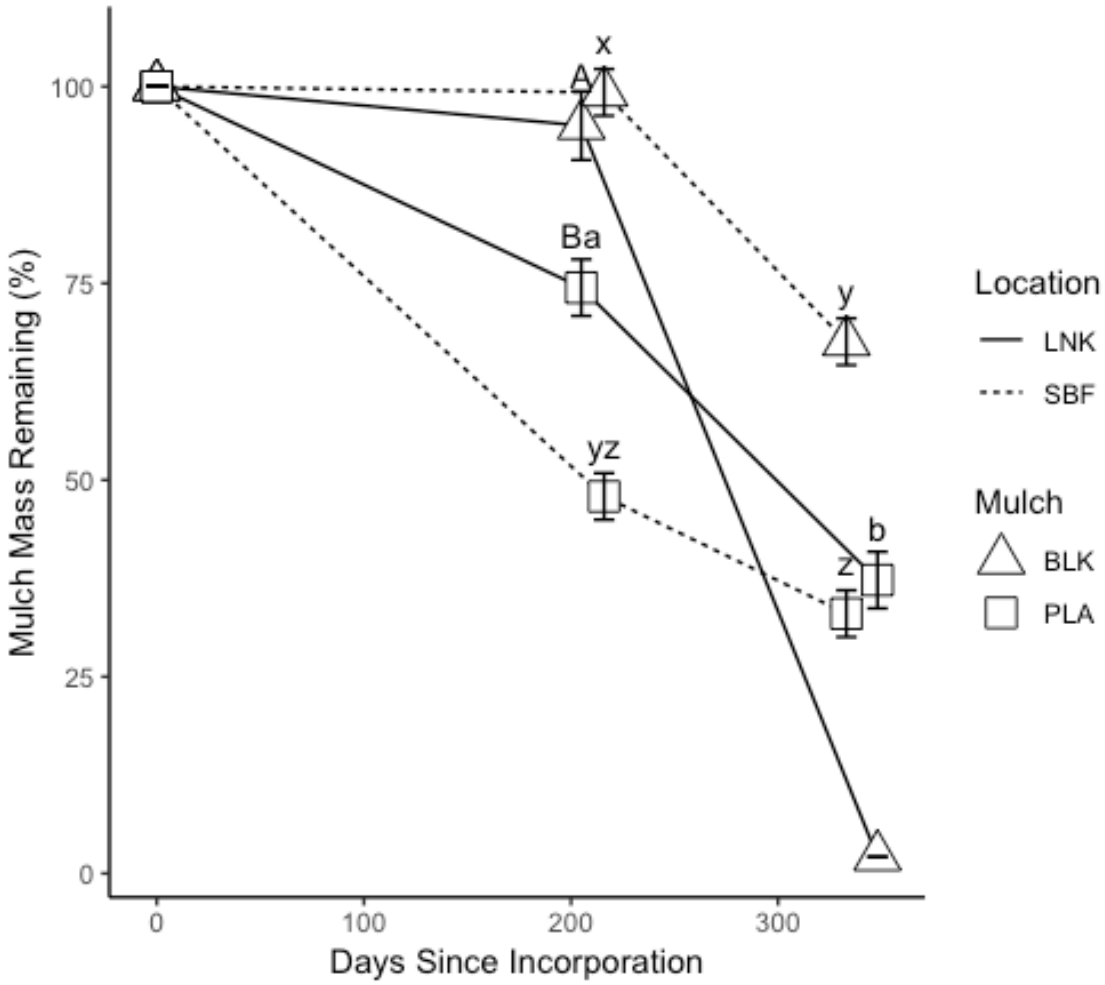


Figure 11: Percent mulch mass loss during one year of burial in litter bags. Values are mean +/- SE (n=18). LNK-Lincoln, Nebraska; SBF-Scottsbluff, Nebraska; BLK-Bio360® black mulch; PLA-prototype poly-lactic acid wood particle mulch. Means sharing lower-case letters "x-z" are not significantly different at Scottsbluff. Means sharing upper-case letters "A-B" are not significantly different at Lincoln 205 days after burial. Means sharing lower case letters "a-b" are not significantly different at Lincoln within PLA mulch. Differences determined by Tukey's test ($p < 0.05$).

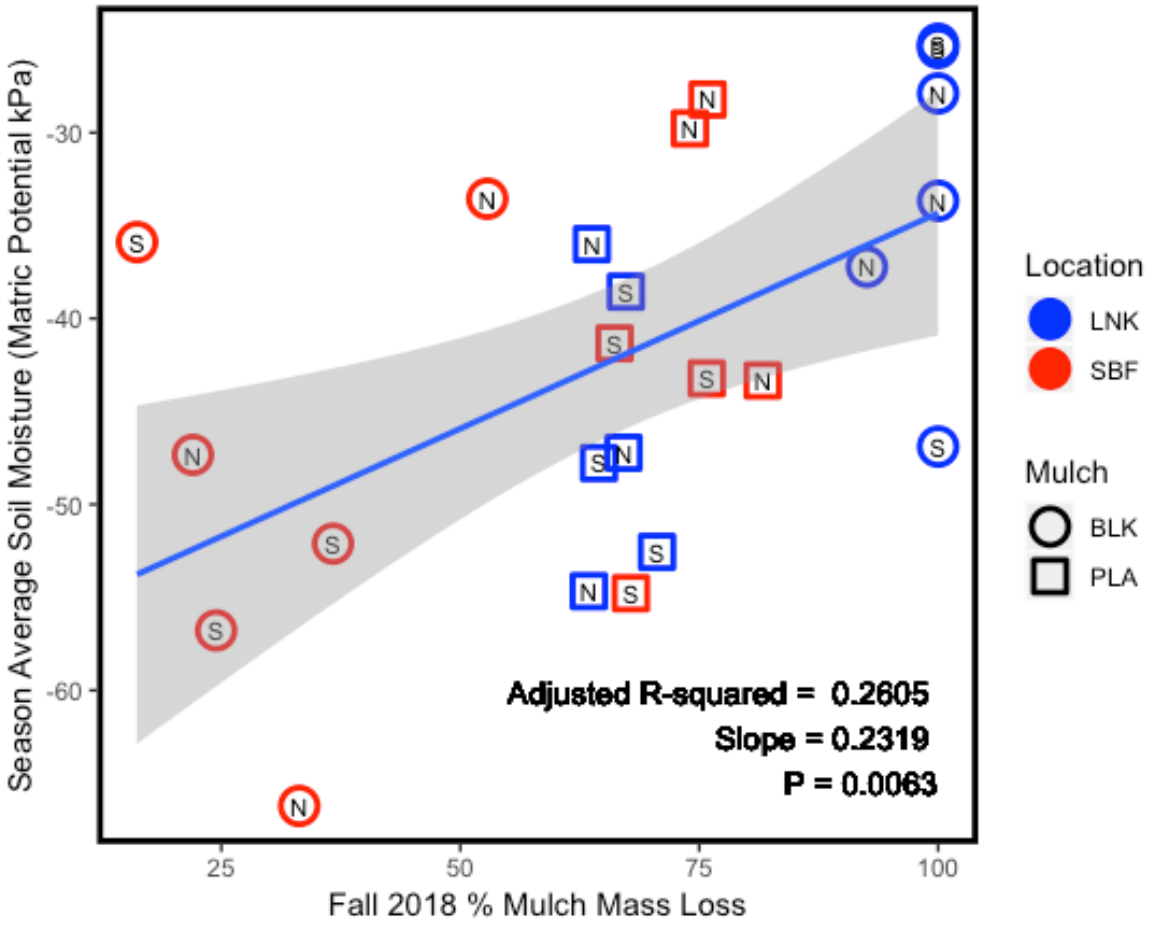


Figure 12: Scatter plot showing correlation of soil moisture with fall 2018 mulch mass loss showing increased mulch mass loss at greater moisture.

Literature Cited

Barel, Janna M., Thomas W. Kuyper, Jos Paul, Wietse de Boer, Johannes H. C. Cornelissen, and Gerlinde B. De Deyn. (2019) "Winter Cover Crop Legacy Effects on Litter Decomposition Act through Litter Quality and Microbial Community Changes." Edited by Lei Cheng. *Journal of Applied Ecology* 56, no. 1: 132–43. https://doi.org/10.1111/1365-2664.13261.

Bastioli, Catia. (1998) "Properties and Applications of Mater-Bi Starch-Based Materials." *Polymer Degradation and Stability* 59, no. 1–3: 263–72. https://doi.org/10.1016/S0141-3910(97)00156-0.Biochem. 39, 1589–1596.

Bhella HS. (1988) "Tomato response to trickle irrigation and black polyethylene mulch." *Amer J Soc Hort Sci* no. 113(4):543–546

Briassoulis, Demetres & Dejean, Cyril. (2010). "Critical Review of Norms and Standards for Biodegradable Agricultural Plastics Part I. Biodegradation in Soil." *J Polym Environ*. 18. 384-400. 10.1007/s10924-010-0168-1.

Bucher, A.E., and L.E. Lanyon. (2005) "Evaluating Soil Management with Microbial Community-Level Physiological Profiles." Applied Soil Ecology 29, no. 1: 59–71. https://doi.org/10.1016/j.apsoil.2004.09.007.

Carrera, L.M., J.S. Buyer, B. Vinyard, A.A. Abdul-Baki, L.J. Sikora, and J.R. Teasdale. (2007) "Effects of Cover Crops, Compost, and Manure Amendments on Soil Microbial Community Structure in Tomato Production Systems." *Applied Soil Ecology* 37, no. 3: 247–55. https://doi.org/10.1016/j.apsoil.2007.08.003.

Chapman, S.S., J.M. Omernik, J.A. Freeouf, D.G. Huggins, IR. McCauley, c.c. Freeman, G. Steinauer, R.T. An- gelo, and R.L. Schlepp. (2001) "Ecoregions of Nebraska and Kansas" (color poster with map, descriptive text, summary tables, and photographs). U.S. Geological Survey (map scale 1:1,950,000), Reston, VA.

Eubeler, Jan P., Sabine Zok, Marco Bernhard, and Thomas P. Knepper. (2009) "Environmental Biodegradation of Synthetic Polymers I. Test Methodologies and Procedures." *TrAC Trends in Analytical Chemistry* 28, no. 9: 1057–72. https://doi.org/10.1016/j.trac.2009.06.007.

Finney, D.M., J.S. Buyer, and J.P. Kaye. (2017) "Living Cover Crops Have Immediate Impacts on Soil Microbial Community Structure and Function." *Journal of Soil and Water Conservation* 72, no. 4: 361–73. https://doi.org/10.2489/jswc.72.4.361.

Ghimire, S., and C. Miles. (2016) "Dimensions and Costs of Paper, Polyethylene and Biodegradable Plastic Mulch." Washington State University Extension. Available

at http://vegetables.wsu.edu/Dimensions%20%26%20costs%20plastic%20and%20biodegradable%20mulch.pdf

Ghimire, Shuresh, Arnold M. Saxton, Annette L. Wszelaki, Jenny C. Moore, and Carol A. Miles. (2017) "Reliability of Soil Sampling Method to Assess Visible Biodegradable Mulch Fragments Remaining in the Field after Soil Incorporation." *HortTechnology* 27, no. 5: 650–58. https://doi.org/10.21273/HORTTECH03821-17.

Ghimire, Shuresh, D. Hayes, J. Cowan, D. Inglis, L. DeVetter, C. Miles. (2018) "Biodegradable Plastic Mulch And Suitability For Sustainable And Organic Agriculture," Washington State University Extension FS103E. http://pubs.cahnrs.wsu.edu/publications/pubs/fs103e/

Goldberger, Jessica R., Robert Emmet Jones, Carol A. Miles, Russell W. Wallace, and Debra A. Inglis. (2015) "Barriers and Bridges to the Adoption of Biodegradable Plastic Mulches for US Specialty Crop Production." *Renewable Agriculture and Food Systems* 30, no. 02: 143–53. https://doi.org/10.1017/S1742170513000276.

Grigera, Maria S., Rhae A. Drijber, Rebecca H. Shores-Morrow, and Brian J. Wienhold. (2007) "Distribution of the Arbuscular Mycorrhizal Biomarker C16:1cis11 among Neutral, Glyco and Phospholipids Extracted from Soil during the Reproductive Growth of Corn." *Soil Biology and Biochemistry* 39, no. 7: 1589–96. https://doi.org/10.1016/j.soilbio.2007.01.009.

Hayes, Douglas G., Sathiskumar Dharmalingam, Larry C. Wadsworth, Karen K. Leonas, Carol Miles, and Debra A. Inglis. (2012). "Biodegradable Agricultural Mulches Derived from Biopolymers." In *Degradable Polymers and Materials: Principles and Practice (2nd Edition)*, 201–23. https://doi.org/10.1021/bk-2012-1114.ch013.

Hakkarainen, M, S Karlsson, and A.-C Albertsson. (2000) "Rapid (Bio)Degradation of Polylactide by Mixed Culture of Compost Microorganisms—Low Molecular Weight Products and Matrix Changes." *Polymer* 41, no. 7: 2331–38. https://doi.org/10.1016/S0032-3861(99)00393-6.

Jeske, Elizabeth S., Hui Tian, Kathryn Hanford, Daniel T. Walters, and Rhae A. Drijber. (2018) "Long-Term Nitrogen Fertilization Reduces Extraradical Biomass of Arbuscular Mycorrhizae in a Maize (Zea Mays L.) Cropping System." *Agriculture, Ecosystems & Environment* 255: 111–18. https://doi.org/10.1016/j.agee.2017.11.014.

Kasirajan, Subrahmaniyan, and Mathieu Ngouajio. (2012) "Polyethylene and Biodegradable Mulches for Agricultural Applications: A Review." *Agronomy for*

Sustainable Development 32, no. 2: 501–29. https://doi.org/10.1007/s13593-011-0068-3.

Karamanlioglu, Mehlika, and Geoffrey D. Robson. (2013) "The Influence of Biotic and Abiotic Factors on the Rate of Degradation of Poly(Lactic) Acid (PLA) Coupons Buried in Compost and Soil." *Polymer Degradation and Stability* 98, no. 10: 2063–71. https://doi.org/10.1016/j.polymdegradstab.2013.07.004.

Karberg, Noah J., Neal A. Scott, and Christian P. Giardina. (2008) "Methods for Estimating Litter Decomposition." In *Field Measurements for Forest Carbon Monitoring*, edited by Coeli M. Hoover, 103–11. Dordrecht: Springer Netherlands. https://doi.org/10.1007/978-1-4020-8506-2_8.

Koitabashi, Motoo, Masako T Noguchi, Yuka Sameshima-Yamashita, Syuntaro Hiradate, Ken Suzuki, Shigenobu Yoshida, Takashi Watanabe, Yukiko Shinozaki, Seiya Tsushima, and Hiroko K Kitamoto. (2012) "Degradation of Biodegradable Plastic Mulch Films in Soil Environment by Phylloplane Fungi Isolated from Gramineous Plants." *AMB Express* 2, no. 1: 40. https://doi.org/10.1186/2191-0855-2-40.

Kyrikou, Ioanna, and Demetres Briassoulis. (2007) "Biodegradation of Agricultural Plastic Films: A Critical Review." *Journal of Polymers and the Environment* 15, no. 2: 125–50. https://doi.org/10.1007/s10924-007-0053-8.

Li, Chenhui, Jennifer Moore-Kucera, Carol Miles, Karen Leonas, Jaehoon Lee, Andrew Corbin, and Debra Inglis. (2014) "Degradation of Potentially Biodegradable Plastic Mulch Films at Three Diverse U.S. Locations." *Agroecology and Sustainable Food Systems* 38, no. 8: 861–89. https://doi.org/10.1080/21683565.2014.884515.

Lim, HoSun. (2010) "A Review of Spun Bond Process" *Journal of Textile and Apparel, Technology and Management.* 6, no. 3: 13.

Lippert LF, Takatori FH, Wilding FL. (1964) "Soil moisture under bands of petroleum and polyethylene mulches." *Proc Amer Soc Hort Sci*, no. 85 :541–546

Liu, E K, W Q He, and C R Yan. (2014) "'White Revolution' to 'White Pollution'—Agricultural Plastic Film Mulch in China." *Environmental Research Letters* 9, no. 9: 091001. https://doi.org/10.1088/1748-9326/9/9/091001.

Liu, Hongfei, Xiaomei Yang, Guobin Liu, Chutao Liang, Sha Xue, Hao Chen, Coen J. Ritsema, and Violette Geissen. (2017) "Response of Soil Dissolved Organic Matter to Microplastic Addition in Chinese Loess Soil." Chemosphere 185: 907–17. https://doi.org/10.1016/j.chemosphere.2017.07.064.

Lowenfels, J., and W. Lewis. (2010) Teaming With Microbes: A Gardener's Guide to the Soil Food Web. Portland, OR: Timber Press.

Lucas, Nathalie, Christophe Bienaime, Christian Belloy, Michèle Queneudec, Françoise Silvestre, and José-Edmundo Nava-Saucedo. (2008) "Polymer Biodegradation: Mechanisms and Estimation Techniques – A Review." *Chemosphere* 73, no. 4: 429–42. https://doi.org/10.1016/j.chemosphere.2008.06.064.

Miles, Carol, Russ Wallace, Annette Wszelaki, Jeffrey Martin, Jeremy Cowan, Tom Walters, and Debra Inglis. (2012) "Deterioration of Potentially Biodegradable Alternatives to Black Plastic Mulch in Three Tomato Production Regions." *HortScience* 47, no. 9: 1270–77. https://doi.org/10.21273/HORTSCI.47.9.1270.

Martín-Closas, L., J. Costa, A. Cirujeda, J. Aibar, C. Zaragoza, A. Pardo, M. L. Suso, M. Moreno, C. Moreno, I. Lahoz, J. I. Mácua, and A. M. Pelacho. (2016) "Above-Soil and in-Soil Degradation of Oxo- and Bio-Degradable Mulches: A Qualitative Approach." *Soil Research* 54 (2): 225–36. https://doi.org/10.1071/SR15133.

Masaki, K., N. R. Kamini, H. Ikeda, and H. Iefuji. (2005) "Cutinase-Like Enzyme from the Yeast Cryptococcus Sp. Strain S-2 Hydrolyzes Polylactic Acid and Other Biodegradable Plastics." *Applied and Environmental Microbiology* 71, no. 11: 7548–50. https://doi.org/10.1128/AEM.71.11.7548-7550.2005.

Moore, Jenny, Annette Wszelaki. (2016) "Plastic Mulch in Fruit and Vegetable Production: Challenges for Disposal." Report No. FA-2016-02, *University of Tennessee Extension*. Available at https://ag.tennessee.edu/biodegradablemulch/Documents/Plastic%20Mulch%20in%20Fruit%20and%20Vegetable%20Production_12_20factsheet.pdf

Ngouajio,M., R.Auras, R.T. Fernandez, M. Rubino, J.W.Counts, and T. Kijchavengkul. (2008) "Field performance of aliphaticaromatic copolyester biodegradable mulch films in a fresh market tomato production system." *HortTechnology* 18:605–610.

Ohkita T, Lee S-H. (2006) "Thermal degradation and biodegradability of poly (lactic acid)/corn starch biocomposites." *Journal of Applied Polymer Science* 100:3009–3017

Samuelson, Ben, Samuel Wortman, Rhae Drijber. (2019) "Assessing the Quality and Possible Functions of Compost Extracts in Organic Systems." *eOrganic extension*. http://eorganic.org/node/33458.

Satti, Sadia Mehmood, Aamer Ali Shah, Terence L. Marsh, and Rafael Auras. (2018) "Biodegradation of Poly(Lactic Acid) in Soil Microcosms at Ambient Temperature: Evaluation of Natural Attenuation, Bio-Augmentation and Bio-Stimulation." Journal of Polymers and the Environment 26, no. 9: 3848–57. https://doi.org/10.1007/s10924-018-1264-x.

Scharenbroch, Bryant C. (2013) “Impacts of Aerated Compost Tea on Containerized Acer Saccharum and Quercus Macrocarpa Saplings and Soil Properties in Sand, Uncompacted Loam, and Compacted Loam Soils.” *HortScience* 48, no. 5: 625–632.

Siwek, P, I Domagała-Świątkiewicz, and A Kalisz. (2015) “The Influence of Degradable Polymer Mulches on Soil Properties and Cucumber Yield.” *Agrochimica* 59 (2). https://doi.org/10.12871/0021857201522.

