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Exploring Crop Management Impacts to Soil Health in Maine Potato Production

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EXPLORING CROP MANAGEMENT IMPACTS TO SOIL HEALTH IN MAINE POTATO PRODUCTION

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UNIVERSITY OF MAINE GRADUATE SCHOOL LAND ACKNOWLEDGMENT

The University of Maine recognizes that it is located on Marsh Island in the homeland of Penobscot people, where issues of water and territorial rights, and encroachment upon sacred sites, are ongoing. Penobscot homeland is connected to the other Wabanaki Tribal Nations—the Passamaquoddy, Maliseet, and Micmac—through kinship, alliances, and diplomacy. The University also recognizes that the Penobscot Nation and the other Wabanaki Tribal Nations are distinct, sovereign, legal and political entities with their own powers of self-governance and self-determination.

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Dissertation Advisor: Dr. Jianjun Hao

An Abstract of the Dissertation Presented in
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The potato, *Solanum tuberosum*, was first brought to Maine in the mid-1700's and has remained economically important since. In 2023, Maine was the ninth largest US producer with a total production of over two billion pounds. However, potato production can be impactful to soil health as the result of crop management practices. The effects of different management factors in potato production (rotation length, variety selection, use of non-grain rotation crops, green manure rotations, chemical fumigation, and compost amendment) were evaluated over four years. No differences were found in the first year of potato planting, but significant differences in soil health parameters were observed in the subsequent potato planting year. In this study, five parameters were found to be related to both management factors and yield, percent organic matter, percent total organic carbon, copper, phosphorus, and zinc. Compost amendments increased organic matter, total organic carbon, zinc, many microbial groups detected via phospholipid fatty acid (PLFA) assays, and was significantly positively related to yields. Rotation length was negatively correlated with organic matter, copper, phosphorus, zinc, and yields, although this could be attributed to few rotation cycles. Fumigation was not positively correlated with yield nor disease incidence reduction compared to standard practices. Other factors resulted in fewer impacts to soil health and yields. The majority of

fungi present in Maine soils were identified as various types of saprotrophs, and the largest phyla of bacteria detected was Proteobacteria. Fungi were found to be more sensitive to management factors than bacteria. Compost amendment appeared to positively impact soil microbial communities and fumigation appeared to negatively impact them. A novel organic soil amendment, post-processed lobster shell, was also explored as a soil amendment. While impacts to microbial communities were not clear, significant reductions in severity of potato early dying was observed in plots which were fumigated and then amended with lobster shell meal, compared to fumigated and unamended plots. The results of these studies could aid Maine potato farmers in developing crop management plans which can improve soil health, reduce disease, and increase yields.

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1.1 Introduction to the potato crop

1.1.1 History of the potato and anthropological importance

The potato, *Solanum tuberosum*, is a unique staple crop with a long and integrated history with humans. Thought to be originally cultivated as early as 3000 B.C.E. in the Altiplano of Western Central South America, also known as the Andean plateau, potatoes were an important food source for the Incan Empire (Mcneill 1999). Potatoes were used as a long-term food that could be stored in the freeze-dried form called chuño or tunta, both of which are still prepared today and have been found in ancient tombs (Hawkes 1992; Peñarrieta et al. 2012). Potatoes are also very culturally significant to the peoples of the Altiplano. Ancient ceremonial vessels over one meter tall were created in the likeness of potatoes (Hawkes 1992). Potatoes still grow wild in the Altiplano, along with cultivated potatoes (Mcneill 1999).

The first time that potatoes were transported out of the Americas is hypothesized to be an importation to the Canary Islands in 1562, where they were likely grown to increase their numbers and further exported to Belgium in 1567, France in 1574, and Spain in 1573 and 1576 (Hawkes and Francisco-Ortega 1993). From there several more rounds of exportations took place and spread the potato around the globe. Within Europe, potatoes are thought to have played a critical role in population growth and provided a consistent food source to prevent food scarcity (Ortiz and Mares 2017). A well-known example of this was seen in Ireland, a country where population increased by five million people over the course of five generations (Ortiz and Mares 2017). However, this reliance on potato production had devastating and tragic effects on the Irish population following the late blight outbreak leading to the infamous Irish potato famine which

resulted in diaspora and death of approximately two million Irish people (Ortiz and Mares 2017). Potatoes could be considered a crop which has had impacts on humans for as long as it has been grown, the surplus of this crop leading to population expansion and success, the absence leading to starvation and diaspora.

Potato production in modern times has seen annual increases of around 4.5% annually, with China and India as the top potato producing countries (Ortiz and Mares 2017). Potatoes are considered a major crop contributing to food security but there is still a lot of room for growth in terms of global consumption and adoption (Ortiz and Mares 2017).

1.1.2 The integration of potatoes into Maine agriculture

Potatoes were first brought to Aroostook County, Maine in the mid-1700's and quickly emerged as an important crop and by 1840, over 10 million total bushels of potatoes were produced in Maine alone (Johnston 1972). With the construction of the railroad in 1891, the bounty of potatoes was able to be dispersed to larger economies in the 1920's as Aroostook County was the leading producer of potatoes in the US (Rose 2003). Over time, the rise of potato production in other states surpassed Maine's production, although Maine has consistently remained a prominent producer and in 2023 was the ninth largest US producer with a total production of over two billion pounds (USDA: NASS 2022a).

1.1.3 Economic and nutritional importance

Potatoes are the fourth most valuable staple food crop behind maize, wheat, and rice (UN FAO 2022). In 2020, 359 million tons of potatoes were produced globally in 150 countries to meet demand (UN FAO 2022). This production not only contributes to global dietary needs but provides a rich source of income for farmers (Devaux et al. 2014). While commonly consumed as a staple, potatoes are often mistakenly considered to be an unhealthy food item (Ortiz and

Mares 2017). However, potatoes are a source of dietary fiber, potassium, and vitamin C (Ortiz and Mares 2017). Additionally, compared to other staple crops, potatoes provide the highest amount of calories, vitamins, and other nutrients relative to the amount of land planted, which is a large reason that potatoes contribute so heavily to food security (Ortiz and Mares 2017).

1.1.4 Potato production, plant development, and biology

While commonly thought of as a root, potatoes are modified underground stems (Stark et al. 2020). The eyes on potatoes contain three undeveloped leaf buds, which allow potatoes to develop into new plants when exposed to light (Stark et al. 2020). Seed tubers are most commonly used in potato production (van Loon 2007). While some farmers may choose to use stored seed tubers from the previous year's harvest, the risk of poor quality and seedborne diseases lead many to prefer the use of certified seed, which must be inspected by a certifying agency (van Loon 2007). It can be summarized that potato seed generally is provided as home-grown certified seed, imported certified seed, home-grown uncertified seed, and self-produced uncertified seed (van Loon 2007). The most important factors to consider for certified seed include lack of pests and diseases, small size of tubers, and vigor which is typically defined by length of dormancy (van Loon 2007). There are several thresholds which vary for each of these factors. Potato plants go through five different growth stages during the growing season, starting with sprout development, vegetative growth, tuber initiation, tuber bulking, and finally maturation (Stark et al. 2020).

Sprout development is the first development stage in which the seed tuber piece begins to produce sprouts (Thornton 2020). During this stage, spout development is highly dependent on soil temperatures being above 45°F, however temperatures above 68°F or seed pieces planted deeper than 6-8 inches can contribute to poor sprout development and thus poor emergence of

the adult plant (Thornton 2020). Vegetative growth is the second stage of development, in which the young plant continues to develop roots and above ground tissues (Thornton 2020). During the vegetative growth stage, the young plants are sensitive to temperatures which are too low or too high and the plant begins to rely on soil nutrition as opposed to the mother seed piece as it did during the sprout development stage (Thornton 2020). Tuber initiation is typically thought to simply coincide with flowering of the potato plant. However, tuber initiation is the stage in which the stolons begin to swell and this stage is once again dependent on weather conditions and nitrogen content of the soil (Thornton 2020). Tuber initiation can also be viewed as the stage in which the plant shifts from entirely vegetative growth to storage of nutrients in the young tubers (Thornton 2020). Some flowers which are produced concurrently with tuber initiation will give rise to fruit and true potato seed, however, because potato plants are tetraploid, seed set is irregular and resulting offspring traits are not uniform (Bradshaw 2007; Stark et al. 2020). Tuber bulking is the fourth growing stage, in which the tubers continue to enlarge, and it is estimated that at harvest approximately 90% of the total yield is the result of photosynthetic activity during this stage (Thornton 2020). The fifth and final stage of potato plant development is maturation, in which the plants begin to senesce and the periderm of the newly developed tubers begins to thicken, which is essential for protecting the tubers from disease and mechanical damage during the harvest (Thornton 2020).