Spaccini, Riccardo, Daniele Todisco, Marios Drosos, Antonio Nebbioso, and Alessandro Piccolo. (2016) “Decomposition of Bio-Degradable Plastic Polymer in a Real on-Farm Composting Process.” *Chemical and Biological Technologies in Agriculture* 3, no. 1. https://doi.org/10.1186/s40538-016-0053-9.

Soil Food Web School. (2019) “Course Manual for Foundation Course #1: Introduction to the Soil Food Web.” Soil Foodweb Inc., Corvallis, OR

Steinmetz, Zacharias, Claudia Wollmann, Miriam Schaefer, Christian Buchmann, Jan David, Josephine Tröger, Katherine Muñoz, Oliver Frör, and Gabriele Ellen Schaumann. (2016) “Plastic Mulching in Agriculture. Trading Short-Term Agronomic Benefits for Long-Term Soil Degradation?” *Science of The Total Environment.* 550: 690–705. https://doi.org/10.1016/j.scitotenv.2016.01.153.

Thompson, Ashley A., Mitchell Benjamin Samuelson, Ignatius Kadoma, Erick Soto-Cantu, Rhae Drijber, and Sam E. Wortman. (2019) “Degradation Rate of Bio-Based Agricultural Mulch Is Influenced by Mulch Composition and Biostimulant Application.” *Journal of Polymers and the Environment* 27, no. 3: 498–509. https://doi.org/10.1007/s10924-019-01371-9.

Toyota, K., and S. Kuninaga. (2006) “Comparison of Soil Microbial Community between Soils Amended with or without Farmyard Manure.” *Applied Soil Ecology* 33, no. 1: 39–48. https://doi.org/10.1016/j.apsoil.2005.09.002.

USDA. CFR Regulatory Text, 7 CFR Part 205, Subpart A — Definitions. § 205.2 *Terms defined.* Current 7/2/2019. *https://www.ecfr.gov/cgi-bin/text-idx?SID=ac13bb030ee7a5c5ded65732f5c8946e&mc=true&node=se7.3.205_12&rgn=div8*

Wortman, Sam E., Ignatius Kadoma, and Michael D. Crandall. (2016) “Biodegradable Plastic and Fabric Mulch Performance in Field and High Tunnel Cucumber Production.” *HortTechnology* 26, no. 2: 148–55.

Yang, Ning, Zhan-Xiang Sun, Liang-Shan Feng, Mu-Zi Zheng, Dao-Cai Chi, Wei-Zhong Meng, Zhi-Yan Hou, Wei Bai, and Kai-Yu Li. (2015) “Plastic Film Mulching for Water-Efficient Agricultural Applications and Degradable Films Materials

Development Research." *Materials and Manufacturing Processes* 30, no. 2: 143–54. https://doi.org/10.1080/10426914.2014.930958.

Zhang, G.S., X.B. Hu, X.X. Zhang, and J. Li. (2015) "Effects of Plastic Mulch and Crop Rotation on Soil Physical Properties in Rain-Fed Vegetable Production in the Mid-Yunnan Plateau, China." *Soil and Tillage Research* 145: 111–17. https://doi.org/10.1016/j.still.2014.09.010.

CHAPTER 2: MICROBIAL RESPONSE TO MANAGEMENT AND MULCH

1. Introduction

Mulch films are used extensively in agriculture worldwide for their dependable improvement of yield and production efficiency. Biodegradable compositions of mulch film may be an important alternative technology to the conventional low density polyethylene (LDPE) film: a single-use, petroleum-based, non-degradable product. Currently available biodegradable mulch films (BDMs) tend to deliver equivalent crop performance compared to LDPE depending on field conditions (Cowan *et* al., 2013; Miles *et* al., 2012). BDMs eliminate the removal and disposal costs associated with LDPE mulch. Postharvest management of BDM is soil incorporation by tillage which is part of a typical vegetable production plan.

All mulch films act as a physical barrier on the soil surface during their working life. This modifies aspects of the soil environment such as temperature, gas exchange, and moisture (Wortman *et* al., 2016; Steinmetz *et* al., 2016). As a result, the action of LDPE mulch film during its functional life can alter soil microbial properties and carbon dynamics (Zhang *et* al., 2015). Various studies have found both that overall soil microbial community can be measurably alerted, or unchanged by use and subsequent removal of mulch film (Carrera *et* al., 2007; Dong *et* al., 2017).

BDMs form a surface barrier during their working life similar to LDPE films, but they are designed to remain in soil and biodegrade along with other crop residues. As

xenobiotic inputs, they may impact soil microbial communities more persistently or dramatically than removed mulches. In developing understanding of ecological outcomes of novel BDM use, it may be useful to discern between the impacts on soil caused by its working life as a surface barrier, versus its soil incorporation.

BDMs can be formulated from a diverse set of polymers, copolymers, and additives. As such, BDMs are expected to biodegrade through diverse microbial pathways depending on their composition. Soil microbial communities are ultimately responsible for the rate and extent of BDM biodegradation. Understanding microbial community shifts on BDM surfaces after soil incorporation may suggest the microbial groups which contribute to biodegradation, while community shifts in surrounding soil will indicate the magnitude of mulch influence and possibly predict long term consequences of BDM use.

Biodegradable copolymer films of thermoplastic starch with petroleum-derived plasticizers are currently the most widely used class of BDMs. These are sometimes the agroeconomic optimum, usually when cost of labor and disposal of LDPE is high. But the necessity of plasticizing and stabilizing starch with petroleum based additives excludes these films from organic production (Brodhagen *et* al., 2015; Shanks and Kong, 2012; Corbin *et* al., 2013). When BDMs are comprised of 100% bio-based feedstock, they are potentially allowable in organic agriculture, provided they also degrade in a reasonable timeframe in diverse field conditions. Polylactic acid is a promising polymer for 100% bio-based BDM production. This polymer is xenobiotic, microbial enzymes have not evolved to be highly effective for hydrolyzing polylactic acid, so it is among the more

recalcitrant substances used in BDMs, but it is ultimately biodegradable (Brodhagen *et* al., 2015; Torres *et* al. 1996; Satti *et* al., 2018). Films of pure polylactic acid are too brittle and UV light sensitive for mulch application (Tachibana *et.* al, 2009; Nuinu *et* al., 2013; Man *et* al., 2012). Instead, nonwoven polylactic acid fabrics are promising formulations for 100% bio-based BDMs. Loading polylactic acid fabrics with plant particles improves several aspects of the mulch, including biodegradability of the polylactic acid polymer component. It has been shown that the choice of organic particle loading influences rate of molecular weight loss of polylactic acid mulch (Thompson *et* al., 2019). Torres *et* al. (1996) found several filamentous fungi to colonize polylactic acid in wild soils and showed that different fungi have variable power for hydrolysis of the polymer and bioassimilation of its degradation products. Additional strategies for manipulating microbial communities around mulches to influence biodegradation rate are worthy of exploration.

The current study investigates the response of soil and mulch-associated microbial communities to two BDMs, a widely used starch/copolymer mulch and a potentially organic-allowable polylactic acid prototype in two distinct ecoregions. We used fatty acid methyl ester (FAME) analysis to characterize microbial biomass and community shifts in several soil microhabitats in a larger experiment tracking performance and degradation of BDMs over two years after soil incorporation.

We hypothesized [1] removal of mulches results in similar microbial communities regardless of mulch type, while distinct soil bacterial FAME profiles develop dependent on mulch type when mulch is incorporated, [2] management

including compost amendment and cover cropping impact soil and mulch bacterial FAME profiles, [3] mesh bag soils and bulk soils will not differ in bacterial FAME profiles, [4] wood-particle loaded polylactic acid mulch will increase saprophytic fungal biomass on mulch surfaces relative to surrounding soil and starch-based mulch, and [5] distinct bacterial FAME profiles will form on mulch surfaces depending on mulch type.

2. Materials and Methods

2.1 Site descriptions

Field trials were established in 2017 at two distinct locations, Lincoln, NE (LNK), and Scottsbluff, NE (SBF) to assess changes in soil microbial communities in response to two potentially biodegradable mulches, management practice, and location-driven climate and soil conditions (Table 1). Sites were chosen to for their dissimilar conditions. LNK is generally characterized by fine textured high organic matter soil with high water holding, high rainfall, and warmer temperatures. SBF is generally characterized by coarse textured, low organic matter soils with low moisture holding, low rainfall, and cooler temperatures. Weather data during the period of study and chronology of key events are shown in Figure 1.

2.2 Crop management

The experiment was cropped uniformly following a typical certified organic vegetable rotation. In 2017 sweet pepper was grown at 0.46 m spacing using the experimental mulches. Fertility was supplied to deliver 160 kg N/ha by fish emulsion (OrganicGem, NPK 2.9-3.5-0.3) and bloodmeal (Earthworks Health LC, 13-1-0), side-

dressed three times during the growing season. Fish emulsion was applied at a total rate of 786 kg/ha, at a 1:15 dilution. Bloodmeal was applied at a total rate of 646 kg/ha.

In 2018 sweet corn was grown at 0.2 m spacing in-row, and 1.1 m spacing between rows. Weeds were controlled by hand cultivation. Organic soybean meal fertilizer (Phyta-Grow, 7-1-2) was applied pre-season at a rate of 2240 kg/ha Fertility to supply at 157 kg N/ha.

During both seasons irrigation was supplied by drip tape as needed to maintain soil water tension moisture below 60 kPa at LNK, and 40 kPa at SBF as measured by Watermark 200SS soil moisture sensors (Irrometer Co., Riverside, CA USA) buried to 30 cm at SBF and 20 cm at LNK.

2.3 Experimental design and mulch treatments

Studies at both locations were established using a split-split-plot, randomized complete block design with three replications. Main plots were 1.83 x 65.84 m extending the length of the experiment, separated by 0.30 m buffers. Main plots were split into two 1.83 x 32.92 m subplots. Subplots were split into six 1.83 x 5.49 m sub-subplots (Figure 2). Main plot treatments were two mulch types: black Bio360® (BLK) biodegradable plastic mulch formulated from the Mater-Bi® polymer (Novamont S.P.A.; Shelton, CT, USA) and a prototype polylactic acid and wood particle mulch (3M Company, St. Paul, MN; abbreviated hereafter as PLA). Mulch input totaled 135 kg/ha and 1743 kg/ha for BLK and PLA, respectively (Table 2).

Subplot treatments were mulch removal status: removal (CTL) and incorporation (INC), in which mulch was either removed or soil-incorporated by tillage at the

completion of the 2017 sweet pepper cropping season. Sub-subplot treatments were management strategies. Six management treatments were included in the project, while this experiment considers only the two extreme treatments: a no amendment control (NA), and a "kitchen sink" management (SNK) in which compost, cover crop, and compost extract were applied (described below). Sub-sub-plots in the field were further split by sample recovery source (bulk soil recovery or mesh bag recovery), sample fraction (mulch or soil), and time (Figure 2). Mesh bags containing soil and a mulch fragment of known weight (described below) were buried only in INC plots, where mulch was incorporated, so only bulk recovered soil was recoverable from within the CTL treatment while bulk recovered and litter bag soil and mulch was recovered from INC plots (Figure 2).

2.4 Management treatments (sub-subplot treatments)

The NA treatment received no additional management beyond normal cropping including fertility input and irrigation in-season, described above in section 2.2.

The "kitchen sink" or SNK treatment was an approach that combined management strategies known or suspected to accelerate microbial activity. Compost, compost extract, and cover crop were applied to SNK plots.

Compost was applied and incorporated by tillage with the mulches in the fall of 2017. In fall 2018 it was top-dressed. Compost rate was adjusted for each batch to supply 504 kg/ha total N. Compost applied at LNK was a municipal yardwaste compost, it was applied at 57 dry Mg/ha in 2017 (and 60 Mg dry/ha in 2018, after the final sampling reported here so not relevant). Compost applied at SBF was a beef feedlot

manure compost, it was applied at 42 Mg dry/ha in 2017 (and 51 Mg dry/ha in 2018, after the final sampling reported here so not relevant).

Compost extract is typically understood to be a suspension of compost in water including fine particulate and soluble fractions of compost. It is not clearly defined by USDA organic regulations, but it is allowable for certified organic production as long as it is included in a producer's organic system plan and approved by their certifier (Samuelson et. al., 2019). It is usually intended as a microbial inoculant, applied at such low rates as to supply negligible amounts of nutrient fertility. Compost extract for this study was prepared by vigorously kneading compost inside of a nylon filter bag with 400 µm openings while submerged in water. 60 g fresh (20 g dry equivalent) compost was used per liter of water. Any compost remaining in the filter bag was discarded. We selected a kitchen and yardwaste vermicompost on criteria recognized by popular sources to be desirable, including presence of darkly colored fungal hyphae, protozoa, and nematodes of diverse feeding groups visible at 400x magnification (Lowenfels and Lewis 2010, Soil Food Web School, 2019). Compost extract was applied to CEX and SNK plots by a coarse spray at a rate of 3742 L/ha every spring and fall. It was applied within 48 hours of tillage in fall 2017, at time of cover crop planting in spring 2018, and after mowing crop residues in fall 2018.

A mustard cover crop (*Brassica juncea* cv. Mighty Mustard® Pacific Gold) from Johnny's Selected Seeds (Winslow, ME, USA) was sown at LNK on 22 Mar. 2018 and re-planted to increase establishment on 20 Apr.2018. The same cover crop was planted at SBF on 23 Apr. 2018. At all three planting events 22.4 kg/ha seed was broadcast and

lightly incorporated by hand raking. Mustard was terminated at a height of approximately 0.3 m by flail mowing and hand hoeing on 23 May 2018 and 30 May 2018 at LNK and SBF, respectively. Sweet corn (*Zea mays* cv. 'Xtra-Tender 2171) was sown within 24 hours at both locations. After 2018 harvest, a cover crop of cereal rye (*Secale cereale*) and hairy vetch (*Vicia villosa*) was sown by broadcast and hand raking at 112 kg/ha rye and 44.8 kg/ha vetch.

2.5 Litter bag preparation

To prepare litter bags, mulch squares measuring 10cm^2 were cut from the gently washed and air dried reserved mulch from the CTL subplot treatment. Each square was weighed and paired with a methanol-washed aluminum label embossed with a unique ID. Mesh bags were 26 x 15 cm, sewn from nylon mesh with 200 µm openings with a hook and loop closure. 250 g of soil sieved to 1 cm from corresponding plots was added to each litter bag, then a mulch square was set on this soil, then another 250g soil was added followed by the label. Eight litter bags were prepared in this way for burial within INC subplots, four of which were used for microbial sampling. Litter bags were buried to occupy a depth of 2-4 inches in a grid pattern within each sub-sub-plot of INC sub-plots, two rows of four bags each spaced 0.61 m between rows and 0.91 m within rows (Figure 3).

2.6 Mulch and soil sampling

Bulk soil and litter bags were sampled on the same day during events at approximately six month intervals after the time of mulch residue incorporation and litter bag burial. A total of four sampling events occurred over two years (Spring '18, Fall

’18, Spring ’19, and Fall ’19). At the time of this thesis, two sampling events have been completed.

At each sampling event, bulk recovery of soil was performed using latex gloves and trowels wiped with ethanol. Four soil samples from 0-10 cm depth were composited into a one-quart plastic bag, mixed, and approximately 250g soil was retained. Bulk recovery of mulch was performed simultaneously, approximately 150cm^2 mulch was recovered from the top 0-10 cm of soil as it was explored for bulk soil recovery. If necessary, we found mulch visible on the surface soil and excavated the buried portions of such pieces. These samples were collected into coolers in the field and refrigerated for one week maximally before processing by block along with the litter bags (described below).

At each sampling event, one litter bag was recovered by random selection from each sub-subplot under the INC treatment for microbial testing. After excavation, litter bags were placed in coolers in the field, then held at 4° C for maximally one week before processing. Bags were cut or carefully ripped open on a 4mm sieve. Mulch fragments were recovered, loosely adhering soil brushed off with gloved hands, and stored at -20° C. Remaining soil was homogenized, 100g was stored at -20° C, and 100g was air dried and stored at room temperature.

2.7 FAME extraction and quantification

Fatty acid methyl esters (FAMEs) were extracted from mulch and soil samples, quantified by gas chromatography, and reported on a nmol/g soil or nmol/g OM by loss on ignition (LOI) basis. To extract FAMEs, approximately 10 g field-moist soil, and 1-5 g

of field-moist mulch plus closely associated soil was added to 50 mL Teflon® tubes. Entire recovered fragments of bag mulch were added to tubes in order to achieve as large a sample as possible. In batches of 24 samples, 20 mL freshly prepared 0.2 M potassium hydroxide in methanol was added to each tube and vortexed for 30 seconds. Tubes were incubated at 37 ^{o}C for one hour shaking at 15 minute intervals to resuspend settled sediment. Then each tube was adjusted to neutral or slightly acid pH, as determined by pH paper, by adding 1-4 mL 1N acetic acid. From each batch one or two samples of each type (i.e. SBF soil and LNK soil) were titrated to neutrality and the same amount of acid was used to adjust the remaining samples of its type. To quantitatively extract hydrophobic FAMEs from the polar methanol solution, 5 mL hexane was added to each tube and tubes was vortexed for 30 seconds ensuring that any sediment pellet was broken up by shaking before vortexing. Tubes were balanced with methanol and centrifuged at 2900 G to separate liquid phases. Hexane supernatant was pipetted into 15 mL glass test tubes. An additional 5 mL hexane was added to the FAME extraction tubes, vortexed, and centrifuged as before. The supernatant was added to its corresponding glass tube. The methanol phase and residue was reserved for dry matter and organic matter determination (described below). Hexane was evaporated to small volume under a stream of N_2, one drop of benzene was added to drive off any water, and samples were evaporated to dryness. Using three washes, samples were dissolved in <2 mL hexane and transferred to small glass vials. Samples were evaporated in these vials and resuspended in hexane containing an internal standard of 0.05 mg/mL methylnonadecanoate (C19:0) for use in gas chromatography.

FAME abundance was reported on the basis of nmol FAME per gram soil or as nmol FAME/g LOI to standardize comparisons between mulch and soil and correct for differing amounts of adhered mineral soil on mulch samples. A combustion approach was used to determine LOI either directly from the sample residue remaining after FAME extraction from mulch samples, or from a subsample in the case of soil samples.

2.8 Dry mass and organic matter determination

To determine the dry mass of mulch samples, solvent and residue was decanted into weighed foil tins after FAME extraction. For each extraction session, 2-5 "blank" tins were filled with 20 mL fresh Methanolic KOH and the same volume of acetic acid used to neutralize samples. Solvent was evaporated, then tins were dried in a 60 °C oven to stable weight. Residue weight was not different due to extraction session, so the averaged residue weight of blanks was subtracted from the oven dry residue weights to determine oven dry sample masses.

For Spring 2018 mulch samples, blanks and sample tins were heated in a muffle furnace to 450 °C over two hours, held at temperature for 4 hours, then cooled to approximately 130 °C with the furnace closed. Tins were removed and weighed. Blanks did not lose significant weight. Spring 2018 soil FAME residues were not reserved, instead subsamples of spring 2018 soils were combusted at 550 ^{o}C separately (temperature change explained below). An OM ratio was calculated and multiplied by the dry sample weight to estimate sample OM mass. Formula 1 was used to calculate organic matter LOI for spring samples.

Formula 1:

$$\mathrm{D} - \mathrm{A} = \mathrm{LOI}$$

D – tin and oven-dry residue (g)

A – tin and ash (g)

LOI – Loss on ignition of organic matter (g) attributable to sample

For Fall 2018 soil samples and mulch samples, blanks and samples were combusted following the same protocol as for Spring 2018 mulch samples, except the furnace temperature was 550 °C because we determined that this temperature volatilized a few percent more mulch mass while volatilizing an insignificantly greater soil mass (data not shown). Again, blanks were prepared for each FAME extraction session to correct for any variance due to difference in solvent concentration or acid volume for pH adjustment. Blanks lost significant mass after combusting under this program. Among the 19 blanks prepared across all extraction sessions, the coefficient of variation was 2.2 and 1.5, for OD and ash residue, respectively. Mean oven and furnace mass loss from blank tins was used as a correction for all Fall 2018 soil and mulch samples. Organic matter LOI mass was determined using Formula 2.