1.1.5 Environmental, nutritional, and erosion concerns and considerations

Potato production is not without environmental concerns which relate to the way in which the crop is grown and harvested. Potato production requires proper plant nutrition for acceptable yields. As a crop, the top nutrients which are utilized include nitrogen, potassium, calcium, and magnesium (Westermann 2005a). However, the application of excess fertilizers can

have environmental impacts and lead to the contamination of water bodies (Westermann 2005a). The issue of excess fertilizer residues can be ameliorated through the employment of rotation crops which have differing nutritional needs from potatoes (Davenport et al. 2005). Cover crops can be beneficial in reducing water erosion and run off while rotation crops, such as sugar beets, can prove useful in scavenging residual fertilizers from the preceding potato crop (Davenport et al. 2005).

Another major concern is erosion of topsoil as the result of harvesting techniques and tillage which is performed before planting seed tubers (Auerswald et al. 2006). Potatoes are also considered a crop with one of the highest risks of erosion damage as the result of tillage, water runoff, and harvesting impacts (Auerswald et al. 2006). In a study comparing cropping systems and their impacts to soil, conventional farming practices resulted in the largest amount of soil loss compared to organic management, which the author indicated could be the result of soil type and the use of a rotation crop preceding the potato crop in the organic fields (Auerswald et al. 2006). This could be in part because conventional agriculture requires intensive tillage, compaction due to the use of heavy farm equipment, little to no crop residues leftover post-harvest, and soil may remain exposed and fallow for long periods of time (Bohl and Johnson 2010b). While these issues are only two of the many concerns to both potato production and agricultural production in general, the use of rotation crops and cover crops can be beneficial in mitigating these challenges. Rotation and cover cropping can have many additional benefits as well, which will be further explored in this review.

1.2 Common diseases of Potatoes in Maine

In addition to the challenges associated with soil chemical and physical health as a result of potato production, potatoes as a food crop are susceptible to numerous diseases caused by and exacerbated by fungi, bacteria, nematodes, viruses, and insects (Fiers et al. 2012). About 40 of these diseases are caused by soilborne pathogens, which can be particularly impactful to the potato industry because of the inherent value of tubers (Fiers et al. 2012). In Maine, some of the most important diseases can be divided into fungal pathogens, including oomycetes, bacterial pathogens, viral pathogens, nematodes, and abiotic disorders. Potato disease can further be distinguished as soilborne diseases which arise from the soil, as opposed to seedborne, vector borne or airborne diseases, and can cause symptoms on tubers or on aboveground portions of the plant (Fiers et al. 2012). There are a number of environmental factors which impact disease severity especially with respect to soilborne diseases (Fiers et al. 2012). A few economically important diseases to potato production in Maine are detailed below.

1.2.1 Fungal pathogens and their diseases

Black scurf is caused by *Rhizoctonia solani* and is typically identified by the presence of dark colored sclerotia on the surface of the tuber (Stevenson et al. 2001). While the sclerotia on the surface of the tubers can be unsightly, they typically do not cause damage to the tuber, although other symptoms of infection can include cracking, deformities, and other blemishes (Stevenson et al. 2001). Rhizoctonia stem canker is also caused by *R. solani* and causes reddish brown to black lesions on vegetative plant tissue which can be severe enough to girdle roots or shoots and result in poor emergence and vigor (Stevenson et al. 2001). This pathogen can overwinter either in decomposing plant tissues or as sclerotia remaining in soil or on tuber surfaces (El Bakali and Martín 2006). As such, black scurf can be considered both a soilborne

and seedborne disease. Cool soil temperatures tend to exacerbate the disease symptoms, and even small amounts of inoculum can significantly reduce yields (Stevenson et al. 2001). Crop rotations are beneficial tools in reducing disease severity of black scurf, especially with disease suppressive crops, such as *Brassica* crops and sudangrass green manures, compared to consecutive potato plantings with no rotations (Larkin et al. 2010). The longer length of crop rotation can also reduce the severity of black scurf especially over multiple seasons of implementation, as was seen in a study comparing two-year and three-year rotations over six years (Carter and Sanderson 2001).

While black scurf can be managed through crop rotations, other cultural practices to reduce disease include ensuring proper potato nutrition and planting less susceptible cultivars, because no high level of resistance to this pathogen seems to exist (Stevenson et al. 2001). Several effective fungicides can be used to control black scurf and stem canker, including captan, iprodione, mancozeb, and fludioxonil (Bains et al. 2002). Additionally, the use of biological control agents, such as *Bacillus subtilis*, have been shown to reduce disease severity and incidence of black scurf and stem canker in field trials (Brewer and Larkin 2005; Larkin and Tavantzis 2013). These biological control agents have also been found to remain in soils after application and may impact disease and other soil microbial communities even if not applied every year (Larkin 2016).

Verticillium wilt and potato early dying (PED) are caused by two different species of fungi; *Verticillium albo-atrum* or *Verticillium dahliae*, the latter of which is responsible for most disease symptoms in Maine (Powelson and Rowe 1993; Johnson 1995). Common aboveground symptoms of an infection by *Verticillium* spp. include acropetal discoloration and chlorosis, necrosis of leaves, and defoliation which are typically unilateral and can be easily confused with

natural senescence of the plant (Powelson and Rowe 1993; Johnson and Dung 2010). Within tubers, necrosis and discoloration of the vascular ring may be present depending on the susceptibility of different cultivars (Powelson and Rowe 1993; Johnson and Dung 2010). Potato early dying typically occurs when a field has simultaneous populations of *Verticillium* spp. and root lesion nematodes, specifically *Pratylenchus penetrans*, through the interaction of these two agents (Powelson and Rowe 1993).

Verticillium dahliae can infect many species of plants globally, and thus it can be challenging to disrupt the fungi's lifecycle through the use of rotation crops as a disease suppressive tool (Ochiai et al. 2008). However, the application of green manures and other soil amendments could prove to be a useful tool, especially as the most commonly used management strategy, soil fumigation, can be costly and hazardous leading to enhanced regulation (Ochiai et al. 2008). Additionally, green manures such as sudangrass have the potential for nematode control, which could prove beneficial in the reduction of early dying (Davis et al. 2010). It is worth noting that while green manures are commonly associated with putting land out of production, many of them may also serve as forage crops or be harvested early as a cash crop and leaving residues to serve as green manures (Davis et al. 2010).

1.2.2 Oomycete pathogens and their diseases

Diseases caused by oomycetes can be highly devastating with examples such as the causal agent of the Irish Potato Famine being of particular economic significance (Rietman et al. 2010). Oomycetes are fungi-like, but not true fungi, and are closely related to diatoms and brown algae (Derevnina et al. 2016). Late blight of potato is caused by *Phytophthora infestans*, an oomycete belonging to a genus which causes symptoms such as wilting, chlorosis, root rots, among other symptoms (Akino et al. 2014). Sporangia of this pathogen are spread aerially from

lesions on diseased plant tissue and give rise to zoospores, which infect new plant tissues directly (Fry 2008). Disease symptoms include necrotic lesions which contain hundreds of thousands of sporangia, which are typically not seen for two days because the pathogen is hemibiotrophic and requires the plant to stay alive during initial infection (Fry 2008). Other important oomycete pathogens of potatoes include pink rot, caused by *Phytophthora erythroseptica*, which causes wilting and leaf loss in the plant, and tuber rot, and pythium leak caused by *Pythium ultimam* var. *ultimum,* mainly exacerbated by wounds to the tubers and symptoms appear in tubers as hollow rotten cores (Stevenson et al. 2001).

1.2.3 Bacterial pathogens and their diseases

There are a large number of bacterial diseases which have large impacts to potato production. Of these, the most common bacterial diseases are ring rot, soft rot, black leg, brown rot, and common or netted scab (Fiers et al. 2012). Common scab is caused by *Streptomyces scabies*, a gram-positive bacterial pathogen and causes scab-like lesions on tuber surfaces, although they have a broad host range including radishes and peanuts 4/30/24 11:51:00 AM. The production of thaxtomin is a requirement of pathogenicity, as this compound causes necrosis and scab formation (Loria et al. 2006). However, this compound may be sensitive to soil physical properties such as temperature and pH, as was demonstrated by reduction of thaxtomin by *Streptomyces scabies* in alkaline environments and elevated temperatures (El-Sheikh et al. 2012). Furthermore, in field trials, soil pH values above 8.5 resulted in a decline in both severity and incidence of common scab in potatoes (Waterer 2002). However, *S. scabies* is one of many other *Streptomyces* spp. which are highly successful in the soil environment because of their production of various enzymes, and there are many saprophytic species which are beneficial to plants due to their antibiotic production and nutrient cycling (Loria et al. 2006).

1.2.4 Viral disorders and diseases

Potatoes are also subject to a number of viral pathogens, and severe cases of potato virus Y (PVY) and Potato leafroll virus (PLRV) can reduce potato yields by an estimated 80% (Kumar et al. 2022). Other viruses can cause less severe reductions in yield, but can co-occur and result in more severe losses, such as Potato virus X (PVX), Potato virus S (PVS), and Potato virus M (PVM) (Kumar et al. 2022). Viruses can result in a wide range of symptoms, from mosaic, stunting, chlorosis, leaf curling, early necrosis, aerial tubers, and death of the plant (Harris 1992; Hane and Hamm 1999). Some viruses are transmitted by insect vectors, such as aphids, whiteflies, or thrips, while other viruses, such as Potato mop top virus, are soilborne (Calvert and Harrison 1966; Kumar et al. 2022). In general, the only tools available for managing viral disorders are through crop destruction, managing insect vectors (if applicable), using certified seed, and using resistant varieties (Kumar et al. 2022).

1.2.5 Nematodes and their disease complexes

Nematodes are unique plant pathogens which can result in potato yield losses estimated at 25% or more as the result of damage to tubers and reduced quality (Youssef 2013). The most commonly associated pathogenic nematodes to potatoes are *Globodera* spp., *Meloidogyne* spp., *Ditylenchus* spp., and *Pratylenchus* spp. (Youssef 2013). In addition to causing destruction of potato crops, they also can serve as vectors of other potato pathogens or form complexes with plant pathogens, as is the case with *V. dahliae*, the causal agent of potato early dying (Powelson and Rowe 1993; Lima et al. 2018). The most efficient management techniques associated with nematode control are the use of resistant varieties, crop rotations, biological controls, and nematicides (Youssef 2013).

1.3 Introduction to the soil health concept

1.3.1 Soil health as measured using soil chemical, physical, and biological parameters

Soil health is a term to describe the capability of soil to sustainably produce high quality crops (USDA NRCS 2023). Soil health is critical to crop production durability, water quality, climate change mitigation, human health, and many other ecosystem services (Lehmann et al. 2020).

In general, soil health criteria can be divided into three categories of soil properties: physical, chemical, and biological (Lehmann et al. 2020). Physical parameters assist plants in physical support and the accessibility of roots to access oxygen, water, and nutrients, while at the same time assisting with water management, aiding in organic carbon accumulation, and in preventing erosion (Doran and Parkin 1994; Allen et al. 2011). Chemical parameters play a role in the cycling and availability of various elements (Karlen and Stott 1994). Chemical soil properties are responsible for plant growth and development, nutrient availability, and cation exchange capacity aids soil in immobilizing toxic metals or contaminants such as pesticides (Allen et al. 2011). Biological properties encompass all of the activities of the living components of soil, including the various macro- and microorganisms, and can be estimated through the measure of such parameters as soil organic matter, microbial activity, Solvita CO_2 burst assay (Solvita), active C as permanganate-oxidizable C (POX-C), and autoclaved citrate-extractable (ACE) soil protein (Chatterjee and Acharya 2018; Karlen et al. 2021). Biological soil health is critical for decomposition of residues, nutrient cycling, and soil architecture maintenance (Turco et al. 1994). Additionally, soil microorganisms can contribute to pathogen suppression through competition for space and nutrients, production of toxic volatiles, utilizing pathogens as a

nutrient source, and promotion of plant defense systems, among other activities (Mehta et al. 2014; Schumann and D'Arcy 2010).

1.3.2 Soil microbial communities, ecosystem services, detection techniques, and niches

Soil microbial communities exist in highly competitive environments which can be nutrient poor, prone to extreme temperatures and moisture levels, and frequently populated with organisms which create antimicrobial substances (Loria et al. 2006). Even given the challenges of existing in this environment, a single gram of soil is estimated to contain thousands of microbial taxa (Fierer 2017). This summation of all existing soil dwelling microorganisms including archaea, bacteria, viruses, fungi, protists, and other microbial eukaryotes is referred to as the soil microbiome (Fierer 2017). While the soil microbiome has been recognized as a highly important and functional tool, until recent technology advances, the massive diversity and complexity of the soil microbiome has remained partially obscured (Fierer 2017). Previously used techniques to estimate microbial diversity in soil relied on in vitro culturing, and it is now known that these techniques, while useful, severely underestimate the diversity of the soil microbiome (Fierer 2017). As culturing only resolves an estimated 1% of all microbes in a given sample, the use of molecular methods have helped to improve the understanding of microbial communities (Youseif et al. 2021).

Most specifically, the use of amplicon sequencing has become a common technique to taxonomically explore microbial communities in various environments, including soil, especially as this service has become less cost prohibitive. Amplicon sequencing targets highly conserved genes, such as the 16S rRNA or internal transcribed spacer (ITS) to detect bacterial or eukaryotic communities, respectively (Lundberg et al. 2013; Alteio et al. 2021). Soil microbial communities are an essential area of further investigations, due to the critical roles that microbial communities

play in the soil environment and agriculture. While there has been more research on the diversity of soil microbial communities, soil microbial communities are still very poorly understood, highly diverse, and functionally redundant (Maron et al. 2011; Fierer 2017).

Soil microbial community analysis is an incredibly useful tool but is not without flaws. Soil contains many diverse microenvironments which differ with respect to pore size, oxygen and water availability, environmental and chemical differences such as pH, and humus content, which can lead to uneven occupation of soil microbes within soil (Daniel 2005). Humus is the natural byproduct of microbial degradation of organic matter in the soil and is mostly composed of humic acid and polysaccharides, which are two types of polymers (Martin and Haider 1971). An initial challenge arises in the DNA extraction process of soil, as soil microbes adhere to soil particles and coextraction of humic substances commonly occurs, which can interfere with restriction enzyme digestion, an essential component of efficient PCR amplicon production (Daniel 2005). A secondary challenge is encountered in the form of bias during the DNA extraction process, although some of this bias may be avoided in part by using cell lytic extraction techniques and avoiding cell separation from soil particles which can increase DNA yield, but also can result in DNA from eukaryotes and other environmental DNA (Daniel 2005). Further challenges arise in the library construction as rare microbes may not be represented (Daniel 2005). While these challenges may be daunting, the power associated with soil metagenomics warrants the efforts to understand these environments.

Soil is rich in not only the diversity of microbes which inhabit it, but also in the diversity of microenvironments which can vary within even centimeters of different soil samples due to variations in moisture, available plant tissue, irregular nutrients, light, presence of soil aggregates, and more (Fierer 2017). These differences reflect in studies of spatial scale diversity

of soil microbial communities and present a challenge to overcome. A study comparing 25 soil samples collected from within a $100cm^2$ area in grasslands indicated heterogeneity of bacterial diversity, no spatial structure, and varying abundances of different taxa was observed (O'Brien et al. 2016). However, when the samples were compared to bacterial communities at a global scale, some congruency was observed and 20% of bacterial taxa were shared between all globally collected soil samples and about 40% of taxa were shared with other samples collected from other grassland soils (O'Brien et al. 2016). This study demonstrated the limitations of small-scale soil microbiome sampling methods and shed light on the potential of a cosmopolitan distribution of soil bacterial communities on a global scale (O'Brien et al. 2016).

Additionally adding to the complexity, soil microbiome analyses are biased towards representing all microorganisms regardless of their physiological state, although active soil dwelling microorganisms, which respond to substrate input immediately, makeup 0.1-2% of the total microbial biomass of soil, but rarely exceed 5%, although microorganisms which are potentially active, or respond to substrate input in a few hours, compose approximately 10-40% of microbial biomass (Blagodatskaya and Kuzyakov 2013). These potential spatial scale biases can cause great challenges to soil microbial community analyses, and while comparative similarities can be seen at a global scale, the need for further research into soil microbial communities is essential to determine spatial and temporal population fluctuations, as well as determining the community make up. The complexity of soil microbial community dynamics must not be understated.

Some soil microorganisms are referred to as beneficial as they contribute to soil health and plant health by providing services such as nutrient cycling, decomposition, soil structure, and pathogen and pest suppression (Larkin 2015). The apparent utility of these beneficial soil

microbes is seen in evolutionary reliance on mycorrhizae, as it was reported that 92% of vascular plants rely on these associations (Ray et al. 2020). Beneficial microbes are thought to play a role in agricultural yield increases as well through plant growth promotion as a result of nutrient uptake assistance or altering plant hormones (Ray et al. 2020). However, there does not seem to be a perfect prescription of microbes to enhance plant growth and disease suppression, and fields inoculated with single strains of nonindigenous soil microbes do not necessarily demonstrate yield increases, potentially due to competition with indigenous soil microbes (Ray et al. 2020). However, the authors argue that consortiums of beneficial microbes could result in enhanced yield and disease suppression because of the synergistic action of compatible microbes (Ray et al. 2020).