Formula 2:

$$(\mathrm{D} - \mathrm{A}) - (B_D - B_A) = \mathrm{LOI}$$

D – tin and oven-dry residue (g)

A – tin and ash (g)

B_D – Dry blank and tin (g)

B_A – Ash blank and tin (g)

LOI – Loss on ignition of organic matter (g) attributable to sample

2.9 Analysis and reporting of chromatograph output

Areas under chromatogram peaks were integrated, standardized to the relative area under the peak of an internal standard (C19:0), converted to nmol FAME/g soil, matched to their fatty acid by retention time relative to the internal standard and identity confirmed by mass spectrometry. The total LOI content of each sample was used to report nmol FAME/g LOI. FAMEs were designated using the IUPAC system (International Union of Pure and Applied Chemistry and the International Union of Biochemistry and Molecular Biology, 1978) where the total number of carbon atoms is listed first and the position of double bonds, branch points, etc., numbered from the carboxyl end of the molecule. The prefixes “a” and “i” indicate anteiso and iso branching, respectively, 10Me indicates a methyl branch on the 10th carbon atom from the carboxyl end of the molecule, and cy(9,10) refers to cyclopropane ring between the ninth and 10th carbon atom.

Of the approximately 60 FAMEs detected, 30 were chosen based on abundance and biomarker significance. The sum of selected biomarker FAMEs were used to represent seven taxonomic groups for analysis by anova. These groups were total

FAMEs (30 FAMEs) as an indicator of total microbial biomass, bacterial FAMEs (16 FAMEs), the signature FAME for saprophytic fungi (C18:2cis9,12), bacterial FAMEs containing cyclopropyl groups (3 FAMEs), 10-methyl branched bacterial FAMEs (5 FAMEs), microeukaryotic FAMES (4 FAMEs), and the signature FAME (C16:1cis11) for arbuscular mycorrhizal fungi (Table 3).

2.10.1 Statistical analysis - ANOVA

Analysis of variance was performed on total FAMEs, the saprophytic fungal FAME, bacterial FAMEs, cyclopropyl FAMEs, 10-methyl FAMEs, microeukaryotic FAMEs, and the arbuscular mycorrhizal (AM) fungal FAME using PROC MIXED on SAS 9.4 (Cary, NC USA; Table 3). Normality was not checked, and no transformations were made to improve normality. LNK and SBF locations were considered separate experiments. Analysis was first performed using repeated measures in time which resulted in many interactions with time. Analysis was then constrained to location by time combinations. The effect of source (bulk recovery vs litter bag) on FAME profile was tested separately for mulch and soil by treating mulch, management, and source as fixed effects (whole plot, split-plot, and split-split-plot respectively). The effect of sample type (mulch vs soil) on FAME profile was tested separately for litter bag and bulk recovered soils within the INC treatment by treating mulch, management, and sample type as fixed effects (whole plot, split-plot, and split-split-plot respectively). The effect of mulch removal (INC vs CTL) on FAME profile was tested in bulk recovered soils by treating mulch, removal status, and management as fixed effects (whole plot, split-plot, and split-split-plot respectively). (Table 4).

In comparisons where BLK mulch at LNK in Fall 2018 was included, it was necessary to further constrain the model. This mulch was not recoverable, so no FAME was recovered from LNK/BLK/Fall 2018 samples. To avoid a violation of assumptions of anova, effects marked with superscripts in Table 4 were eliminated in such cases and a split plot model was used. For the model constructed to detect effect of type, two split plot anovas were performed. One compares soil under each mulch treatment (eliminating the mulch level of the type factor), the other compares PLA mulch to its surrounding soil (eliminating the BLK level of the mulch factor).

In addition to analyzing biomass as nmol FAME/ g LOI, when analysis included only the soil level of the type factor, anova was also performed on biomass as nmol FAME/ g soil. This unit is conventional in the FAME literature and avoids potential error associated with determining LOI. This redundancy offers a kind of protection increasing the trustworthiness main effect significance when it is shared across both analyses, which is helpful given the great number of anovas performed.

2.10.2 Statistical analysis - canonical discriminant analysis

Anovas are useful to compare biomass of taxonomic groupings of lipids, however this approach is not suited to detecting changes in microbial community composition. We used canonical discriminant analysis to test hypotheses regarding treatment effects on microbial community structure. Tests were performed on the 16 bacterial FAMEs, converted to percent of total bacterial FAMEs, as a proxy for community structure (Table 3). This set was used in part because these 16 bacterial FAMEs are largely derived from membrane-associated phospholipid-linked fatty acids (PLFA) while FAMEs

derived from fungi and other eukaryotes include storage, transport and cell-wall associated FAMEs and their contribution may change with cell physiological status. Thus, the set of 16 bacterial FAMEs would be indicative of a large proportion of the quiescent soil bacterial community (i.e. stabilized in soil aggregates and particulate organic matter) and thus a good indicator of overall soil bacterial community structure. Canonical discriminant analysis was performed using PROC CANDISC in SAS. Large differences in microbial community structure are expected between locations and times, so location by sample event combinations were often analyzed separately in order to discern the expectedly less prominent effects of treatments.

One analysis was performed to include all the soil samples measured for FAMEs to date in this project. Four groupings were formed to show SBF and LNK soil in spring and in fall.

To test effects of removal vs incorporation of each mulch, soils were grouped by mulch type (PLA and BLK), recovery source (bulk and bagged), and removal status (INC and CTL), pooling groups across management. Then, to test effects of management, the above soils were grouped by mulch type, management, and recovery source; pooling across removal status. The two above analyses were also used to detect effects of recovery source. To test the effect of management on microbial colonization of mulch surfaces, four groups were constructed from mulch by management treatments, pooled across source. As a reference for magnitude of other drivers of mulch microbial colonization, mulches were also analyzed in groups of mulch by source, pooled across treatment. This allows comparison of mesh bag vs bulk soil recovery against the effect

of managements. In both above mulch analyses, fall 2018 at LNK had no valid comparisons due to the lack of BLK mulch. In some mulch comparisons, one FAME (a10MeC18:0) was below detection levels with enough frequency to cause non-parametric results. In these cases (fall SBF mulch: mulch x mgmt.; and fall SBF mulch: mulch x source), this FAME was eliminated from the analysis. To test the influence of mulch type on recruited microbial community vs surrounding soil, groupings of mulch and soil were set such that mulch was separated by type and season, pooled across management and source; and soil was separated by season only, pooled across mulch type, management, and recovery source. This analysis was performed for each location separately.

3. Results

3.1.1 Effect of mulch removal and mulch type on soil microbial community

Hypothesis [1] was that microbial abundance and community structure of soils with mulch removed before tillage (CTL) will be unaffected by mulch type, and that distinct community structure would result from incorporation of PLA vs BLK mulches, which would also be different from CTL treatments under both mulches. In spring 2018 canonical discriminant analysis of bacterial marker FAMEs found no difference due to mulch type under the bulk recovered soils of the CTL treatments at LNK ($p = 0.40$) or at SBF ($p = 0.38$; Figure 4a).

This pattern continued at LNK in fall 2018 where there was no difference due to mulch type among bulk recovered soils under CTL treatments ($p = 0.32$). At SBF a bacterial community difference between mulch type emerged ($p = 0.02$). Only the first

canonical function was significant (p = 0.002), discrimination was most powerful along the first canonical axis (Can1) which explained 58% of the variation. These two groups did not vary along the Can1, rather their difference was along Can2 which was not significant (p = 0.15) explaining 27% of variation (Figure 4b). So, while it was possible to discriminate between PLA and BLK plots at SBF whose mulch was removed before tillage 12 month prior to sampling, the difference was minor.

No effect of removal was present on biomass of any microbial group at either location or sample time, except on bacterial biomass in spring at SBF in a three-way interaction between mulch type, removal, and management. In this case CTL resulted in greater bacterial biomass under the PLA mulch by SNK management (Table 6a & 6b).

Under INC treatments, mulch type had no significant effect on soil microbial biomass in every microbial group analyzed when the source factor was considered (Tables 7a & 7b). When the removal factor was considered, there were no effects of mulch type at LNK (Table 6a). At SBF there were significant interactions with mulch type influencing bacterial and cyclopropyl bacterial biomass; however, in these cases biomass was much more prominently affected by management than mulch type (Table 6b).

Likewise, soil bacterial community structure was rarely influenced by mulch type. A few exceptions are present in Figure 5a which shows BLK-NA-Bag to discriminate from PLA-NA-Bag at both locations in spring, but this difference becomes non-significant in fall. An apparent overall pattern of mulch influence on soil is present in fall at SBF in which mulch type sorts along DA2. This is present when pooling across management (Figure 4b), and when pooling across removal status (Figure 5b).

3.1.2 Effect of management on soil microbial community

Hypothesis [2] addresses the ability of our management treatments to impact soil microbial communities. We hypothesized that management (NA – no amendment and SNK – cover crop, compost, and compost extract) would result in differences in microbial community and that SNK would result in greater microbial biomass.

Effects of management on microbial biomass were most prominent in spring and at SBF. At LNK the anova model including source detected increases in total, eukaryotic, and AM biomass under the SNK treatment which faded by fall (Table 7a). At SBF the same model detected significant main effects of management indicating an increase in total, bacterial, cyclopropyl bacterial, 10-methyl bacterial, eukaryotic, and AM biomass under the SNK treatment (Table 7b). Again, all of these effects faded to non-significance by fall after cropping with sweet corn.

The pattern of greater effect at SBF was reversed when bacterial community structure is the response. Canonical discriminant analysis was able to significantly discern management groups at LNK in spring, the effect diminished but persisted in fall (Figures 5a & 5b). At SBF, soil bacterial community structure was completely unaffected in both spring and fall when comparisons are made between SNK and NA treatments within a mulch by recovery combination (comparing empty symbols with their filled counterparts; Figures 5a & 5b).

3.1.3 Effect of recovery source on soil microbial community

Of all treatment factors, the greatest driver of soil bacterial community structure was recovery source i.e. mesh bag vs bulk recovery, very much contrary to our

hypothesis [3]. Figures 4 & 5 show recovery source sorting along the first canonical axis, discriminating bagged from bulk soil at both locations and both sampling events in both groupings described above. However, biomass did not reflect these compositional differences. Only AM fungal biomass at LNK was influenced by source with bagged soils containing slightly more AM fungal biomass (Tables 7a & 7b).

3.2.1 Effect of management on mulch-associated microbial community

Differences of biomass or bacterial community structure on mulch fragments reflects differences in recruitment and assembly of organisms from soil as the microbial community adapts to the mulch environment. We performed analysis that pools across recovery method to focus on effect of mulch type and management treatments. Mulch bacterial community structure was not influenced by management at LNK in spring 2018, but BLK mulch was not recoverable in fall at LNK. At SBF, the BLK mulch under SNK management was significantly different from BLK under NA management in the spring ($p = 0.041$), and this difference persisted in the fall ($p = 0.001$). Management did not cause groups to discriminate within PLA mulch (Table 8).

Microbial biomass on mulch was mostly unaffected by management with a few exceptions. The NA management interacted with bulk recovery and PLA mulch to produce more fungal biomass on PLA at SBF in spring (Table 9b). Several interactions with management are present, mostly at SBF and in spring (Tables 6a&b, 7a&b and 9a&b).

3.2.2 Effect of source on mulch-associated microbial community

At LNK in spring 2018, source was not a driver of bacterial community structure, in fall BLK mulch had degraded to the point of being unrecoverable. At SBF in spring 2018, source did affect mulch bacterial community structure of both mulch types. By fall, this difference faded to non-significance (Table 8).

Some microbial biomass groups were affected by source, mostly in the fall. Both LNK and SBF mulches contained greater total biomass on bulk recovered fragments, and several other microbial groups followed this trend at both locations (Table 9a & 9b).

3.2.3 Effect of mulch type on mulch-associated microbial community

We hypothesized [4] that PLA mulch fragments, comprised mostly of wood particles, would enhance fungal biomass relative to soil and BLK mulch. Tables 10a-11b show a consistent trend for PLA to host more fungal FAME per unit organic matter LOI than soil or BLK mulch. There is a significant interaction between mulch type and sample fraction (soil vs mulch) in LNK bagged samples in spring, SBF bagged samples in Fall, LNK bulk samples in spring, and SBF bulk samples in spring and fall (Tables 10a-11b).

On mulch fragments themselves, mulch type was a much stronger driver of bacterial community structure than management or source consistent with hypothesis [5]. Table 8 shows Mahalanobis distances between PLA and BLK under the same source or management treatment are consistently greater with lower p-value than comparisons within mulch type, between source or management. Figures 6 and 7 show that mulch type causes assembly of distinct bacterial community structure when pooled

across treatment and recovery source. These groupings orient similarly over time and between location, reflecting that bacterial community structures associated with BLK mulch, PLA mulch, and soil are similar between ecoregions.

3.3 Assembly of microbial community on mulches from soil recruitment pool.

At LNK, we found that both mulches tended to host greater microbial biomass than surrounding soil per unit of organic matter LOI, but it was roughly equal at SBF (Tables 10 and 11). Furthermore, because BLK and PLA are chemically different, hypothesis [5] states that bacterial community assembly would differ between BLK mulch, PLA mulch, and soil. We found large differences in bacterial community structure on mulches due to mulch type (Table 8). Both mulches reshaped bacterial communities relative to their surrounding soil as well (Figure 6).

Management had very few significant effects on microbial biomass groups associated with mulch fragments, and in these cases, interaction was present with the effect of source i.e. bag vs bulk recovery (Tables 9, 10, and 11). The implications of these effects are unclear. Instead of biomass differences, distinct bacterial consortium assemblies arise between mulch types and surrounding soil, regardless of other treatments (Figures 6 and 7, Table 12). Furthermore, between locations the patterns between groupings are very similar (Figure 6) and the correlations between bacterial FAMEs and the first two discriminant axes are also in close agreement (Figure 7) indicating similar bacterial assembly on mulch surfaces regardless of surrounding soil habitat or microbial recruitment pool.

To confirm that soil bacterial communities are indeed distinct between locations, Figure 8 shows highly significant differences between SBF and LNK soil at both sampling times, pooling across all other treatments.

4. Discussion

4.1 Soil associated microbial community

4.1.1 Mulches did not change soil microbial environment

An early indicator that biodegradable mulch may be leading to possibly undesirable long-term changes in soil would be a marked shift in soil microbial community or biomass. In this study we compare magnitude of impacts on soil microbial community caused by (1) mulch input vs residual effect from mulch during the cropping season, (2) mulch type i.e. BLK or PLA, (3) management treatment i.e. SNK or NA, and (4) soil profile and protection differences arising from mesh bag vs bulk recovered soil. We found minimal or no soil microbial biomass differences due to removal, mulch type, or source factors; while the SNK treatment generally increased biomass at both locations and this effect faded over time. This suggests that xenobiotic mulch residue was much less consequential to biomass than management in the short term. Similarly, source (i.e. bag vs bulk) and management had roughly equal or greater influence on bacterial community structure compared to mulch type. Results of Kapanen *et* al. (2008) and Li *et* al. (2014) also found no significant impact of Mater-Bi® and PLA mulches on soil biota or soil quality in the short term. Our results add to the existing evidence that MaterBi® and PLA residues are less impactful drivers of soil

ecological process than other accepted management practice, and their influence on soil quality is not negative in the short term.

4.1.2 Mulch type and removal status minimally affect soil microbial community

Steinmetz et. al. (2016) reviewed some studies which found residual effects of plastic mulching on soil microbial communities, and other studies in which no residual effect of mulches was detected. In the current study, the removal treatment allowed a comparison between soils subject only to mulch use during the cropping season versus soil subject to this as well as incorporated mulch residue. This comparison is necessary address the question whether soil microbial outcomes are driven by influences created during the working life of the mulch, or but biochemical interaction between soil and mulch residue once it is buried by tillage.

Polyethylene mulch is occasionally reported to cause lasting effects on the soil environment and concomitant microbial structure (Zhang et. al., 2015; Buyer et. al. 2010, Steinmetz et. al., 2016). This is typically attributed to increased soil temperature and reduced soil gas exchange rather than any biochemical influence. Polyethylene mulch is recognized to be a more complete barrier to gases and sometimes more effective in increasing soil temperature than Bio360® mulch (BLK) and similar films which are subject to early perforation during working life due to weathering, while the experimental PLA is a white porous fabric with less soil warming and gas barrier potential (Wortman et. al. 2016; Cowan et. al. 2013). Even so, Kapanen et. al. (2008) found films formulated with MaterBi® (the polymer used in BLK) to resist physical deterioration over 9 months of strawberry production, which resulted in near parity

with polyethylene film with respect to soil microbial community and soil temperature during and after cropping. BLK mulch used in the current study was a thinner membrane than that studied by Kapanen et. al. and sustained some perforation due to weathering through the season.

Residual effect of mulch presence during its working life in the cropping season is assumed to be most prominent at the first sampling event, approximately 225 days after mulch removal. There was no difference in soil microbial community between PLA and BLK mulch plots under the mulch removal (CTL) treatment in spring at either location (Figure 4a). A small difference developed between these groups in the fall at SBF only (Figure 5a). This suggests that any residual impact of mulch during working life on soil microbial community is not influenced by mulch type, notable considering the porosity and soil warming differences between these mulches during their working life (Miles et al., 2012; Wortman *et* al., 2016).

We found minimal difference in residual effect between mulch film type when mulch was removed before incorporation. The difference detected in fall at SBF eludes interpretation or explanation. Absent a bare soil control we can only conclude that if a residual effect of mulch use was present in spring, it is not different between mulch type.

Where biodegradable mulch residues were incorporated in soil, we found a significant soil bacterial community structure differences between mulch types in spring only and in bagged soil only. Soil from bags containing BLK mulch was most distinct from all other soils in this case (Figures 4 & 5). This indicates that any influence that

mulches exert on bulk soil in the short term is eliminated or overwhelmed by microfauna and/or active roots which were suppressed by the mesh bag.

4.1.3 Ranking magnitude of effect on soil microbial community: location > time > source > management > mulch ~ removal

The goal of managing agricultural soil with the intention to increase biodegradation of mulches relies on the ability of a management to alter the microbial condition of the soil, which may in turn influence colonization of mulch. Likewise, monitoring microbial responses to xenobiotic mulch residue offers early warming for any potential threat to soil quality. We rank the magnitude of all factors to place their impact in the broader context of total microbial variability.

Location and time were the prevailing forces driving microbial community structure (Figure 8, Table 6). This is consistent with the general understanding that site-specific edaphic qualities and seasonal nutrient availability, moisture, and temperature variations are major drivers of microbial community (Grandy *et* al., 2009; Kuramae *et* al., 2011; Schutter *et* al., 2001).

Recovery source (bagged vs bulk) was more influential than mulch removal, management, or mulch type in driving soil bacterial community structure, but not biomass. Furthermore, soil profile differences were probably present; bulk samples were collected from 0-7.5 cm, and mesh bags occupied a depth of 5-10 cm. It has been shown that biological communities shift rapidly across depth from 0-10 cm, but this effect should be reduced as both sites have a history of tillage (Eilers *et* al., 2012). Mesh bag differences are known to be substantial due to exclusion of macrofauna, and

alteration of root behavior by girdling invading roots if they expand beyond the diameter of the 200um mesh size (Bradford *et* al., 2002). Furthermore, in our case bags were loaded with 8 mm screened soil. Microbial response to enclosure of soil in the mesh bag is largely irrelevant to real systems, however, it is noteworthy that this intervention influences soil microbial communities more dramatically than mulch residue type, removal status, or management treatments. Among the influences upon mulch environment caused by bagging, burial depth is the only one controllable in real systems by management i.e. tillage depth. We cannot be sure of how microbial communities responded to burial depth specifically, but our results corroborate the idea of microbial habitat variability along a relatively shallow horizon. It may be useful to compare tillage or mulch burial depth effect on mulch biodegradation rate.

Management, as intended, modified microbial communities. Overall, the SNK treatment increased microbial biomass in spring and the effect was reduced by fall. We detected a strong biomass response to treatment at SBF and weaker response at LNK where soil organic matter and baseline microbial biomass is much greater. The relative magnitude of difference in microbial biomass response was damped by this high baseline at LNK. These effects practically disappeared by fall after the cropping season, representing the turnover of spring cover crop and compost additions from the prior fall, as well as the homogenizing influence of an irrigated sweet corn crop.

Despite more prominent biomass changes due to management at SBF, soil bacterial community structure was unaffected by management suggesting that our treatments affected microbial dosage but not recruitment pool. At LNK, where

management changed biomass less dramatically, management did change bacterial community structure in spring, and this effect persisted with slight reduction in fall. In both cases however, mulch mass loss was unaffected by management.

Any shift in soil microbial biomass or bacterial community structure due to management treatment indicates a possible difference in dosage or recruitment pool of mulch decomposers. Response of soil-associated microbes to mulch type indicates sensitivity of the soil microbial community to relatively small inputs of xenobiotic fresh residue, 135 and 1743 kg/ha for BLK and PLA, respectively. If either biodegradable mulch were to cause soil microbial communities to deviate greatly from the variation caused by management or recovery source, this would suggest important alterations due to mulch incorporation.

Overall, we can conclude that mulch type and removal had a minor and roughly equal influence on soil bacterial community structure, while management has a slightly greater impact, and recovery source had a still greater influence often causing significantly distant clusters in canonical discriminant analysis. This result is somewhat striking considering that SNK received compost dry matter input of 57,000 and 42,000 kg/ha at LNK and SBF, respectively, as well as cover crop residue input (biomass not measured), while the mulch treatments only imported 135 and 1743 kg/ha of potentially biodegradable residue. The finding that these two factors influenced soil bacterial community very little in comparison with the recovery source suggests fairly dramatic alteration of the mesh bag environment and illustrates a possible limitation of

the mesh bag approach to simulate true field degradation conditions (Bradford *et* al., 2002).

These results, along with no impact of mulch type on yield and very minor influence on soil aggregate stability (Reid, 2019), suggest that these two biodegradable mulches are not detrimental to overall soil productive potential in the short term.

To the question of bulk soil microbial response to biodegradable mulches, our findings extend those of Li. *et* al. (2014) which found significant impact of both location and mulch type on soil PFLA profiles, but no interaction between the two. We found large differences between soil FAME profiles due to location and very modest differences due to mulch. Our finding of large differences in mulch-associated FAME profiles between mulches that were similar between location (described below) helps elucidate how mulches may shift microbial composition in bulk soil, especially over greater duration of sustained use than studied here.

4.2 Mulch associated microbial community

4.2.1 Mulches create unique microbial consortia that are similar across location regardless of management

It was unknown to what extent soil differences due to location or management would influence mulch microbial colonization. We found distinct site differences and modest soil microbial changes due to management, but these did not result in powerfully divergent microbial outcomes with respect to mulch fragment colonization (Figures 8 & 10). Figure 9 corroborates the similar clustering pattern in Figure 8

indicating that mulch type, independent of location, management or recovery source is the overwhelming driver of microbial community assembly.

The biodegradable mulches in the current study behaved similarly to fresh residues. Mulch residues recruited organisms adapted to survival on the mulch substrate and fitter competitors for this nutrient resource were selected resulting in a mulch-associated community structure dissimilar from surrounding soil. BLK and PLA mulch are divergent in composition and physical properties (Table 2). Many microbes and enzymes have been identified which are effective in hydrolyzing the xenobiotic components of these mulches, although they may not be preferred substrates in a field soil matrix (Janczak et al., 2018; Satti *et* al., 2018; Torres *et* al., 1996). The woody lignocellulosic particle loading of PLA is not xenobiotic. Mechanisms of its degradation are well understood, and present among all soils (Janusz *et* al., 2017).

The current study verifies the expected increase in fugal biomass associated with buried wood particles of PLA, both relative to surrounding soil and BLK mulch. We did not find evidence that the particular fungal consortium recruited by wood particles accelerates PLA degradation, so it is still unknown how microbial consortia selected by wood particle loading will influence with polylactic acid biodegradation rate. Thompson *et* al. (2019) found that the type of organic particle loaded in spunbond PLA mulches influenced molecular weight of PLA after incubation in soil microcosms, but wood particle was not one of the loadings tested.

Most importantly, both mulches recruit similar consortia, not only across management, but even across location. This insight alone suggests that managements

targeting microbial shift to enhance mulch degradation may be ineffective, at least in the short term.

4.2.2 Sequence of microbial behavior similar between locations, but slower at Scottsbluff

Fungal biomass on PLA declined from spring to fall at LNK, while PLA fragments at SBF tended to have similar fungal biomass between spring and fall. This suggests that most of the fungal action on wood at LNK had occurred early in 2018. At SBF surface soils were cooler and more prone to cyclical drying and rewetting by irrigation and rapid evaporation due to coarse soil and dry air. This may have delayed fungal action on wood particles resulting in a longer duration of peak fungal biomass. This pattern is slightly dampened by mesh bag recovery compared to bulk recovered mulch (Table 9b). This may be explained by slightly deeper burial due to placement in the bag resulting in more uniform moisture over time.

The BLK mulch was completely degraded by fall at LNK, but more than 60% of its mass remained at SBF (Chapter 1, Figure 11). Bacterial community structure on BLK mulch was similar across location, but total microbial biomass consistently trends higher at LNK than SBF in spring. Total microbial biomass is also consistently increased on BLK mulch at SBF in fall compared to spring. There is no data for BLK at LNK because it had been fully degraded. These trends suggest that the above described conditions at SBF not only delayed fungal action associate with PLA degradation, but also slowed degradation of BLK.

4.2.3 Management failed to meaningfully influence mulch-associated microbial community

A chief objective of this work was to accelerate the biodegradation of mulch via soil management. In some cases, management affected bacterial FAME profile on BLK mulch surfaces, but never on PLA (Table 9b). This may be due to the powerful influence of saprophytic fungi which was universally increased on the wood-rich PLA mulch. These lignin-degrading fungi are known to form mutualisms between classes of bacteria which may aid in the dissimilation of lignin (Boer *et* al., 2005). The fine details of PLA degradation in soil, and bacterial-fungal competition and mutualism in lignocellulose-rich niches are enigmatic, so reasons for resistance to bacterial community remodeling due to management treatment are open to speculation.

Management treatment did not significantly alter degradation of either mulch at either location (Chapter 1, Figure 11). Consistent with this result, Thompson *et.* al (2019) also found neither compost nor compost extract to influence mass loss or PLA molecular weight.

5. Conclusion

We found no dramatic changes to soil microbial communities in soils exposed to mulch residue, evidence that short-term use of both mulch formulations under study are non-detrimental to soil quality. Minor effects of mulch residue on soil suggest that longer term study is warranted.

The effect of management on the microbial community structure on mulch surfaces was relatively trivial, while the effect of mulch type is profound in shaping

microbial consortia on mulch surfaces during decomposition. Furthermore, unique bacterial compositions assemble on PLA and BLK mulch that are similar across locations.

PLA mulch had strong selection on assembled bacterial community, such that recovery source and management treatment did not cause differences in its bacterial community, while these factors only slightly and inconsistently influenced community structure on the BLK mulch. We found high fungal biomass on PLA mulch during its degradation. Fungi are particularly competitive decomposers of wood, so this strong fungal component of assembled microbial communities is probably attributable to the wood particles in PLA. We suspect that this high-lignin, low-C:N substrate, and the fungi that it recruited, strongly shaped bacterial community structure on PLA (Boer *et* al., 2005). Meanwhile, most known polylactic acid degraders are actinobacteria or bacteria (Qi *et* al., 2017). It may be useful to further characterize the bacterial consortia selected by wood, because particle loading in nonwoven polylactic acid mulch is a young technology. We can conclude that emphasis should be placed on mulch formulation rather than agricultural management to favor polylactic acid degradation in soils.

We found that the mesh bag method, while useful for tracking known mulch fragments during degradation, is a major influence in creating soil microbial environment surrounding mulch fragments. The differences in soil bacterial community composition between bulk recovered and mesh bag recovered soils were greater than those found between mulch type or between managements. This finding agrees with a substantial literature on litter bag and mesh bag methodology (Bradford *et* al., 2002; Outi-Maaria *et* al., 2019). Our results confirm that mesh bags are only approximations

of the surrounding soil microbial conditions. To improve the technique, future studies should use bags of larger mesh size and include less soil in bags to more accurately simulate the soil environment and detect changes in soil attributable to proximity with mulch fragments.

Finally, the technique of combusting residue left after FAME extraction allowed a comparison between samples with widely variable mineral content. By reporting FAME on a g/LOI basis we corrected the systematic error introduced by different mass ratio of adhered soil. This technique may be useful in other settings where it is desirable to remove the influence of variable levels of inert material in biological samples.

Tables

Table 1: General geographic information and soil properties of experimental sites.

	Lincoln, NE (LNK)	Scottsbluff, NE (SBF)
Latitude	40°50'13.2"N	41°53'33.4"N
Longitude	96°39'50.4"W	103°40'54.0"W
Elevation	351 m	1198 m
Mean annual precipitation[a]	78.7 cm	38.1 cm
Soil series[a]	Zook	Tripp
Texture class[a]	Silty clay loam	Very fine sandy loam
Soil subgroup classification[a]	Fine, smectitic, mesic Cumulic Vertic Endoaquolls	Coarse-silty, mixed, mesic Aridic Haplustolls
Soil pH[b] range	6.2-8.2	7.9-8.3
Soil pH[b] mean	7.1	8.1

[a]Soil series, texture, and subgroup classification were obtained from the Web Soil Survey online resource (Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Web Soil Survey. Available online at the following link: https://websoilsurvey.sc.egov.usda.gov/. Accessed [5/1/2019])

[b] Soil pH was measured in a 1:2 soil:water ratio by Ward Laboratories, Kearney NE, USA

Table 2: Mulch background and specifications.

Abbreviation	PLA	BLK
Trade Name	-	Bio360®
Manufacturer	3M Corporation	Novamont
Composition	polylactic acid (38%) and wood (62%)	thermoplastic starch and poly-ε-caprolactone
Roll Length	~74 m	1220 m
Roll Width	1.07 m	1.22 m
Thickness	1.14 mm	0.015 mm
Weight	298.1 g/m^2	20.2 g/m^2

PLA – polylactic acid and wood particle mulch; BLK – Bio360® mulch.

Table 3: Thirty fatty acid methyl esters (FAMEs) quantified by GC/MS used to create biomass groups for analysis by ANOVA. 'Group' column indicates biomass groups, Total FAMEs are the sum of all 30. The 16 FAMEs in a bacterial group were those used in canonical discriminant analysis.

IUPAC name (ME - methyl ester)	Abbreviation	Group
Methyl tetradecanoate ME	iC14:0	Bacterial
Methyl tetradecanoate ME	C14:0	General
13-methyltetradecanoic acid ME	iC15:0	Bacterial
12-methyltetradecanoic acid ME	aC15:0	Bacterial
Pentadecanoic acid ME	C15:0	Bacterial
14-methylpentadecanoic acid ME	iC16:0	Bacterial
9-hexadecanoic acid ME	C16:1c9	General
11-hexadecanoic acid ME	C16:1c11	Arbuscular mycorrhizal fungal
Hexadecanoic acid ME	C16:0	General
10-methylhexadecanoic acid ME	i10MeC17:0	Bacterial (actinomycetes)
16-methylhexadecanoic acid ME	iC17:0	Bacterial
15-methylhexadecanoic acid ME	aC17:0	Bacterial
cis-9,10-methylenehexadecanoic acid ME	cyC17(9,10)	Bacterial (cyclopropyl)
Heptadecanoic acid ME	C17:0	Bacterial
Heptadecanoic acid, 10-methyl-, ME	i10MeC18:0	Bacterial (actinomycetes)
Heptadecanoic acid, 10-methyl-, ME	a10MeC18:0	Bacterial (actinomycetes)
Heptadecanoic acid, 10-methyl-, ME	10MeC18:0	Bacterial (actinomycetes)
cis-9,12-octadecadienoic acid ME	C18:2c9,12	Saprophytic fungal
9-octadecadienoic acid ME	C18:1c9	General
11-octadecadienoic acid ME	C18:1c11	General
Octadecadienoic acid ME	C18:0	General
10-methyloctadecanoic acid, ME	10MeC19:0	Bacterial (actinomycetes)
cis-9,10-methyleneoctadecanoate	cyC19(9,10)	Bacterial (cyclopropyl)
cis-9,10-methyleneoctadecanoate	cyC19(11,12)	Bacterial (cyclopropyl)
5,8,11,14-Eicosatetraenoic acid ME	C20:4	Eukaryotic
Ethyl 5,8,11,14,17-icosapentaenoate ME	C20:5	Eukaryotic
7,10,13-Eicosatrienoic acid ME	C20:3	Eukaryotic
Eicosadienoic acid ME	C20:2	Eukaryotic
11-eicosanoic acid ME	C20:1c11	General
Eicosanoic acid ME	C20:0	General

Table 4: Outline of the five statistical models used to constrain FAME data in order to make statistically valid comparisons by ANOVA within each location by time combination. Isolated effects are in **bold**. When a factor was eliminated from the model by constraining within a single level, this level appears in gray. 'Random' and 'fixed' indicate the type of effect modelled when all levels of the factor are included in analysis.

		model constructed to detect effect of **source**		model constructed to detect effect of **type**		model constructed to detect effect of **removal**	
Factor	Levels Within Factor	within soil	within mulch	within bulk	within bag	within soil	-
block	1,2,3	random	random	random	random	random	-
mulch	BLK,PLA	fixed	fixed[a]	fixed[b]	fixed[b]	fixed	-
removal	INC,CTL	INC	INC	INC	INC	**fixed**	-
management	SNK,NA	fixed	fixed	fixed	fixed	fixed	-
source	bag,bulk	**fixed**	**fixed**	bulk	bag	bulk	-
type	mulch,soil	soil	mulch	**fixed**[c]	**fixed**[c]	soil	-

[a] *In Fall 2018 this effect was removed by constraining within PLA mulch.*
[b] *In Fall 2018 these effects were removed by constraining within PLA mulch.*
[c] *In Fall 2018 these effects were removed by constraining within mulch type.*
BLK – Bio360® mulch; PLA – polylactic acid mulch; INC – incorporated mulch treatment; CTL – mulch removed before tillage in fall 2017; SNK – "kitchen sink" management treatment consisting of compost, cover crop, and compost extract amendment; NA – management treatment receiving no amendment.

Table 5: Factors, levels, and abbreviations. Consider this a legend for Figure 2.

Experimetal Unit	Factor	Levels	Abbreviations
Whole Plot	Mulch	Bio360®	BLK
		Poly-lactic acid	PLA
Split-Plot	Removal	Incorporated	INC
		Control (mulch removed)	CTL
Split-Split-Plot	Management	cover crop, compost & compost extract	SNK
		none	NA
3rd Split Plot	Source	Mesh Bag	Bag
		Bulk Soil	Bulk
4th Split Plot	Type	Mulch	mulch
		Soil	soil

BLK – Bio360® mulch; PLA – polylactic acid mulch; INC – incorporated mulch treatment; CTL – mulch removed before tillage in fall 2017; SNK – "kitchen sink" management treatment consisting of compost, cover crop, and compost extract amendment; NA – management treatment receiving no amendment

Table 6a: Lincoln Nebraska (LNK). Effects of mulch type, removal status, and management on biomass of microbial taxonomic groups in soil. Units are nmol FAME/ g soil.

Lincoln Nebraska - Soil			Total Biomass		Bacterial Biomass		Saprophytic Fungal Biomass		Eukaryotic Biomass		Cyclopropyl Bacterial Biomass		10-methyl Bacterial Biomass		Arbuscular Mycorrhizal Biomass	
Mulch	Removal	Mgmt	S	F	S	F	S	F	S	F	S	F	S	F	S	F
BLK	CTL	SNK	116	150	47.3	58.3	6.2	9.5	1.4	3.9	8.0	9.3	9.4	11.1	4.2	5.5
		NA	108	124	45.9	50.9	5.7	7.7	1.2	2.3	8.2	8.7	9.1	9.6	3.9	4.4
	INC	SNK	116	131	46.3	55.2	6.7	6.9	1.6	1.8	8.7	9.3	8.8	11.0	3.8	4.5
		NA	109	141	44.4	51.9	6.3	11.2	1.4	2.2	8.3	8.9	9.1	9.2	3.7	5.3
PLA	CTL	SNK	109	134	41.6	55.2	7.5	8.9	2.5	1.9	6.8	8.9	7.8	10.9	4.3	4.4
		NA	100	123	42.2	50.1	5.3	7.5	1.1	2.0	7.3	8.5	8.7	9.3	3.6	4.5
	INC	SNK	133	136	51.2	58.3	9.7	6.6	1.6	1.7	8.7	9.8	10.0	10.8	4.7	5.5
		NA	106	109	40.8	40.4	7.0	7.5	1.4	1.8	6.6	6.5	8.0	7.3	3.9	4.0
S. E. of Means			*8.8*	*14.9*	*4.1*	*5.1*	*1.0*	*1.8*	*0.4*	*0.8*	*0.9*	*1.0*	*1.0*	*1.0*	*0.5*	*0.9*
Source of variation			Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F
Mulch (M)			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Removal (R)			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Mgmt (G)			ns	ns	ns	0.01	0.05	ns	ns	ns	ns	ns	ns	<0.01	ns	ns
M x R			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
M x G			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
R x G			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
M x G x R			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Columns are sampling period: S – spring 2018, F – fall 2018. Mgmt - management fixed effect. BLK – Bio360® mulch, PLA - polylactic acid and wood mulch, SNK - kitchen sink (compost, compost extract and cover crop), NA - no amendment. CTL – mulch removed after cropping, INC – mulch incorporated by tillage after cropping. *Upper portion is mean microbial biomass (nmol FAME/ g soil), FAME - fatty acid methyl ester, lower portion is Anova of type III fixed effects.*

Table 6b: Scottsbluff Nebraska (SBF). Effects of mulch type, removal status, and management on biomass of microbial taxonomic groups in soil. Units are nmol FAME/ g soil.

Scottsbluff Nebraska - Soil			Total Biomass		Bacterial Biomass		Saprophytic Fungal Biomass		Eukaryotic Biomass		Cyclopropyl Bacterial Biomass		10-methyl Bacterial Biomass		Arbuscular Mycorrhizal Biomass	
Mulch	Removal	Mgmt	S	F	S	F	S	F	S	F	S	F	S	F	S	F
BLK	CTL	SNK	79	88	20.8	21.3	8.0	8.2	1.4	1.9	3.7	3.5	3.1	3.2	2.9	8.0
		NA	56	94	13.6	20.9	6.8	10.0	1.2	1.9	2.7	3.2	2.4	3.2	2.4	7.6
	INC	SNK	85	110	21.7	25.3	8.4	10.0	1.5	2.1	4.0	4.0	3.1	3.7	3.7	6.9
		NA	53	94	13.3	23.0	4.9	8.3	2.1	1.8	2.5	4.0	2.4	3.4	2.2	8.4
PLA	CTL	SNK	82	111	21.2	25.3	8.2	11.6	1.5	2.2	3.6	4.0	3.1	3.8	3.2	8.9
		NA	56	88	14.6	20.2	5.5	10.0	1.1	1.9	2.8	3.4	2.6	3.2	2.6	7.3
	INC	SNK	64	84	17.0	20.6	7.2	9.3	1.1	1.4	2.9	3.4	2.7	3.2	3.1	6.1
		NA	61	98	15.1	21.3	7.5	12.0	1.2	1.8	2.6	3.4	2.4	3.1	2.6	9.1
S. E. of Means			*6.8*	*13.7*	*1.6*	*2.9*	*1.1*	*2.3*	*0.4*	*0.3*	*0.3*	*0.4*	*0.2*	*0.3*	*0.4*	*1.9*
Source of variation			Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F
Mulch (M)			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Removal (R)			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Mgmt (G)			<0.01	ns	<0.01	ns	0.04	ns	ns	ns	<0.01	ns	<0.01	ns	<0.01	ns
M x R			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
M x G			ns	ns	0.02	ns	ns	ns	ns	ns	0.04	ns	ns	ns	ns	ns
R x G			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
M x G x R			ns	ns	0.04	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Columns are sampling period: S – spring 2018, F – fall 2018. Mgmt - management fixed effect. BLK – Bio360® mulch, PLA - polylactic acid and wood mulch, SNK - kitchen sink (compost, compost extract and cover crop), NA - no amendment. CTL – mulch removed after cropping, INC – mulch incorporated by tillage after cropping. *Upper portion is mean microbial biomass (nmol FAME/ g soil), FAME - fatty acid methyl ester, lower portion is Anova of type III fixed effects.*

Table 7a: Lincoln Nebraska (LNK). Effects of mulch type, sample source, and management on biomass of microbial taxonomic groups in soil. Units are nmol FAME/ g soil.