1.3.3 Management and environmental impacts to soil microbial communities

Agricultural cropping strategy plays a role in microbial community composition. This was seen in microbial community shifts and selection in response to long term organic or conventional management, and an increase in microbial richness was seen in response to organic management (Hartmann et al. 2015). However, the authors argue that simply observing these changes in richness does not properly capture the complexity of the systems and that specific factors, such as mineral or organic fertilizer selection, could also play a large role (Hartmann et al. 2015). Organic agriculture in the short term has been shown to reduce yield, but after several years yields could increase to levels comparable to conventional agriculture, as was seen in a study contrasting potato and rotation crops grown organically, conventionally with pig slurry, or conventionally with mineral fertilizer (Schrama et al. 2018). This was thought to occur due to the efficiency of nitrogen application and integration in organic treatments as opposed to nitrogen loss in conventional agriculture, which was detected in groundwater with nitrate levels 50%

higher than organic treatments (Schrama et al. 2018). The application of mineral nitrogen was also seen to influence microbial communities as a result of soil acidification and loss over fertility overtime in switchgrass plots fertilized with various levels of nitrogen fertilizer (Chen et al. 2019). Microbes can be very sensitive to these variations in nutrients, pH, and especially temporal scale, as was also seen in this study in which bacterial and fungal phyla differed significantly depending on the time of year of sampling (Chen et al. 2019).

1.3.4 Soil health for potato production

Potato crops are adaptable to growing in many different types of soils ranging in pH from acidic to alkaline soils, with the most important factor being that the soils are well drained and friable (Bohl and Johnson 2010b). Potato production is possible in soil with high clay contents as well as high sand contents, with unique field management techniques specific to the soil types (Bohl and Johnson 2010b). Tillage of the soil is also critical for potato production, to reduce weed pressure and ensure that potato roots can effectively move through the soil (Bohl and Johnson 2010b). However, tillage must be performed on dry ground and sparingly so as to not degrade soil structure quality, as excess tillage can increase compaction (Bohl and Johnson 2010b).

Soil fertility is an essential component of potato production, as removal of the potato plants and tubers continually depletes soil nutrients, which must be replenished to ensure future production (Bohl and Johnson 2010b). Nutrient deficiencies and excesses can also cause a wide variety of abiotic issues, such as misshapen tubers, hollow heart, low specific gravity, and brown centers (Bohl and Johnson 2010b). There are six macronutrients and eight micronutrients which potatoes require for proper growth and nutrition, categorized as primary macronutrients, secondary macronutrients, and micronutrients (Bohl and Johnson 2010b). In general, both

primary and secondary macronutrients are required in large amounts for potato productions, but primary macronutrients, nitrogen, phosphorus, and potassium, are poor in agricultural soils and must be added for successful potato production, as opposed to the secondary macronutrients, sulfur, calcium, and magnesium, which are commonly already present in the soil (Bohl and Johnson 2010b). The micronutrients, zinc, manganese, iron, copper, boron, chloride, molybdenum, and nickel, while required in smaller volumes, may also need to be added to soil depending on their presence or absence (Bohl and Johnson 2010b). In order to determine soil nutritional needs, regular soil testing is suggested to ensure that potato plants are neither deficient or in excess of required nutrients (Bohl and Johnson 2010b).

Potato roots are fibrous and weak and are thus poor at nutrient and water uptake and potatoes as a crop are consequently not drought tolerant and can be impacted by poor soil structure (Struik 2007). Root development declines during tuber bulking and roots which develop on the stolons and tubers are particularly important during this developmental stage (Struik 2007). Potato roots also require oxygen to uptake both water and nutrients, and for this reason, well-drained soil, as mentioned previously, is critical to potato production (Bohl and Johnson 2010b).

1.4 Management impacts to soil health

1.4.1 Rotation length and variety selection

When a farmer initially plants a potato crop, two of the first decisions to make are the length of crop rotation and the variety which will be selected for use. The industry standard of a potato rotation length in Maine is 2 years, although longer rotation lengths have demonstrated benefits (Larkin et al. 2021b, 2021a). Longer rotations have been found to significantly increase

substrate utilization patterns by microorganisms, reduce soilborne disease pressure, and improve soil health metrics (Bucher and Lanyon 2005; Larkin et al. 2010; Wright et al. 2017). On the other hand, variety selection can be an important tool to increase yields and provide resistance to plant pathogens (Tessema et al. 2020).

1.4.2 Rotation and cover crops and their use in potatoes

Cover crops are alternative rotation crops which are planted when a field would otherwise be left fallow. They can be very beneficial in improving soil health in many ways, including reducing erosion, increasing water retention, breaking pathogen lifecycles, increasing organic matter, and many other ways (Clark 2008). The main techniques of cover crop use include during winter fallow, summer fallow, and full-year fallow (Clark 2008). Winter fallow involves sowing a cover crop following harvest at least 6-weeks before frost conditions or undersowing or interplanting a shade tolerant crop (Clark 2008). Summer fallow cover crops can be utilized between cash crops planted in the spring and fall seasons (Clark 2008). Full-year fallow cover crops can be utilized over the course of a growing season when a cash crop is not planted and can be used to improve and conserve soil (Clark 2008). This type of cover crop is also referred to as a rotation crop, and can be harvested as an alternative source of income for farmers. Rotation crops are often utilized to reduce disease through interrupting the lifecycle of pathogens, altering the soil environment, or producing inhibitory compounds (Larkin and Honeycutt 2006).

In potatoes, rotation crops are typically utilized because of the invasive nature of producing this crop. Potato production requires tillage of the soil and potatoes require large amounts of nutrients in order to produce high yields and quality tubers (Harris 1992). The industry standard of a rotation length is 2 years, although benefits have been seen in increased

length of crop rotation (Larkin et al. 2021b). During this two-year rotation, following potato production in year one, a grain, such as barley or oats is sown, occasionally underseeded or followed by another winter cover crop, such as clover, although this may vary based on the unique needs and problems of a particular field (Bohl and Johnson 2010b).

Rotation crops can have impacts on soil structure and soil microbial communities depending on the types of crops selected for rotations. A long-term study of the impacts of various rotations crops on disease incidence, yield of potatoes, and microbial communities indicated that rotation crop selection is an important factor when disease suppression is the goal (Larkin et al. 2010). Seven various crops in two-year rotations were evaluated and contrasted to continuously planted potato crop, and the authors found that the use of canola or rapeseed rotations significantly reduced black scurf, common scab, and *Rhizoctonia* canker when disease severity was averaged across the eight-year study (Larkin et al. 2010). Additionally, canola planted as a rotation crop significantly increased potato yields and reduced the percentage of misshapen tubers compared to the continuous potato plantings (Larkin et al. 2010). However, in this same study, none of the tested rotation crops reduced long term disease incidence of Verticillium wilt and common scab, and the authors argue that the need for long term studies investigating disease impacts is essential to fully capture these changes (Larkin et al. 2010). Microbial populations were also altered through the use of various rotation crops. Rapeseed followed by winter rye as a fall cover crop had the largest bacterial population and soil samples had 2.5 million more colony forming units per gram of soil compared to continuously planted potato soil bacterial populations (Larkin et al. 2010). When no fall cover crop was planted, barley had the largest bacterial populations and contained 6.08 million more colony forming

units per gram of soil than soil collected from plots containing continuously planted potato (Larkin et al. 2010).

When selecting rotation crops, it is essential to select crops which are not reservoirs for disease because of their ability to be infected by pathogens which also infect potatoes, thus enhancing disease pressure. An example of this is if white mold disease pressure is present, as both potatoes, beans, and canola can be infected, so beans and canola should be avoided as rotation crops if this disease is a concern (Bohl and Johnson 2010b). Another consideration when planting rotation crops is the type of equipment and skills needed to grow alternative non-grain rotation crops which may be less familiar or profitable, the need for highly organized farms and record keeping, and the potential economic loss of producing a less profitable crop (Zegada-Lizarazu and Monti 2011). However, this familiarity with alternative non-grain rotation crops can lead to another potential benefit of crop rotations in that farmers can diversify production for more economic opportunities and climate resiliency as a result of diversified plantings (Zegada-Lizarazu and Monti 2011).

1.4.3 Green manures, biofumigants, and their use in potatoes

Allelopathy is a tool utilized by plants to inhibit other plants through the production of harmful substances (Taiz et al. 2018). While allelopathy is a natural adaptation, farmers can utilize plants with allelopathic properties to overcome various problems in their crops. When these plants are used as a tool to combat various soilborne pathogens or pests, they are referred to as biofumigants (Kirkegaard and Sarwar 1998). Typically, biofumigation can take place either by planting allelopathic plants as a rotation crop, utilizing seed meal as an amendment, or as green manure in which plants are planted, tilled and incorporated into the soil (Kirkegaard and Sarwar 1998).

The most commonly utilized biofumigants are derived from members of the Brassicaceae family because of the production of glucosinolates, organic compounds containing sulfur which are formed from glucose and an amino acid, which are hydrolyzed into various products including different volatile isothiocyanates (Kirkegaard and Sarwar 1998; Taiz et al. 2018). Isothiocyanates are nonspecific biocides, and can impact organisms ranging from insects, nematodes, bacteria, fungi, and other plants (Kirkegaard and Sarwar 1998).

In potato production, the use of *Brassica* green manures, especially *Brassica juncea*, planted before potato crops contributed to reductions of black scurf (*Rhizoctonia solani*), common scab (*Streptomyces scabies*), and powdery scab (*Spongospona subterranea*) (Larkin and Griffin 2007). While green manures were thought by the authors to only be partially responsible for the reduction in soilborne pathogens in their field trial, reductions in viable inoculum in greenhouse trials and inhibition of growth of various pathogens following exposure to volatiles produced from *Brassica* crops indicates the potential that further research could bring (Larkin and Griffin 2007).

1.4.4 Soil chemical fumigation

Before planting to reduce disease and weed pressure, soil may be fumigated with volatile, toxic chemicals. Fumigation occurred historically with methyl bromide, which began to be phased out of use in the United State in 1993 with the phase out complete by 2005 (Duniway 2002). Since the phase out of methyl bromide, alternative chemicals, such as chloropicrin, 1,3-decholoroproperene, and methyl isothiocyanate generators, such as metam sodium, are more commonly used presently (Duniway 2002). In Maine, methyl isothiocyanate (MITC) generating soil fumigants, such as Vapam HL, are commonly used (Aaron Buzza, personal communication). These chemical fumigants rapidly degrade into MITC, which is not very mobile in soil and

requires careful application with respect to weather conditions, such as temperature and amount of rainfall, to ensure successful application (Duniway 2002). Chemical fumigation is commonly performed in the United States and has demonstrated reduction in soilborne diseases, increases of beneficial microbial communities, and enhanced yields (Neilson et al. 2020). It is important to note that chemical fumigants do not completely sterilize soil, and that microbial communities can persist and grow following fumigation (Duniway 2002). However, often results are conflicting and may indicate bacterial diversity declines, increases, or changes annually (Neilson et al. 2020).

1.4.5 Organic soil amendments, including compost and shellfish byproducts

Composting is a process by which thermophilic aerobic microorganisms degrade and convert waste into a stable product which is rich in nutrients and beneficial microorganisms (Agnew and Leonard 2003). It is a useful tool in transforming food and agricultural wastes into a valuable soil amendment. The composting process mostly occurs through the services of cellulose degrading microorganisms which can decompose plant residues and wastes (Singh and Nain 2014). The proper production of compost relies on the appropriate environment for these microorganisms, including temperatures above 40°C, high moisture, and adequate airflow (Agnew and Leonard 2003). As a soil amendment, compost improves soil stability and water retention, although the effectiveness and quality of compost depends on the precision of the process of maintaining moisture, providing oxygen, and ensuring proper warmth (Agnew and Leonard 2003). The massive consortium of microorganisms in compost can also be useful inhibitors of plant pathogens (Gong et al. 2005; Singh and Nain 2014).

Compost as a plant disease suppressive tool functions through a few different mechanisms (Mehta et al. 2014). The first mechanism is through the competition for both space

and available nutrients within the soil environment (Mehta et al. 2014). This process is not specific to reduction of pathogens but can result in their suppression. The second method is through the production of toxic substances such as metabolites, volatiles, and degrading enzymes (Mehta et al. 2014). There are a number of different organisms common in composts which are capable of producing these substances including microorganisms in the *Pseudomonas* and *Bacillus* genera (Mehta et al. 2014). The third mechanism of pathogen suppression by compost dwelling microorganisms is hyperparasitism, in which a pathogen is directly killed (Mehta et al. 2014). The fourth strategy is through the induction of a plant's natural defense mechanisms including induced systemic resistance (ISR) which contributes to enhanced protection by the plant from pathogens (Mehta et al. 2014). ISR is separate from systemic acquired resistance (SAR), which occurs when a plant is directly challenged by a pathogen and detects the presence of pathogenesis-related proteins which triggers the production of various chemicals and defense responses (Schumann and D'Arcy 2013). SAR typically begins with the walling off and death of tissue which was initially infected (hypersensitive response) followed by the sending of signals throughout the plant's entire system to resist further attack (Schumann and D'Arcy 2013). On the other hand, ISR occurs through the communication of root dwelling bacteria with the plant to promote the production of physical and chemical defense mechanisms by the plant (Schumann and D'Arcy 2013). This does not follow physical damage to the plant similarly to SAR and can be promoted by different amendments and chemicals, including the microorganisms and other components of compost which can trigger ISR (Schumann and D'Arcy 2013; Mehta et al. 2014).

Organic soil amendments are also derived from other natural sources, including shellfish byproducts. The state of Maine is uniquely situated with 3,478 miles of shoreline, shorter than only Alaska, Florida, and Louisiana, respectively (NOAA 2017). This unique location of cold

coastal waters encourages shellfish production, including bivalves, such as oysters and clams, and crustaceans, such as crabs and the renowned American Lobster. Shellfish industries produce substantial amounts of byproducts, including shells of both bivalves and crustaceans. Disposing of lobster shell waste is highly costly, for example, a facility processing 15,000 pounds of lobster per day can expect to pay upwards of $4000 per month for disposal services, and while a small portion of lobster shell waste is composted, the majority is sent to landfills (Fulton et al. 2013). However, the shells of shellfish may also have potential for use in agriculture as soil amendments.

Crustaceans are a diverse group of arthropods which include lobsters, crabs, and shrimp (Urry et al. 2017). While their exoskeletons are made of chitin, the shells of crustaceans are reinforced with calcium carbonate (Urry et al. 2017). Chitin content varies between different organisms, and in the *Homarus* genus, which includes the American Lobster, the chitin content of the cuticle ranges from 60-75% (Tharanathan and Kittur 2003).

Chitin as a soil amendment may be capable of promoting plant growth and could be a useful tool in disease suppression. The first pathway is through the triggering of plant defenses by the presence of chitin. An example of this was seen through the production of phytoalexins, antimicrobial molecules which are one of many plant defenses, by rice callus following the introduction of N-acetylchitooligosaccharides, which are oligosaccharides of chitin (Yamada et al. 1993). In a study of cabbage and strawberry plants infected with *Alternaria brassicicola* and *Colletotrichum fructicola*, respectively, the application of chitin nanofibers elicited systemic disease-resistance and had a growth-promoting effect in both hosts (Parada et al. 2018).

The second pathway is through the promotion of native communities of beneficial soil dwelling microbes. In a study of chitin as a soil amendment for fusarium yellows of celery,

bacterial populations were higher following the addition of chitin compared to unamended soil and fewer fungi were present following chitin amendments (Bell et al. 1998). In the same study, it was found that actinomycete populations were significantly increased in treatments with chitin amendments (Bell et al. 1998). Actinomycetes are known to be plant growth promoting rhizobacteria and also have many other traits such as nutrient cycling, antibiosis, and potential as biological control agents (Jog et al. 2016). In a study of various soil amendments in greenhouse production of cucumber, chitin amendments from shrimp and crab shells were found to promote growth of seedlings, however when inoculated with *Fusarium oxysporum f.* sp. *radiciscucumerinum*, the seedlings planted one day post inoculation were more susceptible to disease pressure (Rose et al. 2003). The authors speculated that this could be due to the rapid increase of nitrogen, promoting seedling growth, but without the time to allow antagonistic populations to build and suppress pathogenic populations (Rose et al. 2003).

In a separate study of chitin from crab shell meal against root rots in ginseng, actinomycete populations exceeded 25 times the amount in the untreated soil and populations of *Cylindrocarpon destructans*, the causal agent of ginseng root rots, were reduced to about half the population in untreated soil (Chung and Kim 1978). In a study of chili plants, chitin in the form of ground prawn shells was added to soil and both reduced the incidence of *Rhizoctonia solani* in inoculated plants and resulted in longer shoot growth when used as a soil amendment (Hussain et al. 2013b). Pathogens are not the only organism which may be inhibited by chitin soil amendments, and in plants inoculated with viable root-knot nematode eggs, *Meloidogyne javanica*, the root-knot index score of 4.3 was seen in control plants compared to the root-knot index score of 1.2-2.3 in plants amended with chitin (Hussain et al. 2013b). While chitin is available both commercially and as a raw byproduct post shellfish processing, in a study of chitin

from commercial sources and as an industry byproduct, no difference was seen in between the two as a tool in suppression of *Colletotrichum* spp. of cucumber plants (Dodgson and Dodgson 2017). In summary, the use of chitin-based soil amendments have been demonstrated to be useful tools in pathogen suppression, plant growth promotion, and enhancing beneficial microbial communities.