Lincoln Nebraska - Soil			Total Biomass		Bacterial Biomass		Saprophytic Fungal Biomass		Eukaryotic Biomass		Cyclopropyl Bacterial Biomass		10-methyl Bacterial Biomass		Arbuscular Mycorrhizal Biomass	
Mulch	Source	Mgmt	S	F	S	F	S	F	S	F	S	F	S	F	S	F
BLK	Bulk	SNK	116	131	46.3	55.2	6.7	6.9	1.6	1.8	8.7	9.3	8.8	11.0	3.8	4.5
		NA	109	141	44.4	51.9	6.3	11.2	1.4	2.2	8.3	8.9	9.1	9.2	3.7	5.3
	Bag	SNK	139	145	55.6	58.7	10.1	10.4	2.0	2.5	9.5	9.9	10.2	10.6	5.9	6.2
		NA	106	105	45.5	47.1	6.1	5.8	1.4	1.4	8.1	8.3	8.7	8.8	3.8	3.6
PLA	Bulk	SNK	133	136	51.2	58.3	9.7	6.6	1.6	1.7	8.7	9.8	10.0	10.8	4.7	5.5
		NA	106	109	40.8	40.4	7.0	7.5	1.4	1.8	6.6	6.5	8.0	7.3	3.9	4.0
	Bag	SNK	127	131	50.3	52.5	8.9	11.8	1.7	1.9	8.4	8.8	9.8	9.4	5.4	5.0
		NA	109	116	43.2	48.5	7.6	7.4	1.4	1.6	7.7	8.2	9.0	9.1	3.9	4.0
S. E. of Means			*9.2*	*14.6*	*4.3*	*5.2*	*1.3*	*2.4*	*0.2*	*0.3*	*0.9*	*1.0*	*1.0*	*1.0*	*0.5*	*0.9*
Source of variation			Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F
Mulch (M)			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Source (S)			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.04	ns
Mgmt (G)			0.03	ns	ns	ns	ns	ns	0.03	ns	ns	ns	ns	ns	0.02	ns
M x S			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
M x G			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
G x S			ns	ns	ns	ns	ns	ns	ns	0.02	ns	ns	ns	ns	ns	ns
M x G x S			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.05

Columns are sampling period: S – spring 2018, F – fall 2018. Mgmt - management fixed effect. BLK – Bio360®, PLA - polylactic acid and wood mulch, SNK - kitchen sink (compost, compost extract and cover crop), NA - no amendment. Upper portion is mean microbial biomass (nmol FAME/ g soil), FAME - fatty acid methyl ester, lower portion is Anova of type III fixed effects.

Table 7b: Scottsbluff Nebraska (SBF). Effects of mulch type, sample source, and management on biomass of microbial taxonomic groups in soil. Units are nmol FAME/ g soil.

Scottsbluff Nebraska - Soil			Total Biomass		Bacterial Biomass		Saprophytic Fungal Biomass		Eukaryotic Biomass		Cyclopropyl Bacterial Biomass		10-methyl Bacterial Biomass		Arbuscular Mycorrhizal Biomass	
Mulch	Source	Mgmt	S	F	S	F	S	F	S	F	S	F	S	F	S	F
BLK	Bulk	SNK	85	110	21.7	25.3	8.4	10.0	1.5	2.1	4.0	4.0	3.1	3.7	3.7	6.9
		NA	53	94	13.3	23.0	4.9	8.3	2.1	1.8	2.5	4.0	2.4	3.4	2.2	8.4
	Bag	SNK	68	92	19.1	22.2	8.7	8.3	1.1	2.7	3.2	3.9	2.8	3.3	2.7	6.6
		NA	53	79	13.6	17.0	8.6	8.7	1.1	2.0	2.5	3.1	2.3	2.8	2.5	11.7
PLA	Bulk	SNK	64	84	17.0	20.6	7.2	9.3	1.1	1.4	2.9	3.4	2.7	3.2	3.1	6.1
		NA	61	98	15.1	21.3	7.5	12.0	1.2	1.8	2.6	3.4	2.4	3.1	2.6	9.1
	Bag	SNK	78	96	19.9	24.4	9.9	8.0	1.3	2.5	3.4	4.4	2.9	3.5	3.2	9.5
		NA	64	67	13.1	17.4	16.0	6.7	1.0	1.3	2.4	3.1	2.3	2.9	2.3	6.6
S. E. of Means			*7.8*	*11.1*	*1.6*	*2.4*	*3.1*	*2.5*	*0.4*	*0.3*	*0.3*	*0.4*	*0.2*	*0.2*	*0.4*	*1.9*
Source of variation			Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F
Mulch (M)			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Source (S)			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Mgmt (G)			0.03	ns	<0.01	ns	ns	ns	ns	ns	<0.01	ns	0.01	ns	0.02	ns
M x S			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
M x G			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
G x S			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
M x G x S			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Columns are sampling period: S – spring 2018, F – fall 2018. Mgmt - management fixed effect. BLK – Bio360®, PLA - polylactic acid and wood mulch, SNK - kitchen sink (compost, compost extract and cover crop), NA - no amendment. Upper portion is mean microbial biomass (nmol FAME/ g soil), FAME - fatty acid methyl ester, lower portion is Anova of type III fixed effects.

Table 8: Pairwise squared Mahalanobis distances for mulch fragment associated bacterial FAMEs between mulch type by source treatments (top), and mulch type by management treatments (bottom).

Mulch type by recovery source groupings, pooled across management

Spring

Scottsbluff	BLK_bulk	BLK_bag	PLA_bulk	PLA_bag
	Squared Moholonobis distance (Probability > Moholonobis distance)			
BLK_bulk	0	196	128	273
	-	(0.0112)	(0.0289)	(0.0053)
BLK_bag	196	0	321	587
	(0.0112)	-	(0.0036)	(0.0009)
PLA_bulk	128	321	0	118
	(0.0289)	(0.0036)	-	(0.0339)
PLA_bag	273	587	118	0
	(0.0053)	(0.0009)	(0.0339)	-

Lincoln	BLK_bulk	BLK_bag	PLA_bulk	PLA_bag
BLK_bulk	0	47	110	193
	-	(NS)	(0.0398)	(0.0117)
BLK_bag	47	0	74	129
	(NS)	-	(ns)	(0.028)
PLA_bulk	110	74	0	47
	(0.0398)	(ns)	-	(NS)
PLA_bag	193	129	47	0
	(0.0117)	(0.028)	(NS)	-

Fall

	BLK_bulk	BLK_bag	PLA_bulk	PLA_bag
BLK_bulk	0	65	145	187
	-	(ns)	(0.0069)	(0.0035)
BLK_bag	65	0	237	249
	(ns)	-	(0.0018)	(0.0016)
PLA_bulk	145	237	0	45
	(0.0069)	(0.0018)	-	(NS)
PLA_bag	187	249	45	0
	(0.0035)	(0.0016)	(NS)	-

Mulch type by management groupings, pooled across recovery source

Spring

	BLK_NA	BLK_SNK	PLA_NA	PLA_SNK
BLK_NA	0	108	259	297
	-	(0.0407)	(0.0059)	(0.0043)
BLK_SNK	108	0	616	659
	(0.0407)	-	(0.0008)	(0.0006)
PLA_NA	259	616	0	69
	(0.0059)	(0.0008)	-	(NS)
PLA_SNK	297	659	69	0
	(0.0043)	(0.0006)	(NS)	-

	BLK_NA	BLK_SNK	PLA_NA	PLA_SNK
BLK_NA	0	58	120	109
	-	(NS)	(0.033)	(0.0402)
BLK_SNK	58	0	90	77
	(NS)	-	(ns)	(ns)
PLA_NA	120	90	0	12
	(0.033)	(ns)	-	(NS)
PLA_SNK	109	77	12	0
	(0.0402)	(ns)	(NS)	-

Fall

	BLK_NA	BLK_SNK	PLA_NA	PLA_SNK
BLK_NA	0	259	753	545
	-	(0.0014)	(<0.0001)	(0.0002)
BLK_SNK	259	0	1,737	1,419
	(0.0014)	-	(<0.0001)	(<0.0001)
PLA_NA	753	1,737	0	20
	(<0.0001)	(<0.0001)	-	(NS)
PLA_SNK	545	1,419	20	0
	(0.0002)	(<0.0001)	(NS)	-

BLK – Bio360® mulch; PLA – polylactic acid and wood particle mulch; NA – no amendment management; SNK – "kitchen sink" compost, cover crop, and compost extract management.

Table 9a: Lincoln Nebraska (LNK). Effects of mulch type, sample source, and management on biomass of microbial taxonomic groups on mulch fragments. Fall analysis was constrained within PLA mulch because no BLK mulch was recoverable at fall sampling. Units are nmol FAME/ g LOI.

Lincoln Nebraska - Mulch			Total Biomass		Bacterial Biomass		Saprophytic Fungal Biomass		Eukaryotic Biomass		Cyclopropyl Bacterial Biomass		10-methyl Bacterial Biomass		Arbuscular Mycorrhizal Biomass	
Mulch	Source	Mgmt	S	F	S	F	S	F	S	F	S	F	S	F	S	F
BLK	Bulk	SNK	1986	-	566	-	159	-	25	-	74	-	93	-	37	-
		NA	2024	-	611	-	168	-	34	-	73	-	111	-	36	-
	Bag	SNK	2646	-	570	-	434	-	26	-	83	-	78	-	35	-
		NA	2170	-	520	-	299	-	22	-	81	-	68	-	28	-
PLA	Bulk	SNK	2577	3497	595	969	592	560	40	46	101	150	69	140	100	112
		NA	2411	2915	454	729	618	572	36	38	75	110	47	104	64	88
	Bag	SNK	3148	1490	536	411	1037	223	29	20	85	75	36	31	102	51
		NA	2489	1654	532	417	602	294	27	22	87	73	38	40	88	51
S. E. of Means			*370*	*395*	*41*	*69*	*174*	*122*	*8*	*5*	*10*	*7*	*10*	*10*	*10*	*13*
Source of variation			Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F
Mulch (M)			ns	-	ns	-	ns	-	ns	-	ns	-	ns	-	0.02	-
Source (S)			ns	0.01	ns	<0.01	ns	ns	ns	0.01	ns	<0.01	<0.01	<0.01	ns	0.02
Mgmt (G)			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
M x S			ns	-	ns	-	ns	-	ns	-	ns	-	ns	-	ns	-
M x G			ns	-	ns	-	ns	-	ns	-	ns	-	ns	-	ns	-
G x S			ns	ns	ns	ns	ns	ns	ns	ns	ns	0.02	ns	ns	ns	ns
M x G x S			ns	-	ns	-	ns	-	ns	-	ns	-	ns	-	ns	-

Columns are sampling period: S – spring 2018, F – fall 2018. Mgmt - management fixed effect. BLK – Bio360®, PLA - polylactic acid and wood mulch, SNK - kitchen sink (compost, compost extract and cover crop), NA - no amendment. Upper portion is mean microbial biomass (nmol FAME/ g LOI), FAME - fatty acid methyl ester, LOI - loss on ignition, lower portion is Anova of type III fixed effects.

Table 9b: Scottsbluff Nebraska (SBF). Effects of mulch type, sample source, and management on biomass of microbial taxonomic groups on mulch fragments. Units are nmol FAME/ g LOI.

Scottsbluff Nebraska - Mulch			Total Biomass		Bacterial Biomass		Saprophytic Fungal Biomass		Eukaryotic Biomass		Cyclopropyl Bacterial Biomass		10-methyl Bacterial Biomass		Arbuscular Mycorrhizal Biomass	
Mulch	Source	Mgmt	S	F	S	F	S	F	S	F	S	F	S	F	S	F
BLK	Bulk	SNK	1007	5805	324	1624	98	584	2	49	33	215	34	118	14	118
		NA	1583	4209	472	1129	123	388	13	51	55	185	51	110	24	187
	Bag	SNK	1307	2785	297	720	208	276	12	48	70	185	16	37	20	147
		NA	1486	3037	349	634	252	355	12	64	78	159	21	50	25	317
PLA	Bulk	SNK	3481	5495	695	864	632	1186	42	82	119	124	70	91	96	183
		NA	4395	6062	483	914	1339	1433	44	89	60	156	51	81	105	143
	Bag	SNK	3798	3447	551	741	820	564	41	47	128	174	37	45	99	169
		NA	2867	3504	447	735	678	769	21	47	93	192	16	30	61	128
S. E. of Means			*399*	*1285*	*65*	*128*	*122*	*303*	*10*	*20*	*7*	*25*	*8*	*10*	*21*	*64*
Source of variation			Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F
Mulch (M)			0.02	ns	ns	ns	0.03	ns	ns	ns	0.02	ns	ns	ns	ns	ns
Source (S)			ns	0.02	ns	<0.01	ns	ns	ns	ns	<0.01	ns	<0.01	<0.01	ns	ns
Mgmt (G)			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
M x S			ns	ns	ns	0.02	0.03	ns	ns	ns	ns	ns	ns	ns	ns	ns
M x G			ns	ns	ns	ns	ns	ns	ns	ns	<0.01	ns	ns	ns	ns	ns
G x S			0.05	ns	ns	ns	0.01	ns	ns	ns	ns	ns	ns	ns	ns	ns
M x G x S			ns	ns	ns	ns	0.01	ns	ns	ns	0.03	ns	ns	ns	ns	ns

Columns are sampling period: S – spring 2018, F – fall 2018. Mgmt - management fixed effect. BLK – Bio360®, PLA - polylactic acid and wood mulch, SNK - kitchen sink (compost, compost extract and cover crop), NA - no amendment. Upper portion is mean microbial biomass (nmol FAME/ g LOI), FAME - fatty acid methyl ester, LOI - loss on ignition, lower portion is Anova of type III fixed effects.

Table 10a: Lincoln Nebraska (LNK). Effects of mulch type, sample type (soil/mulch), and management on biomass of microbial taxonomic groups on mulch and soil recovered from mesh bags. Because no BLK mulch was recoverable at fall sampling, fall analysis was constrained within PLA mulch and then within soil type. Results of each analysis are separated by "/" with PLA at left and soil at right. Means corresponding to PLA-constrained and soil-constrained analyses are marked by solid and broken lines, respectively, under the Total FAMEs column to emphasize analyzed groups. Units are nmol FAME/ g LOI.

Lincoln Nebraska - Mesh bag recovered

			Total Biomass		Bacterial Biomass		Saprophytic Fungal Biomass		Eukaryotic Biomass		Cyclopropyl Bacterial Biomass		10-methyl Bacterial Biomass		Arbuscular Mycorrhizal Biomass	
Mulch	Fraction	Mgmt	S	F	S	F	S	F	S	F	S	F	S	F	S	F
BLK	Mulch	SNK	2646	-	570	-	434	-	26	-	83	-	78	-	35	-
		NA	2170	-	520	-	299	-	22	-	81	-	68	-	28	-
	Soil	SNK	1918	2128	769	862	137	152	28	37	131	145	141	155	81	92
		NA	1676	1709	708	759	99	99	22	23	126	133	135	140	61	59
PLA	Mulch	SNK	3148	1490	536	411	1037	223	29	20	85	75	36	31	102	51
		NA	2489	1654	532	417	602	294	27	22	87	73	38	40	88	51
	Soil	SNK	1920	2077	751	827	138	189	26	31	127	139	145	146	82	79
		NA	1670	1881	660	780	117	121	21	27	117	131	137	146	60	65
S. E. of Means			*343*	*133/130*	*45*	*37 / 37*	*152*	*45 / 41*	*5*	*3 / 4*	*10*	*7 / 8*	*9*	*6 / 7*	*11*	*6 / 10*
Source of variation			Pr>F	Pr>F within PLA/Soil	Pr>F	Pr>F within PLA/Soil	Pr>F	Pr>F within PLA/Soil	Pr>F	Pr>F within PLA/Soil	Pr>F	Pr>F within PLA/Soil	Pr>F	Pr>F within PLA/Soil	Pr>F	Pr>F within PLA/Soil
Mulch (M)			ns	- /ns	ns	- /ns	ns	- /ns	ns	- /ns	ns	- /ns	ns	- /ns	ns	- /ns
Fraction (F)			<0.01	0.04 /-	<0.01	<0.01 /-	<0.01	ns /-	ns	ns /-	<0.01	<0.01 /-	<0.01	<0.01 /-	ns	0.03 /-
Mgmt (G)			ns	ns / 0.03	ns	ns /ns	ns	ns /ns	ns	ns / 0.02	ns	ns /ns	ns	ns /ns	ns	ns / 0.01
M x F			ns	- /-	ns	- /-	0.04	- /-	ns	- /-	ns	- /-	0.01	- /-	<0.01	- /-
M x G			ns	- /ns	ns	- /ns	ns	- /ns	ns	- /ns	ns	- /ns	ns	- /ns	ns	- /ns
F x G			ns	ns /-	ns	ns /-	ns	ns /-	ns	ns /-	ns	ns /-	ns	ns /-	ns	ns /-
M x F x G			ns	- /-	ns	- /-	ns	- /-	ns	- /-	ns	- /-	ns	- /-	ns	- /-

Columns are sampling period: S – spring 2018, F – fall 2018. Mgmt - management fixed effect. BLK – Bio360® mulch, PLA - polylactic acid and wood mulch, SNK - kitchen sink (compost, compost extract and cover crop), NA - no amendment. Upper portion is mean microbial biomass (nmol FAME/ g LOI), FAME - fatty acid methyl ester, LOI - loss on ignition, lower portion is Anova of type III fixed effects.

Table 10b: Scottsbluff Nebraska (SBF). Effects of mulch type, sample type, and management on biomass of microbial taxonomic groups on mulch and soil recovered from mesh bags. Units are nmol FAME/ g LOI.

Scottsbluff Nebraska - Mesh bag recovery			Total Biomass		Bacterial Biomass		Saprophytic Fungal Biomass		Eukaryotic Biomass		Cyclopropyl Bacterial Biomass		10-methyl Bacterial Biomass		Arbuscular Mycorrhizal Biomass	
Mulch	Fraction	Mgmt	S	F	S	F	S	F	S	F	S	F	S	F	S	F
BLK	Mulch	SNK	1307	2785	297	720	208	276	12	48	70	185	16	37	20	147
		NA	1486	3037	349	634	252	355	12	64	78	159	21	50	25	317
	Soil	SNK	2411	3101	671	744	309	283	37	92	113	132	99	111	96	220
		NA	2038	2739	520	593	330	298	41	70	95	106	89	98	96	410
PLA	Mulch	SNK	3798	3447	551	741	820	564	41	47	128	174	37	45	99	169
		NA	2867	3504	447	735	678	769	21	47	93	192	16	30	61	128
	Soil	SNK	2736	3036	694	776	345	250	45	77	118	139	102	110	113	298
		NA	2409	2396	492	622	591	242	39	48	90	112	85	103	86	237
S. E. of Means			*224*	*291*	*40*	*37*	*134*	*88*	*3*	*12*	*7*	*17*	*5*	*4*	*10*	*67*
Source of variation			Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F
Mulch (M)			0.02	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.04	ns
Fraction (F)			ns	ns	<0.01	ns	ns	<0.01	<0.01	0.03	0.03	<0.01	<0.01	<0.01	<0.01	0.02
Mgmt (G)			ns	ns	0.02	<0.01	ns	ns	0.04	ns	0.02	ns	0.04	ns	ns	ns
M x F			<0.01	ns	0.01	ns	ns	0.01	<0.01	ns	<0.01	ns	ns	ns	<0.01	ns
M x G			ns	ns	ns	ns	ns	ns	0.02	ns	ns	ns	ns	ns	ns	ns
F x G			ns	ns	0.03	0.03	ns	ns	0.03	ns	ns	ns	ns	ns	ns	ns
M x F x G			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	<0.01	ns	ns

Columns are sampling period: S – spring 2018, F – fall 2018. Mgmt - management fixed effect. BLK – Bio360® mulch, PLA - polylactic acid and wood mulch, SNK - kitchen sink (compost, compost extract and cover crop), NA - no amendment. Upper portion is mean microbial biomass (nmol FAME/ g LOI), FAME - fatty acid methyl ester, LOI - loss on ignition, lower portion is Anova of type III fixed effects.

Table 11a: Lincoln Nebraska (LNK). Effects of mulch type, sample type (soil/mulch), and management on biomass of microbial taxonomic groups on mulch and soil recovered from bulk soil. Because no BLK mulch was recoverable at fall sampling, fall analysis was constrained within PLA mulch and then within soil type. Results of each analysis are separated by "/" with PLA at left and soil at right. Means corresponding to PLA-constrained and soil-constrained analyses are marked by solid and broken lines, respectively, under the Total FAMEs column to emphasize analyzed groups. Units are nmol FAME/ g LOI.