CHAPTER 2

SHORT-TERM MANAGEMENT IMPACTS TO SOIL PROPERTIES, POTATO YIELD, AND DISEASE PRESSURE IN MAINE POTATO PRODUCTION

Abstract: Enhanced soil health can provide protection for plants from diseases, enhance nutritional fertility, prevent erosion through improved physical structure, and consequently result in higher crop yields. Agricultural management practices can significantly impact soil health. Potato production can be particularly taxing on soil health due to intensive tillage, short crop rotations, and soilborne diseases. To better understand the impacts of different management strategies on soil health, potato cropping system treatments incorporating varying rotation lengths, rotation crops, green manures, compost amendments, and a soil fumigant were evaluated over four years from 2019 to 2022 with two potato varieties, 'Caribou Russet' and 'Russet Burbank'. Management impacts were analyzed using various soil health and production parameters, including soil properties, nematode populations, soilborne disease incidence, and potato yield data. While no differences among treatments were observed in the first year of potato planting, significant differences in soil health parameters were observed in the subsequent potato planting year. Compost amendment increased potato yields, whereas yield was negatively affected by rotation length. Management practices also impacted various soil properties, including compost amendments, which increased organic matter, soil respiration, soil organic carbon, zinc levels, and many microbial groups detected via phospholipid fatty acid (PLFA) assays. Rotation length was negatively correlated with organic matter, copper, phosphorus, and zinc, although this could be attributed to the small number of rotation cycles. Fumigation was not

positively correlated with yield nor a reduction in disease incidences compared to standard practices, and microbial stress was indicated. Biofumigation with green manures did not indicate any significant differences in yield or disease incidences. The use of non-grain rotation crops reduced the incidences of black scurf (caused by *Rhizoctonia solani*) compared to standard practice with grain rotation crops, but soil properties were not altered by their usage. Variety selection also did not impact any soil health metrics, but a significantly reduced number of *Pratylenchus penetrans* males were found to be associated with 'Caribou Russet'. While long-term effects were not explored in this study, these results may aid farmers in developing crop management plans to improve soil health, reduce disease, and increase yields.

2.1 Introduction to Potatoes and Soil Health

Potato (*Solanum tuberosum* L.) is one of the most important food crops around the world. In the United States, over 38.9 billion pounds of potatoes were produced in 2022 (USDA: NASS 2022a). Potato is the top agricultural commodity for the state of Maine, which consistently ranks within the top ten potato producing states (USDA: NASS 2022a; Rose 2003). Potatoes are highly susceptible to diseases caused and exacerbated by fungi, bacteria, nematodes, and viruses, about forty of which are caused by soilborne pathogens (Fiers et al. 2012; Hao and Ashley 2021). The soilborne potato pathogens that are most important in Maine include *Rhizoctonia solani* and *Verticillium dahliae*, fungal pathogens that are responsible for black scurf and potato early dying, respectively, and the bacterial pathogen *Streptomyces scabies,* the causal agent of common scab.

In addition to disease pressure, potato production can be very impactful to soils and the environment. Major concerns related to potato production include the potential for fertilizer runoff and the loss of soils to erosion, especially as a result of harvesting techniques and tillage (Auerswald et al. 2006; Westermann 2005b). Consequently, potato production in many states throughout the US has led to a decline in soil structure, loss of organic matter, lower biological activity, and ultimately reduced crop productivity (Ninh et al. 2015).

Many of these issues can be considered related to soil health either directly or indirectly. Soil health can be broadly described as how capable soil is of producing high quality crops and how sustainably it can serve this purpose (USDA NRCS 2023). Soil health is critical to the longevity of crop production, water quality, climate change mitigation, human health, and many other ecosystem services (Lehmann et al. 2020).

In general, soil health criteria can be divided into three categories of soil properties: physical, chemical, and biological (Lehmann et al. 2020). Physical parameters of soil health

include texture, topsoil depth, aggregate stability, porosity, soil bulk density, and water holding capacity (Karlen and Stott 1994). These physical criteria assist plants in physical support and the ease of roots to access oxygen, water, and nutrients, while at the same time storing and moving water, aiding in organic carbon accumulation, and resisting erosion (Doran and Parkin 1994; Allen et al. 2011). Chemical parameters play a role in the cycling of various elements, and encompass pH, cation exchange capacity, and macro and micronutrient content (Karlen and Stott 1994). Chemical soil properties are responsible for plant growth and development nutritionally, nutrient cycling and availability, and cation exchange capacity aids soil in immobilizing toxic metals or contaminants such as pesticides (Allen et al. 2011). Biological properties encompass such things as soil organic matter and the diversity and activity of macro- and microorganisms. Biological services provided by microorganisms include decomposition of residues and manures, nutrient cycling, and contributions to soil architecture (Turco et al. 1994). Additionally, soil microorganisms can contribute to pathogen suppression through competition for space and nutrients, production of toxic volatiles, utilizing pathogens as a nutrient source, and promotion of plant defense systems, among other techniques (Mehta et al. 2014; Schumann and D'Arcy 2010).

When planting a new potato field, there are many different management decisions to make. Management techniques are utilized to reduce disease pressure, increase yields, build or maintain soil health, or simply be based on grower preference. Versions of these management practices have been performed since the inception of potato production by Andean potato farmers and include soil preparation and nutrition, crop rotations, irrigation, pest control, and cultivar selection (Stark et al. 2020). Some management practices, such as adding organic amendments, have been shown to improve soil properties including water availability, soil carbon and nitrogen, and numerous micronutrients (Larkin et al. 2021a). Overall, management

techniques that can improve soil health and lead to more productive crops in potato production include lengthening crop rotations, using cover crops and green manures, reducing tillage as much as possible, and the use of organic amendments such as manures and composts (Stark and Porter 2005).

This study was established to gain perspective on the impacts of various management techniques on soil properties, potato yields, and disease incidences. As soil health is highly important for soil conservation, nutrition, and potato crop productivity, it is hypothesized that the treatments will impact yield as well as soil physical, biological, and chemical properties (Westermann 2005b; USDA NRCS 2023; Magdoff and Van Es 2021). Building on other Maine-based studies indicating management practices which improve soil health, it is hypothesized that compost amendment, longer rotation lengths, and the use of green manures, diverse rotation crops, and cover crops would result in enhanced soil properties (Larkin et al. 2021a, 2021b, 2010). It was also hypothesized that disease severity and incidence would be suppressed through the use of green manures, the addition of compost, and chemical fumigation by either directly suppressing soilborne pathogens or indirectly promoting disease suppressive beneficial microorganisms or inducing plant resistance (Mehta et al. 2014; Larkin and Lynch 2018; Larkin et al. 2017; MacGuidwin et al. 2012). Six different management factors were investigated: rotation length (2 or 3 years), variety of potato used (‘Caribou Russet’ and ‘Russet Burbank’), the usage of green manure crops in rotations, compost integration (once, twice, or not amended), fumigation usage, and rotations which included non-grain cash crops (broccoli, garlic, legumes, or corn).

In this study, the short-term impacts of adopting various management techniques relative to a standard practice in either two-year or three-year rotation lengths were analyzed. The goals

are to provide information which can aid farmers in increasing yields, reducing disease pressure, and enhancing soil health to provide sustainability and longevity into potato production.

2.2 Materials and Methods

2.2.1 Field design and plot layout

Over the course of four years from 2019-2022, two adjacent fields oriented from south-north were established in Presque Isle, Maine at the University of Maine Aroostook Research Farm (46.653704, -68.020309). The fields had been planted to oats for the last 25 years prior to this experiment. Field plots were 12.2 meters long and 5.5 meters wide to allow space for six rows of potatoes during potato production years. Each field contained six different cropping system treatments. The treatments were designed to incorporate different management practices grouped into management factors of rotation length, potato variety, rotation crop, green manure usage, compost integration, and soil fumigation. The 12 individual treatments are described in Table 2.1a and 2.1b. The northern field was configured with a rotation length of three years, and the southern field was configured with a two-year rotation length, and the treatment plots were arranged in a randomized complete block (RCB) design with five replicate blocks for each field. The two-year rotations were planted in potatoes in 2020 and 2022, and three-year rotations were planted in potatoes in 2019 and 2022. Two different potato varieties were used, ‘Russet Burbank’, which is the industry standard potato for processing and the predominant potato variety grown in commercial potato production in Maine, and ‘Caribou Russet’, a newer variety developed by the University of Maine breeding program that may provide some improved characteristics (earlier maturity, less defects, larger size) relative to Russet Burbank. Rotation crops which were included in standard practice treatments were barley underseeded with red

clover or ryegrass in two-year rotations and barley underseeded with red clover or ryegrass followed by canola with or without winter rye as a cover crop in three-year rotations. Non-grain crops which were investigated in this study were field peas 'Maxum', buckwheat, garlic 'German Extra Hardy', soybean, and corn 'X52519GR.0', see Tables 2.1a and 2.1b for details on planting schedule. Seeds were procured from Johnny's Selected Seeds, Deer Creek Seed, and Dyna-Gro (Winslow, Maine; Windsor, Wisconsin; Richmond, California). The green manure crops used were yellow mustard and rapeseed 'Dwarf Essex'. Compost was procured from Pineland Farms and applied at a rate of 2.13 Mg ha^{-1} through incorporation by tilling with a tractor in the spring in 2019 in treatment 3, spring of 2020 in treatment 9 and 11, and the fall of 2021 in treatments 3 and 9 (New Gloucester, Maine; Table 2.1a and 2.1b). The compost was composed of cattle manure, and plant and crop residues, including potato residues. Soil was fumigated with metam sodium at a rate of 50gal $acre^{-1}$ in the fall of 2019 and 2021 when weather conditions were appropriate and following safety protocol (AMVAC, Newport Beach, California).

All plots were managed by standard techniques for weed and insect management. As is common in Maine potato production, plots were not irrigated, and relied on rainfall for irrigation. Additionally, potato plots were fertilized at planting using a 14-14-14 (N-P-K) fertilizer applied at a rate of 1.23 Mg ha^{-1} produced by McCain Fertilizers (Presque Isle, Maine), which consisted of ammonium sulfate, diammonium phosphate, and potash, contributing N, Ca, S, P, K, and Cl to the soil. All plots were tilled, planted, prepared, and harvested using standard practices for northern Maine potato production.

2.2.2 Field soil sampling

Field soil was collected from each plot and bulked together by using 40 cores collected to a depth of approximately six inches using a one-inch soil probe. The soil was placed into individual Ziploc bags labeled with plot number and transported on ice to the lab where it was stored at 4°C and processed as quickly as feasible, but within one week maximum. The soil was homogenized by hand mixing. Depending on the tests required, soil was collected and assessed at the time of potato planting (typically May), mid-season (typically July), and a fall sampling in plots that would be in potato the following year (typically September). Exact dates of soil collection varied based on weather and soil conditions.

2.2.3 Soil fertility and pathogen tests

Each plot bulk soil sample was air-dried on a bench below 35°C before sending approximately 0.75 liters of soil for physiochemical analysis by Agvise Laboratories (Northwood, North Dakota). Soil moisture content was measured by weighing soil before and after air-drying. To measure the presence of nematodes and *Verticillium* spp. propagules, 750 ml of fresh homogenized soil was sent to Pest Pros (Allied Cooperative, Plainfield, Wisconsin). Approximately 50 grams of soil was air-dried, labeled, and stored at room temperature as archival soils retained in case additional fertility tests were needed.

To establish a baseline, soil texture was quantified at the beginning of the study. During the years when plots were planted with potatoes, all soil fertility metrics were measured in the spring including cation exchange capacity, pH, base saturation, soil organic matter, soil organic carbon, NO_3, NH_4, phosphorus, potassium, calcium, magnesium, sulfur, iron, manganese, zinc, boron, and sodium, as well as the biological indicators of microbial respiration as determined by Solvita CO_2 burst assay (Solvita), active C as permanganate-oxidizable C (POX-C), and

autoclaved citrate-extractable (ACE) soil protein. In addition, wet aggregate stability was estimated as an indicator of soil structure using a separate soil sample collected by cutting a 1 in by 6 in by 1 in slice from the side of a 6-inch-deep hole from each plot and sending intact to be processed by Agvise Laboratories (Benson, Minnesota). In the summer, the same chemical properties were measured, but not the biological indicators or aggregate stability. During the years when non-potato crops were planted, the full panel of measurements were taken only in the fall.

In July of the final year of the study when all plots were planted in potatoes, phospholipid fatty acid (PLFA) analyses were performed to measure soil biological activity. PLFA analyses rely on the detection of phospholipid biomarkers to characterized and broadly describe the structure of a microbial community and were performed by Ward Laboratories (Ward Laboratories, Kearney, Nebraska) (Willers et al. 2015). Established PLFA biomarkers were used to estimate total microbial biomass and relative quantities of microbial groups such as gram-positive and gram-negative bacteria, actinomycetes, fungi, and arbuscular mycorrhizal fungi (AMF).

2.2.4 Potato yield and disease evaluations

The entire length of the middle two rows of each plot planted to potato were harvested and used for the determination of potato yield and disease parameters. Harvested potatoes were separated by plot and washed. They were then weighed to determine total yield (weight of all tubers harvested) for each plot, with yield expressed as Mg/ha.

To determine the incidence and severity of black scurf caused by *Rhizoctonia solani* or common scab caused by *Streptomyces scabies*, 50 randomly selected tubers were visually inspected for the presence of sclerotia or scab, respectively, on the surface. The percent coverage

of the surface by sclerotia or scab was separately recorded to describe respective disease severity. Disease incidence was determined as the number of tubers showing the respective disease symptoms or signs out of the 50 tubers evaluated and expressed as a percentage. To determine the incidence and severity of *Verticillium dahliae*, 50 randomly selected tubers from each plot were dissected lengthwise parallel with the stem end to reveal the vascular tissue. The percentage of the entire vascular ring of tissue within the tuber which was discolored as a result of *V. dahliae* infection was recorded to represent disease severity. Similarly, the incidence was the proportion of the fifty tubers which expressed symptoms due to infection by *V. dahliae*. Incidence of hollow heart, an abiotic disorder that represents an internal tuber defect, and which typically follows rapid tuber growth, and is related to high nitrogen or irregular watering (McCann and Stark 1989), was also assessed on the same 50 tubers cut for vascular discoloration detection.

2.2.5 Statistical Analyses

A baseline of soil properties was established by comparing soil metrics at the initial sampling timepoints (Table 2.2). However, compost amendment was performed prior to sampling, and found to significantly impact many soil health metrics (potassium, salts, sodium, sulfur, zinc, total organic carbon, total carbon, organic matter, and POXC). Fumigation was also performed before initial soil sampling occurred, and differences with respect to cation exchange capacity and percent of macroaggregates were shown to be different. Additionally, due to the differences in field preparations between the two different rotation lengths, as the result of planting either potatoes or rotation crops, the initial soil sampling results of the two fields were evaluated separately. To establish a preliminary analysis based on these challenges, compost and fumigation treatments were removed and the two rotation lengths were evaluated separately. To

determine if there were any initial differences in soil physical, biological and chemical metrics, as well as existing levels of root lesion nematodes and propagules of *V. dahliae,* standard analysis of variance (ANOVA) for RCB design was conducted in R using the package Agricolae version 1.3-4 (de Mendiburu 2020). All assumptions, including normality and equal variances were verified, or corrected for in R before performing these ANOVAs and all subsequent tests. After these adjustments, there were no significant differences among plots for any measured soil properties at the initiation of the study, except for pH in the 3-year rotation plots, which could have been the result of historically uneven liming ($p = 0.0145$). Averages of baseline soil health metrics separated by rotation length are described in in Table 2.2.

To determine the effect of management on yield, disease incidence and severity, and soil physical and chemical metrics in the final sampling timepoint of the final year of the study, standard analysis of variance (ANOVA) for RCB design was conducted and means separated using Fisher's protected LSD tests performed in R using the package Agricolae version 1.3-4 (de Mendiburu 2020). Linear regressions were performed using base R to determine significant relationships between soil health metrics and total yields. The results of PLFA analyses, nematode assays, and propagules of *V. dahliae* were also analyzed and compared statistically. The final soil sampling timepoint for sampling was approximately 60 days after planting, and all treatments were planted in potatoes. This sampling timepoint was selected as it allowed the maximum length since the project's initiation and was used for nearly all physical, chemical, and biological soil health metrics, except in the case of those metrics which were only collected at planting (percent organic matter, total organic carbon, and total carbon).