Lincoln Nebraska - Bulk recovered

			Total Biomass		Bacterial Biomass		Saprophytic Fungal Biomass		Eukaryotic Biomass		Cyclopropyl Bacterial Biomass		10-methyl Bacterial Biomass		Arbuscular Mycorrhizal Biomass	
Mulch	Fraction	Mgmt	S	F	S	F	S	F	S	F	S	F	S	F	S	F
BLK	Mulch	SNK	1986	-	566	-	159	-	25	-	74	-	93	-	37	-
		NA	2024	-	611	-	168	-	34	-	73	-	111	-	36	-
	Soil	SNK	1583	1968	633	830	92	105	22	27	119	139	121	166	52	67
		NA	1570	2138	640	796	92	173	21	33	119	137	132	142	52	78
PLA	Mulch	SNK	2577	3497	595	969	592	560	40	46	101	150	69	140	100	112
		NA	2411	2915	454	729	618	572	36	38	75	110	47	104	64	88
	Soil	SNK	1767	1924	682	825	128	93	22	24	116	139	134	153	63	78
		NA	1732	2220	662	817	120	158	23	37	106	131	128	147	64	83
S. E. of Means			*189*	*393 / 193*	*36*	*65 / 47*	*87*	*121 / 32*	*8*	*6 / 5*	*8*	*7 / 9*	*9*	*12 / 11*	*6*	*14 / 14*
Source of variation			Pr>F	Pr>F within PLA/Soil	Pr>F	Pr>F within PLA/Soil	Pr>F	Pr>F within PLA/Soil	Pr>F	Pr>F within PLA/Soil	Pr>F	Pr>F within PLA/Soil	Pr>F	Pr>F within PLA/Soil	Pr>F	Pr>F within PLA/Soil
Mulch (M)			ns	- / ns	ns	- / ns	ns	- / ns	ns	- / ns	ns	- / ns	ns	- / ns	0.03	- / ns
Fraction (F)			<0.01	0.04 / -	<0.01	ns / -	<0.01	0.02 / -	ns	ns / -	<0.01	ns / -	<0.01	ns / -	ns	ns / -
Mgmt (G)			ns	ns / ns	ns	ns / ns	ns	ns / ns	ns	ns / ns	ns	ns / ns	ns	ns / ns	ns	ns / ns
M x F			ns	- / -	ns	- / -	<0.01	- / -	ns	- / -	ns	- / -	<0.01	- / -	<0.01	- / -
M x G			ns	- / ns	ns	- / ns	ns	- / ns	ns	- / ns	ns	- / ns	ns	- / ns	ns	- / ns
F x G			ns	ns / -	ns	ns / -	ns	ns / -	ns	ns / -	ns	ns / -	ns	ns / -	0.03	ns / -
M x F x G			ns	- / -	ns	- / -	ns	- / -	ns	- / -	ns	- / -	ns	- / -	0.05	- / -

Columns are sampling period: S – spring 2018, F – fall 2018. Mgmt - management fixed effect. BLK – Bio360® mulch, PLA - polylactic acid and wood mulch, SNK - kitchen sink (compost, compost extract and cover crop), NA - no amendment. Upper portion is mean microbial biomass (nmol FAME/ g LOI), FAME - fatty acid methyl ester, LOI - loss on ignition, lower portion is Anova of type III fixed effects.

Table 11b: Scottsbluff Nebraska (SBF). Effects of mulch type, sample type, and management on biomass of microbial taxonomic groups on mulch and soil recovered from bulk soil. Units are nmol FAME/ g LOI.

Scottsbluff Nebraska - Bulk recovery

			Total Biomass		Bacterial Biomass		Saprophytic Fungal Biomass		Eukaryotic Biomass		Cyclopropyl Bacterial Biomass		10-methyl Bacterial Biomass		Arbuscular Mycorrhizal Biomass	
Mulch	Fraction	Mgmt	S	F	S	F	S	F	S	F	S	F	S	F	S	F
BLK	Mulch	SNK	1007	5805	324	1624	98	584	2	49	33	215	34	118	14	118
		NA	1583	4209	472	1129	123	388	13	51	55	185	51	110	24	187
	Soil	SNK	2855	3388	726	784	282	309	51	66	135	124	104	113	123	215
		NA	2050	2913	510	710	191	258	83	57	98	122	91	106	83	255
PLA	Mulch	SNK	3481	5495	695	864	632	1186	42	82	119	124	70	91	96	183
		NA	4395	6062	483	914	1339	1433	44	89	60	156	51	81	105	143
	Soil	SNK	2243	2666	592	656	251	298	37	46	102	108	95	102	108	195
		NA	2350	3248	583	704	291	383	47	60	102	112	93	104	102	319
S. E. of Means			*415*	*1281*	*71*	*133*	*97*	*299*	*17*	*19*	*10*	*20*	*9*	*10*	*22*	*61*
Source of variation			Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F
Mulch (M)			ns	ns	ns	ns	0.04	ns	ns	ns	ns	ns	ns	ns	ns	ns
Fraction (F)			ns	0.01	0.04	<0.01	<0.01	<0.01	0.03	ns	<0.01	<0.01	<0.01	ns	0.01	0.04
Mgmt (G)			ns	ns	ns	ns	0.05	ns	ns	ns	ns	ns	ns	ns	ns	ns
M x F			<0.01	ns	0.04	0.01	<0.01	0.05	0.03	ns	<0.01	ns	ns	ns	0.02	ns
M x G			ns	ns	ns	ns	0.03	ns	ns	ns	ns	ns	ns	ns	ns	ns
F x G			ns	ns	ns	ns	0.01	ns	ns	ns	ns	ns	ns	ns	ns	ns
M x F x G			ns	ns	0.01	ns	ns	ns	ns	ns	<0.01	ns	ns	ns	ns	ns

Columns are sampling period: S – spring 2018, F – fall 2018. Mgmt - management fixed effect. BLK – Bio360® mulch, PLA - polylactic acid and wood mulch, SNK - kitchen sink (compost, compost extract and cover crop), NA - no amendment. Upper portion is mean microbial biomass (nmol FAME/ g LOI), FAME - fatty acid methyl ester, LOI - loss on ignition, lower portion is Anova of type III fixed effects.

Table 12: Mahalanobis distances and p-values corresponding with canonical discriminant analysis of 16 bacterial FAME abundance ratios at both locations, shown in Figure 6. Groupings are soil, PLA mulch, and BLK mulch at spring and fall sampling events, pooled across removal status, recovery source, and management treatment.

Lincoln	BLK_Spring	PLA_Fall	PLA_Spring	Soil_Fall	Soil_Spring
BLK_Spring	0	73	49	95	95
	-	(<.0001)	(<.0001)	(<.0001)	(<.0001)
PLA_Fall	73	0	14	36	46
	(<.0001)	-	(<.0001)	(<.0001)	(<.0001)
PLA_Spring	49	14	0	54	62
	(<.0001)	(<.0001)	-	(<.0001)	(<.0001)
Soil_Fall	95	36	54	0	3
	(<.0001)	(<.0001)	(<.0001)	-	(0.0041)
Soil_Spring	95	46	62	3	0
	(<.0001)	(<.0001)	(<.0001)	(0.0041)	-

Scottsbluff	BLK_Fall	BLK_Spring	PLA_Fall	PLA_Spring	Soil_Fall	Soil_Spring
BLK_Fall	0	37	65	53	110	125
	-	(<.0001)	(<.0001)	(<.0001)	(<.0001)	(<.0001)
BLK_Spring	37	0	149	120	195	204
	(<.0001)	-	(<.0001)	(<.0001)	(<.0001)	(<.0001)
PLA_Fall	65	149	0	53	96	125
	(<.0001)	(<.0001)	-	(<.0001)	(<.0001)	(<.0001)
PLA_Spring	53	120	53	0	78	94
	(<.0001)	(<.0001)	(<.0001)	-	(<.0001)	(<.0001)
Soil_Fall	110	195	96	78	0	6
	(<.0001)	(<.0001)	(<.0001)	(<.0001)	-	(<.0001)
Soil_Spring	125	204	125	94	6	0
	(<.0001)	(<.0001)	(<.0001)	(<.0001)	(<.0001)	-

BLK – Bio360® mulch, PLA - polylactic acid and wood mulch.

Figures

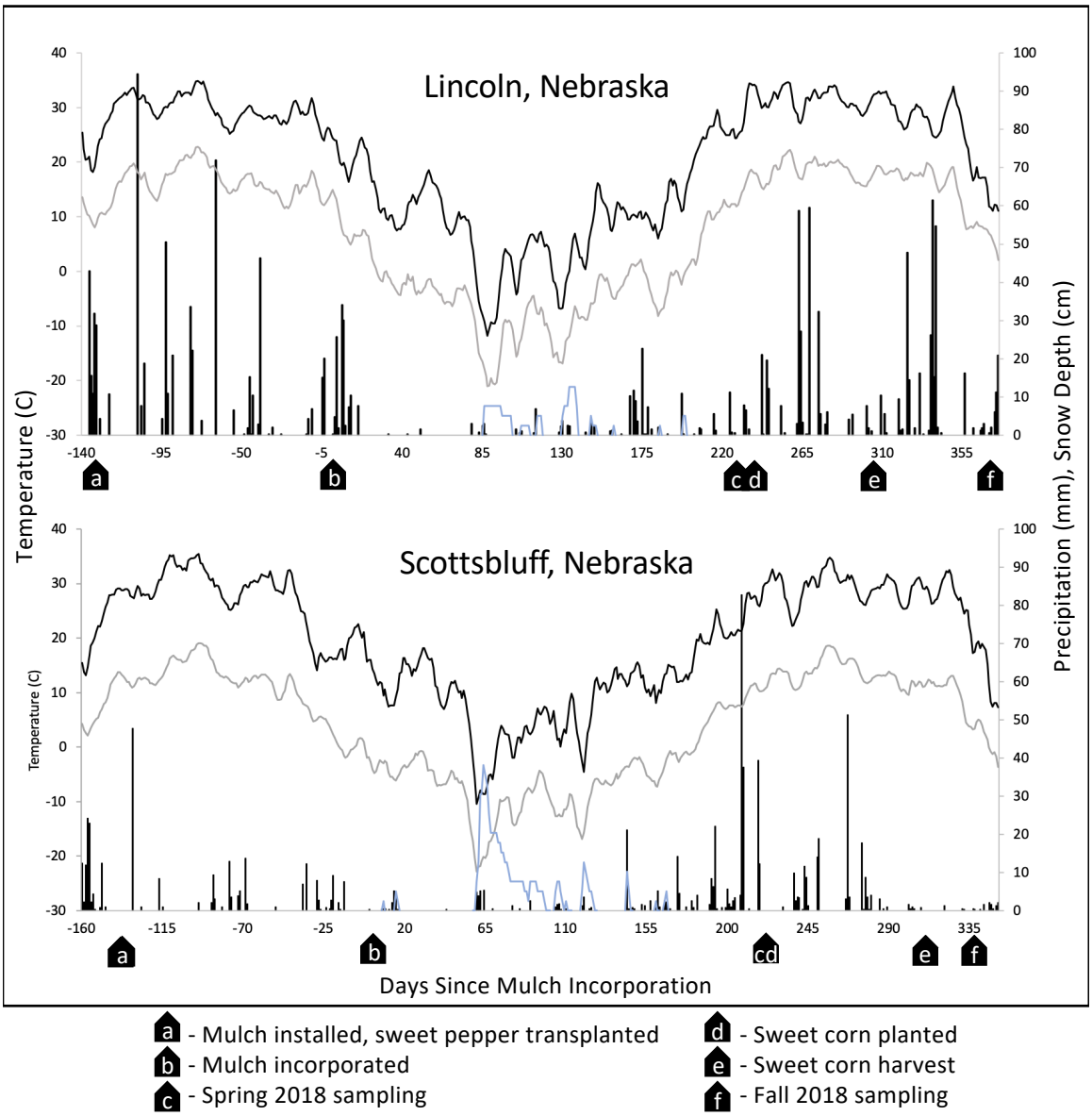


Figure 1: Climate from 16 May 2017 to 9 Oct 2018 at LNK and SBF locations. Black and gray lines are weekly rolling averages of daily high and low temperature. Bars are precipitation. Blue line is depth of snow accumulation. Data courtesy of the High Plains Regional Climate Center stations within 12 m elevation of sites, located 10.5 and 4.7 km from LNK and SBF sites, respectively. Cumulative precipitation before soil incorporation at LNK was 61.6 cm, and 90.2 cm after incorporation. Cumulative precipitation before soil incorporation at SBF was 26.6 cm, and 53.5 after incorporation (irrigation was also applied during the growing seasons).

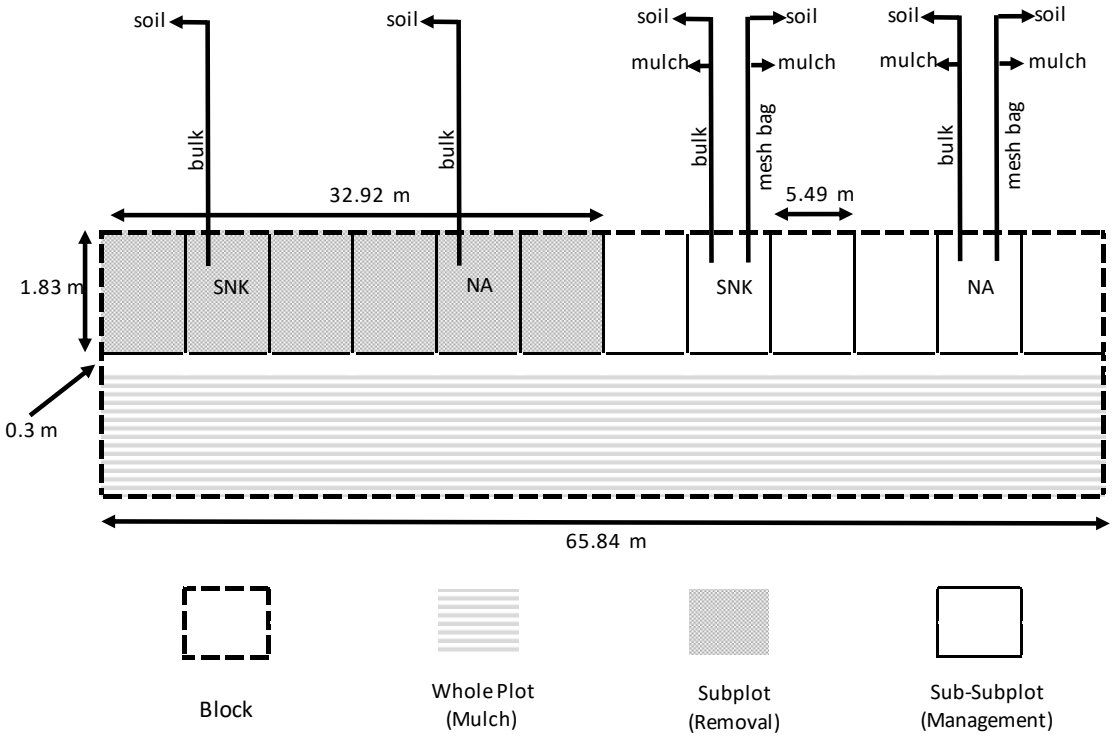


Figure 2: Field layout of each block including schematic of soil and mulch sampling for FAME analysis. SNK – "kitchen sink" management including compost, compost tea, and cover crop. NA – no amendment management.

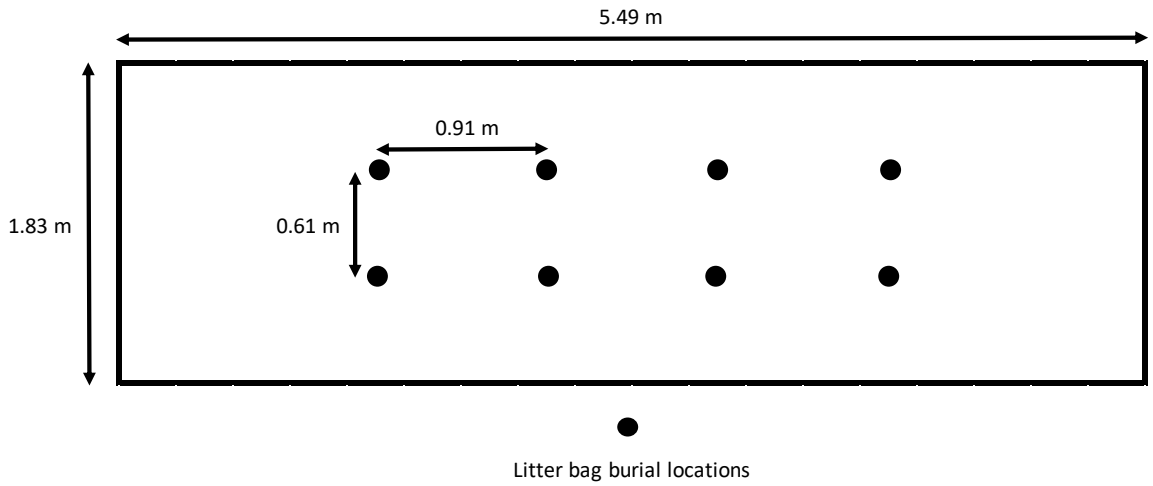
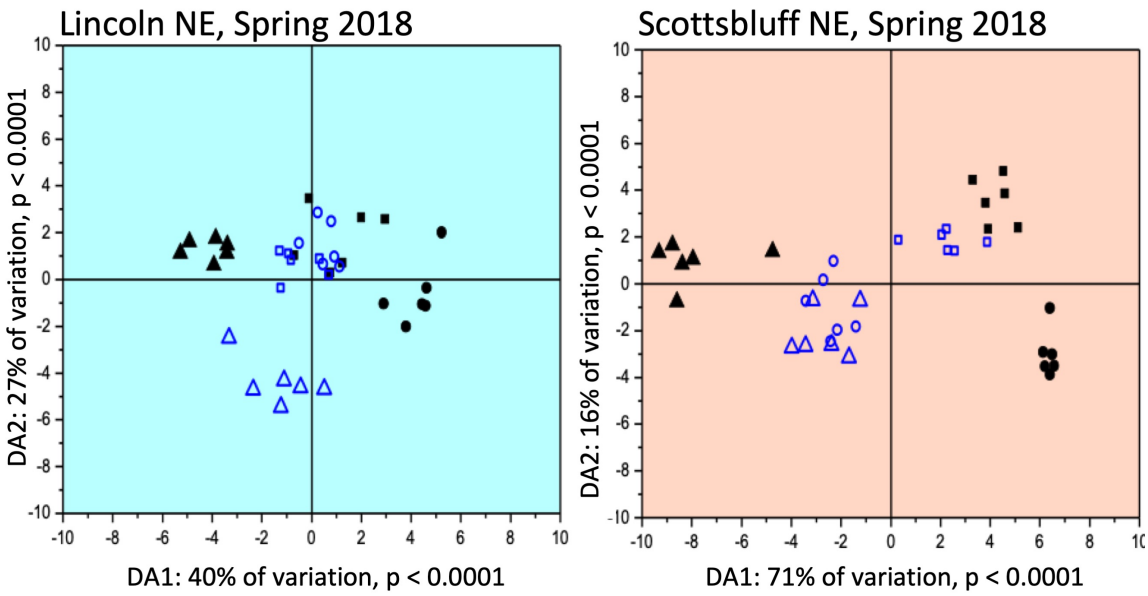


Figure 3. Scale diagram of each sub-subplot showing burial pattern of mesh bags.



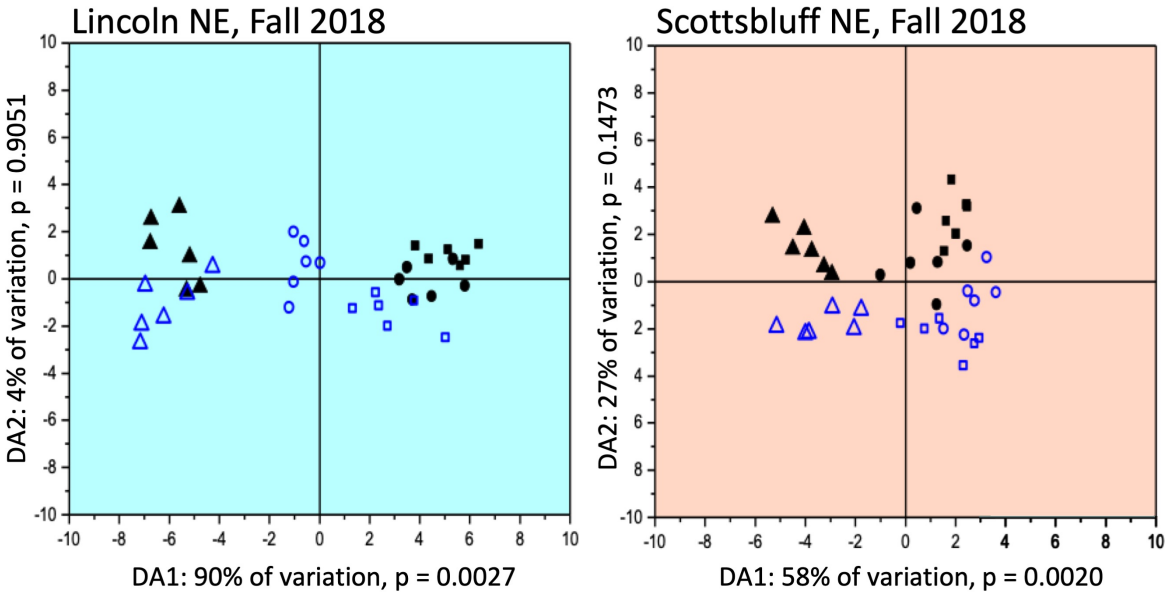
LNK Spring: Prob > Mahalanobis Distance for Squared Distance to MSM

From MSM	BLK - CTL - Bulk ■	BLK - INC - Bulk ●	BLK - INC - Bag ▲	PLA - CTL - Bulk □	PLA - INC - Bulk ○	PLA - INC - Bag △
■ BLK - CTL - Bulk	1	0.0304	0.0086	0.4001	0.0327	0.0029
● BLK - INC - Bulk	0.0304	1	0.0002	0.0175	0.0036	0.0016
▲ BLK - INC - Bag	0.0086	0.0002	1	0.0779	0.0027	0.0031
□ PLA - CTL - Bulk	0.4001	0.0175	0.0779	1	0.0554	0.0237
○ PLA - INC - Bulk	0.0327	0.0036	0.0027	0.0554	1	0.0028
△ PLA - INC - Bag	0.0029	0.0016	0.0031	0.0237	0.0028	1

SBF Spring: Prob > Mahalanobis Distance for Squared Distance to MSM

From MSM	BLK - CTL - Bulk ■	BLK - INC - Bulk ●	BLK - INC - Bag ▲	PLA - CTL - Bulk □	PLA - INC - Bulk ○	PLA - INC - Bag △
■ BLK - CTL - Bulk	1	0.0015	<.0001	0.3823	0.0003	0.0002
● BLK - INC - Bulk	0.0015	1	<.0001	0.0028	<.0001	<.0001
▲ BLK - INC - Bag	<.0001	<.0001	1	<.0001	0.0003	0.0019
□ PLA - CTL - Bulk	0.3823	0.0028	<.0001	1	0.0057	0.0054
○ PLA - INC - Bulk	0.0003	<.0001	0.0003	0.0057	1	0.69
△ PLA - INC - Bag	0.0002	<.0001	0.0019	0.0054	0.69	1

Figure 4a: Canonical discriminant analysis of bacterial FAMEs from bulk and bagged soils sampled from both locations at the spring 2018 sampling event. BLK – Bio360®, PLA - polylactic acid and wood mulch, INC – mulch incorporated by fall 2017 tillage, CTL – mulch removed before fall 2017 tillage



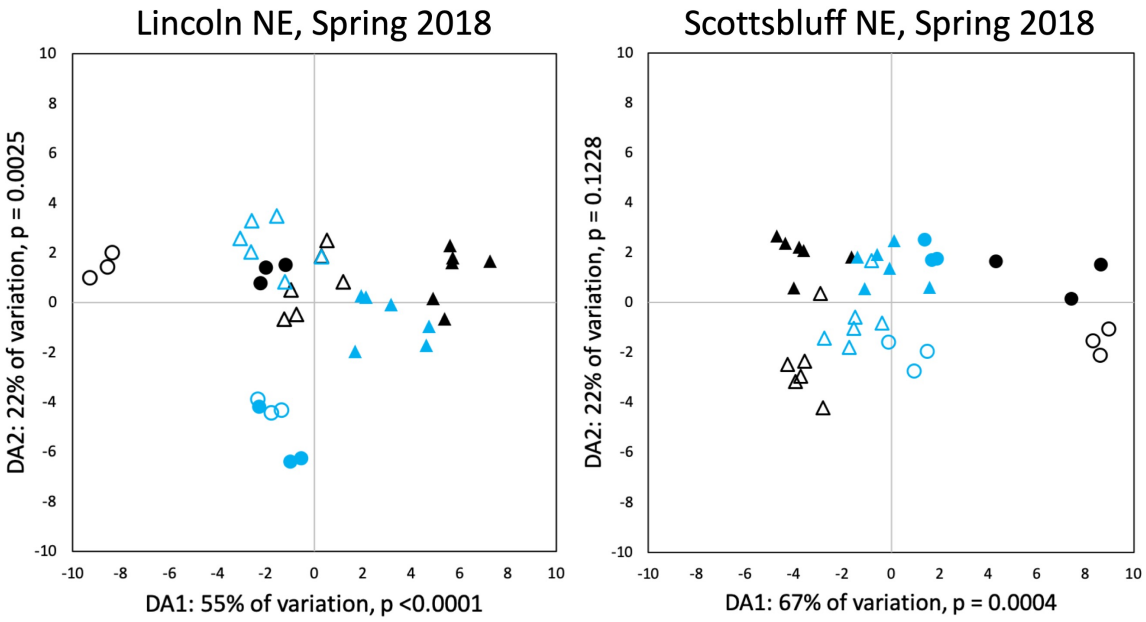
LNK Fall: Prob > Mahalanobis Distance for Squared Distance to MSM

From MSM	BLK - CTL - Bulk ■	BLK - INC - Bulk ●	BLK - INC - Bag ▲	PLA - CTL - Bulk □	PLA - INC - Bulk ○	PLA - INC - Bag △
■ BLK - CTL - Bulk	1	0.9308	<.0001	0.3218	0.0082	<.0001
● BLK - INC - Bulk	0.9308	1	<.0001	0.7353	0.0186	<.0001
▲ BLK - INC - Bag	<.0001	<.0001	1	0.0001	0.022	0.6739
□ PLA - CTL - Bulk	0.3218	0.7353	0.0001	1	0.1123	<.0001
○ PLA - INC - Bulk	0.0082	0.0186	0.022	0.1123	1	0.0108
△ PLA - INC - Bag	<.0001	<.0001	0.6739	<.0001	0.0108	1

SBF Fall: Prob > Mahalanobis Distance for Squared Distance to MSM

From MSM	BLK - CTL - Bulk ■	BLK - INC - Bulk ●	BLK - INC - Bag ▲	PLA - CTL - Bulk □	PLA - INC - Bulk ○	PLA - INC - Bag △
■ BLK - CTL - Bulk	1	0.1652	0.0058	0.0295	0.2222	0.0024
● BLK - INC - Bulk	0.1652	1	0.0303	0.1525	0.37	0.0181
▲ BLK - INC - Bag	0.0058	0.0303	1	0.0032	0.002	0.2525
□ PLA - CTL - Bulk	0.0295	0.1525	0.0032	1	0.8895	0.0283
○ PLA - INC - Bulk	0.2222	0.37	0.002	0.8895	1	0.0084
△ PLA - INC - Bag	0.0024	0.0181	0.2525	0.0283	0.0084	1

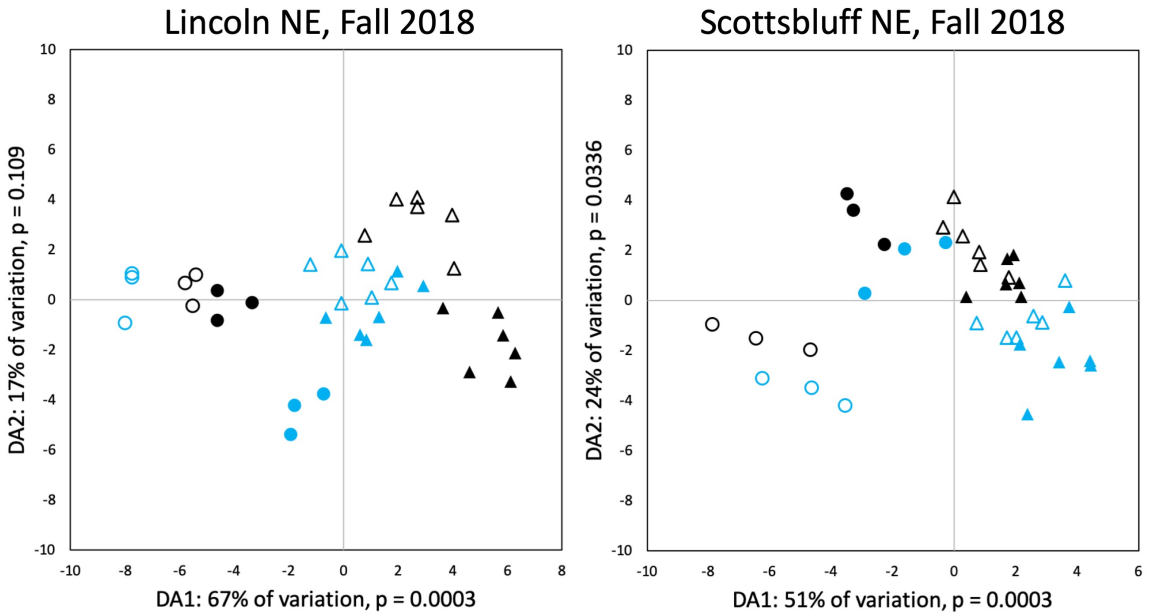
Figure 4b: Canonical discriminant analysis of bacterial FAMEs from bulk and bagged soils sampled from both locations at the fall 2018 sampling event. BLK – Bio360®, PLA - polylactic acid and wood mulch, INC – mulch incorporated by fall 2017 tillage, CTL – mulch removed before fall 2017 tillage



spring LNK soil	BLK_NA_AE	BLK_NA_bag	BLK_SNK_AE	BLK_SNK_bag	PLA_NA_AE	PLA_NA_bag	PLA_SNK_AE	PLA_SNK_bag
BLK_NA_AE	0 -	84 (0.0031)	45 (0.0087)	53 (0.0236)	24 (ns)	39 (ns)	33 (0.0308)	60 (0.0141)
BLK_NA_bag	84 (0.0031)	0 -	212 (<.0001)	71 (0.0226)	58 (0.0161)	92 (0.0079)	149 (0.0002)	110 (0.0034)
BLK_SNK_AE	45 (0.0087)	212 (<.0001)	0 -	81 (0.0037)	66 (0.0015)	94 (0.0019)	19 (NS)	100 (0.0013)
BLK_SNK_bag	53 (0.0236)	71 (0.0226)	81 (0.0037)	0 -	35 (NS)	76 (0.0171)	47 (0.0369)	63 (0.0363)
PLA_NA_AE	24 (ns)	58 (0.0161)	66 (0.0015)	35 (NS)	0 -	52 (0.0258)	38 (0.0169)	71 (0.0066)
PLA_NA_bag	39 (ns)	92 (0.0079)	94 (0.0019)	76 (0.0171)	52 (0.0258)	0 -	53 (0.0228)	23 (NS)
PLA_SNK_AE	33 (0.0308)	149 (0.0002)	19 (NS)	47 (0.0369)	38 (0.0169)	53 (0.0228)	0 -	47 (0.0367)
PLA_SNK_bag	60 (0.0141)	110 (0.0034)	100 (0.0013)	63 (0.0363)	71 (0.0066)	23 (NS)	47 (0.0367)	0 -

spring SBF soil	BLK_NA_AE	BLK_NA_bag	BLK_SNK_AE	BLK_SNK_bag	PLA_NA_AE	PLA_NA_bag	PLA_SNK_AE	PLA_SNK_bag
BLK_NA_AE	0 -	154 (0.0001)	22 (NS)	123 (0.0005)	24 (ns)	32 (NS)	33 (0.031)	61 (0.0132)
BLK_NA_bag	154 (0.0001)	0 -	167 (<.0001)	26 (NS)	112 (0.0008)	72 (0.0215)	93 (0.0019)	70 (0.0239)
BLK_SNK_AE	22 (NS)	167 (<.0001)	0 -	116 (0.0006)	24 (ns)	45 (0.0434)	20 (NS)	40 (ns)
BLK_SNK_bag	123 (0.0005)	26 (NS)	116 (0.0006)	0 -	94 (0.0018)	63 (0.0358)	63 (0.0114)	52 (ns)
PLA_NA_AE	24 (ns)	112 (0.0008)	24 (ns)	94 (0.0018)	0 -	13 (NS)	16 (NS)	22 (NS)
PLA_NA_bag	32 (NS)	72 (0.0215)	45 (0.0434)	63 (0.0358)	13 (NS)	0 -	20 (NS)	27 (NS)
PLA_SNK_AE	33 (0.031)	93 (0.0019)	20 (NS)	63 (0.0114)	16 (NS)	20 (NS)	0 -	17 (NS)
PLA_SNK_bag	61 (0.0132)	70 (0.0239)	40 (ns)	52 (ns)	22 (NS)	27 (NS)	17 (NS)	0 -

Figure 5a: Canonical discriminant analysis of 16 bacterial FAMEs from bulk and bagged soils sampled from both locations at the spring 2018 sampling event. Eight groupings are presented to illustrate relative influence of mulch, management, and recovery on bacterial community structure. BLK – Bio360®; PLA - polylactic acid and wood mulch; SNK – compost, cover crop, and compost extract applied fall 2018; NA – no amendment; AE – 'aseptic excavation' bulk recovered soil; bag – litter bag recovered soil. Tables show Mahalanobis distances and Prob > Mahalanobis distance for squared distance to MSM.



fall LNK soil

	BLK_NA_AE	BLK_NA_bag	BLK_SNK_AE	BLK_SNK_bag	PLA_NA_AE	PLA_NA_bag	PLA_SNK_AE	PLA_SNK_bag
BLK_NA_AE	0 -	85 (0.0031)	35 (0.0233)	69 (0.0075)	16 (NS)	127 (0.0004)	20 (NS)	77 (0.0047)
BLK_NA_bag	85 (0.0031)	0 -	131 (0.0003)	30 (NS)	41 (ns)	18 (NS)	53 (0.0243)	52 (ns)
BLK_SNK_AE	35 (0.0233)	131 (0.0003)	0 -	110 (0.0008)	38 (0.0182)	182 (<.0001)	25 (ns)	64 (0.011)
BLK_SNK_bag	69 (0.0075)	30 (NS)	110 (0.0008)	0 -	44 (0.0459)	42 (NS)	52 (0.0252)	40 (NS)
PLA_NA_AE	16 (NS)	41 (ns)	38 (0.0182)	44 (0.0459)	0 -	77 (0.0046)	6 (NS)	38 (ns)
PLA_NA_bag	127 (0.0004)	18 (NS)	182 (<.0001)	42 (NS)	77 (0.0046)	0 -	88 (0.0025)	77 (0.0164)
PLA_SNK_AE	20 (NS)	53 (0.0243)	25 (ns)	52 (0.0252)	6 (NS)	88 (0.0025)	0 -	29 (NS)
PLA_SNK_bag	77 (0.0047)	52 (ns)	64 (0.011)	40 (NS)	38 (ns)	77 (0.0164)	29 (NS)	0 -

fall SBF soil

	BLK_NA_AE	BLK_NA_bag	BLK_SNK_AE	BLK_SNK_bag	PLA_NA_AE	PLA_NA_bag	PLA_SNK_AE	PLA_SNK_bag
BLK_NA_AE	0 -	75 (0.0052)	20 (NS)	31 (NS)	24 (ns)	68 (0.0081)	36 (0.0228)	33 (NS)
BLK_NA_bag	75 (0.0052)	0 -	74 (0.0058)	46 (NS)	80 (0.0039)	23 (NS)	104 (0.0011)	63 (0.0357)
BLK_SNK_AE	20 (NS)	74 (0.0058)	0 -	35 (NS)	8 (NS)	75 (0.0053)	23 (NS)	45 (0.0447)
BLK_SNK_bag	31 (NS)	46 (NS)	35 (NS)	0 -	48 (0.0336)	60 (0.043)	79 (0.0041)	26 (NS)
PLA_NA_AE	24 (ns)	80 (0.0039)	8 (NS)	48 (0.0336)	0 -	65 (0.0098)	7 (NS)	37 (ns)
PLA_NA_bag	68 (0.0081)	23 (NS)	75 (0.0053)	60 (0.043)	65 (0.0098)	0 -	74 (0.0058)	57 (ns)
PLA_SNK_AE	36 (0.0228)	104 (0.0011)	23 (NS)	79 (0.0041)	7 (NS)	74 (0.0058)	0 -	54 (0.0217)
PLA_SNK_bag	33 (NS)	63 (0.0357)	45 (0.0447)	26 (NS)	37 (ns)	57 (ns)	54 (0.0217)	0 -

Figure 5b: Canonical discriminant analysis of 16 bacterial FAMEs from bulk and bagged soils sampled from both locations at the fall 2018 sampling event. Eight groupings are presented to illustrate relative influence of mulch, management, and recovery on bacterial community structure. BLK – Bio360®; PLA - polylactic acid and wood mulch; SNK – compost, cover crop, and compost extract applied fall 2018; NA – no amendment; AE – 'aseptic excavation' bulk recovered soil; bag – litter bag recovered soil. Tables show Mahalanobis distances and Prob > Mahalanobis distance for squared distance to MSM.

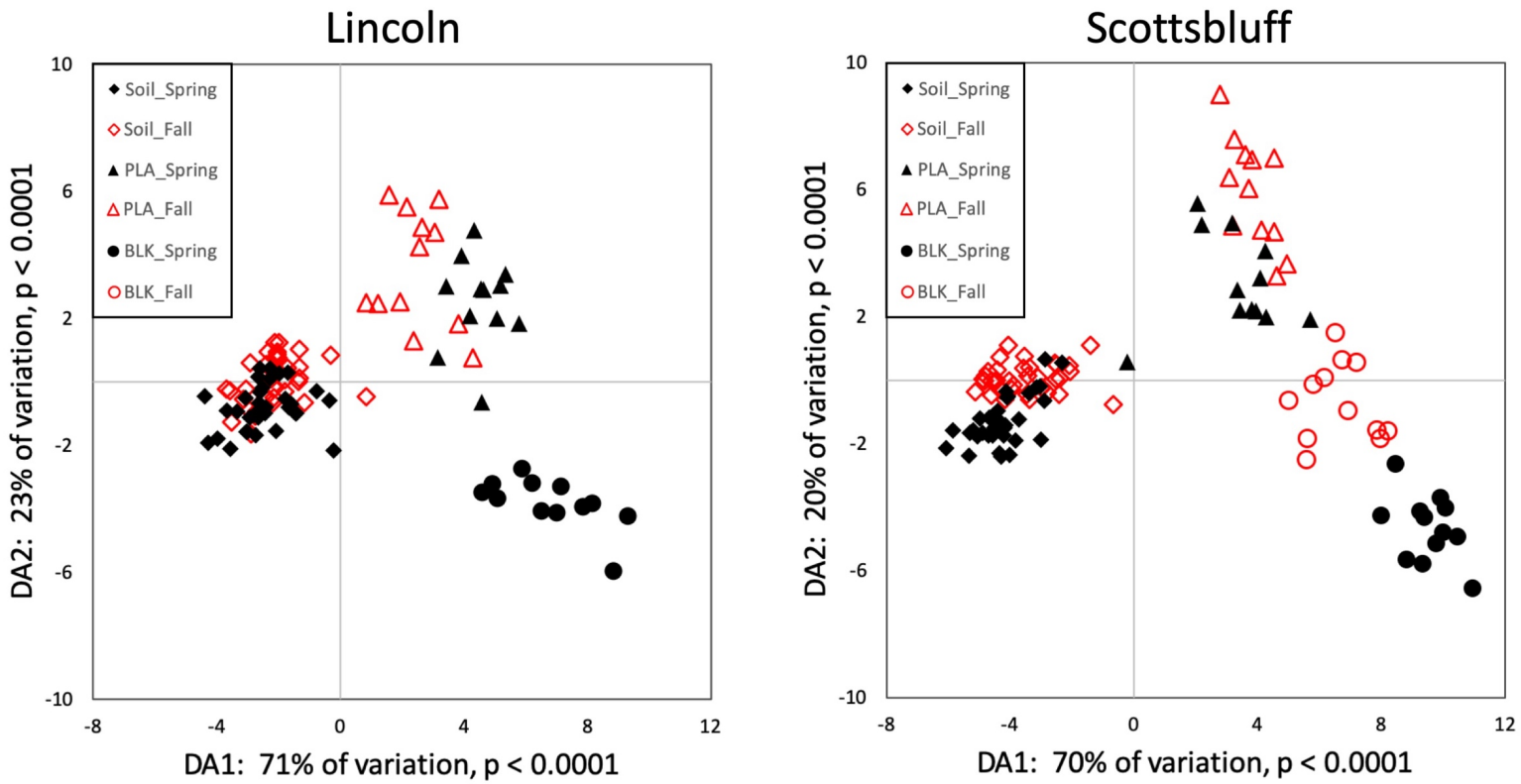


Figure 6: Discriminant scores of BLK mulch, PLA mulch and soil for the first two discriminant functions, DA1 and DA2, at each sampling time, pooled across removal status, recovery source, and management treatment. PLA – polylactic acid and wood mulch, BLK – Bio360® mulch, DA – discriminant axis. All groupings are significantly different from one another, Mahalanobis distances and p-values are shown in Table 12.

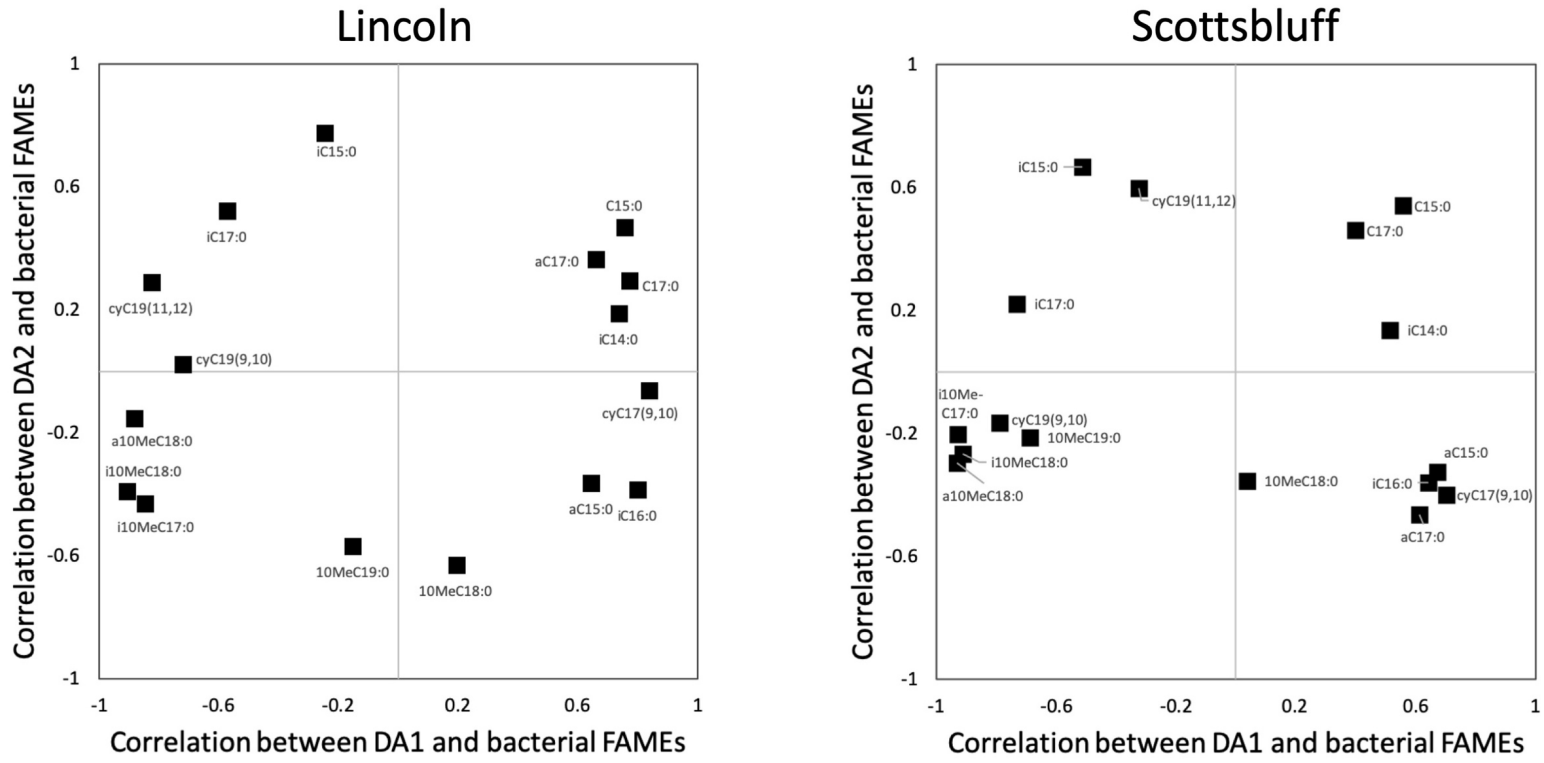


Figure 7: Companion plots to Figure 6. Correlations of bacterial FAMEs with the first two discriminant functions.

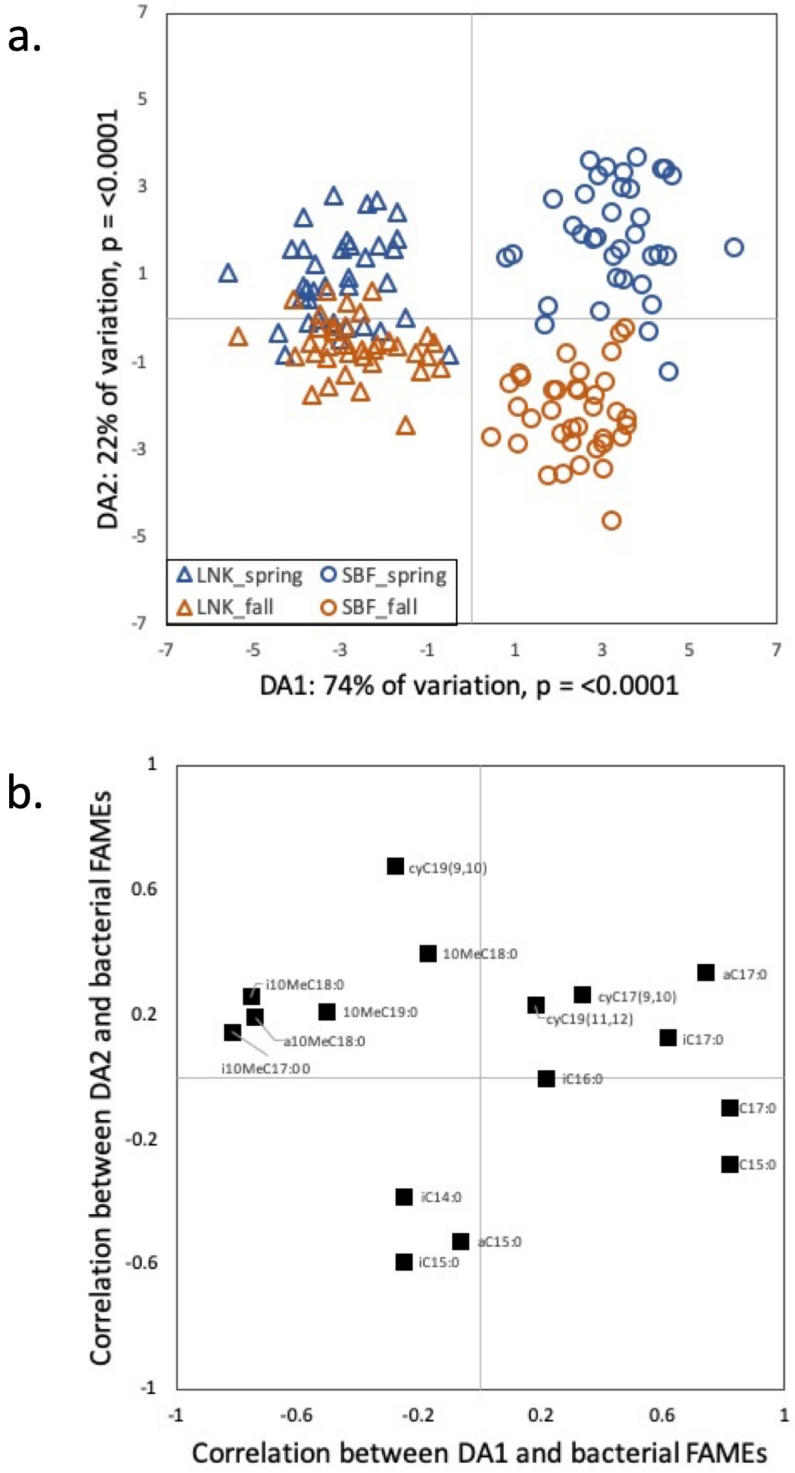


Figure 8: Discriminant scores of location by season groups of soil bacterial FAMEs for (a) and correlation of soil bacterial FAMEs with (b) the first two discriminant functions, DA1 and DA2. LNK – Lincoln, NE; SBF – Scottsbluff, NE. All pairwise differences between centroids are highly significant ($p < 0.0001$).

Literature Cited

Boer, Wietse de, Larissa B. Folman, Richard C. Summerbell, and Lynne Boddy. (2005) "Living in a Fungal World: Impact of Fungi on Soil Bacterial Niche Development." *FEMS Microbiology Reviews* 29, no. 4: 795–811. https://doi.org/10.1016/j.femsre.2004.11.005.

Bradford, Mark A., George M. Tordoff, Till Eggers, T. Hefin Jones, and John E. Newington. (2002) "Microbiota, Fauna, and Mesh Size Interactions in Litter Decomposition." *Oikos* 99, no. 2: 317–23. https://doi.org/10.1034/j.1600-0706.2002.990212.x.

Brodhagen, Marion, Mark Peyron, Carol Miles, and Debra Ann Inglis. (2015) "Biodegradable Plastic Agricultural Mulches and Key Features of Microbial Degradation." *Applied Microbiology and Biotechnology* 99, no. 3: 1039–56. https://doi.org/10.1007/s00253-014-6267-5.

Buyer, Jeffrey S., John R. Teasdale, Daniel P. Roberts, Inga A. Zasada, and Jude E. Maul. (2010) "Factors Affecting Soil Microbial Community Structure in Tomato Cropping Systems." *Soil Biology and Biochemistry* 42, no. 5: 831–41. https://doi.org/10.1016/j.soilbio.2010.01.020.

Carrera, L.M., J.S. Buyer, B. Vinyard, A.A. Abdul-Baki, L.J. Sikora, and J.R. Teasdale. (2007) "Effects of Cover Crops, Compost, and Manure Amendments on Soil Microbial Community Structure in Tomato Production Systems." *Applied Soil Ecology* 37, no. 3: 247–55. https://doi.org/10.1016/j.apsoil.2007.08.003.

Corbin, A. T., C. Miles, J. Cowan, D. Hayes, J. Moore-Kucera, and D. Inglis. (2013) "Current and future prospects for biodegradable plastic mulch in certified Degradation of Potentially Biodegradable Plastic Mulch Films" Oregon State University Extension – eOrganic. http://www.extension.org/pages/67951/currentand-future-prospects-for-biodegradable-plastic-mulch-in-certified-organicproduction-systems (accessed July 31, 2019).

Cowan, Jeremy S., Debra A. Inglis, and Carol A. Miles. (2013) "Deterioration of Three Potentially Biodegradable Plastic Mulches Before and After Soil Incorporation in a Broccoli Field Production System in Northwestern Washington." *HortTechnology*, 849–58. https://doi.org/10.21273/HORTTECH.23.6.849.

Dong, Wenyi, Pengfei Si, Enke Liu, Changrong Yan, Zhe Zhang, and Yanqing Zhang. (2017) "Influence of Film Mulching on Soil Microbial Community in a Rainfed Region

of Northeastern China." *Scientific Reports* 7, no. 1 https://doi.org/10.1038/s41598-017-08575-w.

Drijber, R.A., J.W. Doran, A.M. Parkhurst, and D.J. Lyon. (2000) "Changes in Soil Microbial Community Structure with Tillage under Long-Term Wheat-Fallow Management." *Soil Biology and Biochemistry* 32, no. 10: 1419–30. https://doi.org/10.1016/S0038-0717(00)00060-2.

Eilers, K. G., Debenport, S., Anderson, S., and Fierer, N. (2012). "Digging deeper to find unique microbial communities: the strong effect of depth on the structure of bacterial and archaeal communities in soil." *Soil Biol. Biochem.* 50, 58–65. doi: 10.1016/j.soilbio.2012.03.011

Grandy, A. Stuart, Michael S. Strickland, Christian L. Lauber, Mark A. Bradford, and Noah Fierer. (2009) "The Influence of Microbial Communities, Management, and Soil Texture on Soil Organic Matter Chemistry." *Geoderma* 150, no. 3–4: 278–86. https://doi.org/10.1016/j.geoderma.2009.02.007.

Janczak, Katarzyna, Katarzyna Hrynkiewicz, Zuzanna Znajewska, and Grażyna Dąbrowska. (2018) "Use of Rhizosphere Microorganisms in the Biodegradation of PLA and PET Polymers in Compost Soil." *International Biodeterioration & Biodegradation* 130:65–75. https://doi.org/10.1016/j.ibiod.2018.03.017.

Kapanen, Anu, Evelia Schettini, Giuliano Vox, and Merja Itävaara. (2008) "Performance and Environmental Impact of Biodegradable Films in Agriculture: A Field Study on Protected Cultivation." *Journal of Polymers and the Environment* 16, no. 2: 109–22. https://doi.org/10.1007/s10924-008-0091-x.

Li, Chenhui, J. Moore-Kucera, J. Lee, A. Corbin, M. Brodhagen, C. Miles, and D. Inglis. (2014) "Effects of Biodegradable Mulch on Soil Quality." *Applied Soil Ecology* 79: 59–69. https://doi.org/10.1016/j.apsoil.2014.02.012.

Man, Changzhen, Chunmei Zhang, Yiming Liu, Weiwei Wang, Wuyang Ren, Long Jiang, Frederic Reisdorffer, Thien Phap Nguyen, and Yi Dan. (2012) "Poly (Lactic Acid)/Titanium Dioxide Composites: Preparation and Performance under Ultraviolet Irradiation." *Polymer Degradation and Stability* 97, no. 6: 856–62. https://doi.org/10.1016/j.polymdegradstab.2012.03.039.

Miles, Carol, Russ Wallace, Annette Wszelaki, Jeffrey Martin, Jeremy Cowan, Tom Walters, and Debra Inglis. (2012) "Deterioration of Potentially Biodegradable Alternatives to Black Plastic Mulch in Three Tomato Production Regions." *HortScience* 47, no. 9: 1270–77. https://doi.org/10.21273/HORTSCI.47.9.1270.

Moreno, M.M., and A. Moreno. (2008) “Effect of Different Biodegradable and Polyethylene Mulches on Soil Properties and Production in a Tomato Crop.” *Scientia Horticulturae* 116, no. 3: 256–63. https://doi.org/10.1016/j.scienta.2008.01.007.

Nuinu, Pranee, Kittikorn Samosorn, Kittisak Srilatong, Siripa Tongbut, and Sayant Saengsuwan. (2013) “Thermal and Mechanical Properties of Mulch Film from Poly(Lactic Acid)/Expoxidized Natural Rubber Blends Filled with Rutile TiO_2 as Fillers.” *Advanced Materials Research* 844: 65–68. https://doi.org/10.4028/www.scientific.net/AMR.844.65.

Outi-Maaria Sietiö, Minna Santalahti, Anuliina Putkinen, Sylwia Adamczyk, Hui Sun, Jussi Heinonsalo. (2019) “Restriction of plant roots in boreal forest organic soils affects the microbial community but does not change the dominance from ectomycorrhizal to saprotrophic fungi” *FEMS Microbiology Ecology*, Volume 95, Issue 9, fiz133, https://doi.org/10.1093/femsec/fiz133

Qi, Xiang, Yiwei Ren, and Xingzu Wang. (2017) “New Advances in the Biodegradation of Poly(Lactic) Acid.” International Biodeterioration & Biodegradation 117: 215–23. https://doi.org/10.1016/j.ibiod.2017.01.010.

Reid, Elise. (2019) “Legacy effects of biodegradable mulch and soil amendments on vegetable crops and soil.” Masters Thesis. University of Nebraska – Lincoln

Samuelson, Ben, Samuel Wortman, Rhae Drijber. (2019) “Assessing the Quality and Possible Functions of Compost Extracts in Organic Systems.” Oregon State University - eOrganic. http://eorganic.org/node/33458.

Satti, Sadia Mehmood, Aamer Ali Shah, Terence L. Marsh, and Rafael Auras. (2018) “Biodegradation of Poly(Lactic Acid) in Soil Microcosms at Ambient Temperature: Evaluation of Natural Attenuation, Bio-Augmentation and Bio-Stimulation.” *Journal of Polymers and the Environment* 26, no. 9: 3848–57. https://doi.org/10.1007/s10924-018-1264-x.

Schutter ME, Sandeno JM & Dick RP. (2001) “Seasonal, soil type, and alternative management influences on microbial communities of vegetable cropping systems”. *Biol Fertil Soils* 34: 397–410.

Shanks R, Kong I. (2012) “Thermoplastic starch.” In: El-Sonbati A (ed) Thermoplastic elastomers. ISBN: 978-953-51-0346-2. *InTech*, doi:10.5772/36295

Steinmetz, Zacharias, Claudia Wollmann, Miriam Schaefer, Christian Buchmann, Jan David, Josephine Tröger, Katherine Muñoz, Oliver Frör, and Gabriele Ellen Schaumann. (2016) “Plastic Mulching in Agriculture. Trading Short-Term Agronomic

Benefits for Long-Term Soil Degradation?" Science of The Total Environment. 550: 690–705. https://doi.org/10.1016/j.scitotenv.2016.01.153.

Tachibana, Yuya, Takuya Maeda, Osamu Ito, Yasukatsu Maeda, and Masao Kunioka. (2009) "Utilization of a Biodegradable Mulch Sheet Produced from Poly(Lactic Acid)/Ecoflex®/Modified Starch in Mandarin Orange Groves." *International Journal of Molecular Sciences* 10, no. 8: 3599–3615. https://doi.org/10.3390/ijms10083599.

Thompson, Ashley A., Mitchell Benjamin Samuelson, Ignatius Kadoma, Erick Soto-Cantu, Rhae Drijber, and Sam E. Wortman. (2019) "Degradation Rate of Bio-Based Agricultural Mulch Is Influenced by Mulch Composition and Biostimulant Application." *Journal of Polymers and the Environment* 27, no.3: 498–509. https://doi.org/10.1007/s10924-019-01371-9.

Torres, A, S M Li, S Roussos, and M Vert. (1996) "Screening of Microorganisms for Biodegradation of Poly(Lactic Acid) and Lactic Acid-Containing Polymers." *Appl. Environ. Microbiol.* 62: 5.

Wortman, Sam E., Ignatius Kadoma, and Michael D. Crandall. (2016) "Biodegradable Plastic and Fabric Mulch Performance in Field and High Tunnel Cucumber Production." *HortTechnology* 26, no. 2: 148–55.

Zhang, G.S., X.B. Hu, X.X. Zhang, and J. Li. (2015) "Effects of Plastic Mulch and Crop Rotation on Soil Physical Properties in Rain-Fed Vegetable Production in the Mid-Yunnan Plateau, China." Soil and Tillage Research 145: 111–17. https://doi.org/10.1016/j.still.2014.09.010.

Janusz, Grzegorz, Anna Pawlik, Justyna Sulej, Urszula Świderska-Burek, Anna Jarosz-Wilkołazka, and Andrzej Paszczyński. (2017) "Lignin Degradation: Microorganisms, Enzymes Involved, Genomes Analysis and Evolution." *FEMS Microbiology Reviews* 41, no. 6: 941–62. https://doi.org/10.1093/femsre/fux049.