Soil properties that had both a significant relationship with yield and were significantly different by the treatments were further investigated through the use of correlation analyses

which provided correlation coefficients and were visualized using the R package corrplot, FactoMineR, and factoextra (Husson et al. 2016; Kassambara and Mundt 2017; Wei et al. 2017). To visualize these relationships and identify the factors which were the most important with respect to yield, principal components analyses (PCA) were performed.

Many of the management factors overlapped within the treatment layout. After the initial preliminary analysis, because of the risk of overfitting the dataset and losing resolution when attempting to analyze the impacts of individual management factors on soil properties, disease, and yield, the dataset was subset before performing further statistical analysis. These subsets compared treatments with the six factors of interest compared to standard practice treatments of the same variety and ideally the same rotation length in a balanced design to improve resolution of the impact of these treatments. This aided in reducing noise within the dataset and highlighted specific management factors and their impacts to potato yields and disease, and soil health metrics.

Correlations of significant soil properties with management factors, yield, and disease incidences and severities were also evaluated in R using the package Corrplot version 0.92, factoextra version 1.0.7, and FactoMineR version 2.8 (Wei et al. 2017; Kassambara and Mundt 2017; Lê et al. 2008; Husson et al. 2016). To corroborate the ANOVA results, and due to the complexity of the large dataset, a series of multivariate analyses of variance (MANOVA) were performed on the full dataset using base R (R Core Team 2018).

2.3 Results

2.3.1 Potato yield responses to short-term management practices

In the initial two years of this study there were no significant differences between total or marketable yields of potatoes by the treatments (Table 2.3). This was the first time in 25 years that potatoes had been grown in both fields. Treatment effects were marginal in the initial potato years for both the two-year and three-year rotations, due to a lack of full implementation of the rotations and management factors at this early stage.

In the initial potato crops, yields were higher overall in treatments which were in three-year rotations and produced a first potato crop in 2019 compared to treatments in two-year rotations which produced a first potato crop in 2020. This is likely due to environmental differences, as 2020 was much warmer and drier than 2019. This could especially be seen from May to September, a period critical in Maine potato production, as in 2019, temperatures only exceeded 90°F in July and cumulative rainfall was 15.97 inches. Comparatively, in 2020 from May to September, maximum temperatures exceeded 90°F every month and cumulative rainfall totaled only 9.69 inches (NOAA National Weather Service n.d.).

However, treatment effects on yields were evident in the final (fourth) year of the study, as indicated in the total and marketable yields observed for both the two-year and three-year fields in 2022 (Table 2.3). Across all treatments, highest yields were seen in the two-year rotation amended with compost and in the three-year rotation with compost (Table 2.3). Within marketable yields, which included tubers larger than 1-7/8 inches in diameter per USDA size classes, the highest yields were in the two-year rotation with compost amendment, and in the three-year rotation with compost amendment (Table 2.3).

When data was subset, some trends were seen within the various management factors. Within the two rotation lengths, two-year rotations had significantly higher yields than three-year rotations when compared within standard treatments only. Within the two varieties tested, yield was not significantly different between varieties. The same was true for the use of non-grain rotation crops, fumigation, and green manure usage as yields were not significantly different when subset and compared to standard practices. Yield was not correlated with either variety nor green manures when subset and compared to standard treatments.

Of the compost amended plots, yields were significantly higher with compost compared to unamended plots or when compared to all treatments. Compost amendment was also significantly positively correlated with total yield in the final year of the study and was the most positively correlated with total yield compared to all other management factors. While yields were only significantly different within compost amended plots and with two-year rotations over three-year rotations, general trends of management factors compared to standard practice on yields described as z-scores can be seen in Figure 2.3.

2.3.2 Tuber disease response to management practices

In the initial potato growing seasons, no significant differences were seen in soilborne tuber disease incidences nor severities, as represented by black scurf, common scab, and vascular discoloration from potato early dying (PED; Table 2.3). However, soilborne disease varied among the treatments in the final year of the study, as indicated by the incidence and severity data from the 2022 field season (Table 2.3). Over all twelve treatments in the full dataset, significant differences were seen in the incidences and severities of black scurf and PED, with standard practices with ‘Caribou Russet’ in two-year rotations and standard practices with ‘Russet Burbank’ having the highest incidences of black scurf and fumigation and standard

practice with ‘Caribou Russet’ treatments having the highest incidences of PED in two-year and three-year rotations, respectively (Table 2.3). The non-grain rotation crops in three-year rotations (with soybeans and corn) and Midwest standard practice had the highest severities of both diseases in three-year rotations, and in two-year rotations, the disease suppressive treatment with green manure and standard practice with ‘Caribou Russet’ had the highest severities of black scurf and PED, respectively (Table 2.3). Significant differences were not observed between treatments for common scab incidence or severity in the final year of the study. Similarly, incidences of hollow heart, a physiological disorder, were not significantly different based on the treatments.

Unsurprisingly, when subset there were once again differences based on various individual management factors. Rotation lengths were not significantly different with respect to disease incidences in the subset dataset, however, rotation length was negatively correlated with PED incidence and positively correlated with hollow heart incidence when compared to all other treatments.

Of the two potato varieties evaluated, there were significantly more total *Pratylenchus* sp. males detected in plots planted with ‘Russet Burbank’ compared to ‘Caribou Russet’. However, this did not increase the incidence or severity of disease symptoms caused by *Verticillium dahliae*, and fungal propagules were also not significantly different. For the *Pratylenchus* sp. male populations and propagules of *Verticillium* spp., only fumigation resulted in significantly lower amounts, as no other factors were significantly different.

No disease symptoms were correlated with either variety when compared to all treatments or when subset and compared within standard treatments only. Fumigation or the use of green manures in treatments were not significantly different from standard treatments with

respect to disease incidences. Green manure usage was not correlated with any disease metric, but fumigation was significantly negatively correlated with PED and common scab incidences when compared to all treatments, but significantly positively correlated to PED incidence when compared to the two-year standard practice only. Hollow heart incidence and scab severity was significantly positively correlated to compost amended plots when subset and only compared to standard treatments. However, compost amendment was negatively correlated with PED incidence when compared to all other treatments. The use of non-grain rotation crops resulted in lower black scurf incidences than in standard grain rotations. Consequently, the use of non-grain rotation crops was negatively corelated with black scurf incidence when compared to only standard treatments or all other treatments.

2.3.3 Soil physiochemical metrics preliminary analysis

As a preliminary investigation to determine if the treatments had specific impacts to soil health metrics, a panel of 32 different physical, chemical, and biological soil health metrics collected in the final year of the study in which all plots were planted in potatoes were initially analyzed (Tables 2.4a and 2.4b). Three metrics were not significantly different initially or in the final sampling point and are not displayed in Table 2.4 (boron (ppm), salts (mmhos/cm), and sodium (ppm); see averages of these metrics in Table 2.2). Of those, 15 were found to be significantly different by the treatments, however, consistent patterns were not observed. To further determine which individual management factors had the largest effects on soil health metrics, the dataset was subset into a series of balanced comparisons to isolate the six management factors (rotation length, variety, green manure usage, compost amendment, fumigation, and the use of non-grain rotation crops) and compare them to or within standard practice treatments.

2.3.4 Soil property responses to management practices

Overall, soil physical, chemical, and biological properties were affected to various degrees by the management practice treatments, as indicated in the results from the summer soil sampling from the 2022 field season (Tables 2.4 and 2.5; Supplemental Table 2.1). In general, there were no consistent patterns within soil chemical properties, however rotation length was responsible for the largest number of significantly different metrics. Soil pH was significantly higher in in three-year rotations compared to two-year rotations, regardless of if the data was subset or all treatments were analyzed together ($p < 0.001$). Cation exchange capacity was also significantly higher in in three-year rotations compared to two-year rotations, however the disease suppressive treatments with green manure and fumigation within the two-year rotations did not have significantly different cation exchange capacities from treatments in the three-year rotations ($p = 0.029$). Alternatively, copper and manganese were both significantly higher in two-year rotations compared to three-year rotations ($p = 0.001$ and 0.003, respectively). Olsen phosphorus was also significantly higher in two-year rotations compared to three-year rotations, with the exception of the compost amended treatment in the three-year rotations, which was not significantly different from the two-year rotation treatments ($p = 0.04$). Of all treatments, those amended with compost or fumigated resulted in the highest organic matter, total carbon, and total organic carbon ($p < 0.001$, $p = 0.018$, and 0.009, respectively). Compost amended and fumigated treatments also resulted in the highest soil respiration with the exception of the treatment which was only compost amended once ($p = 0.006$; Table 2.4). Treatments amended with compost twice had significantly higher zinc compared to all other treatments ($p < 0.001$).

Of the PLFA data, the treatment within three-year rotations which was amended with compost consistently had the highest biomasses of fungi, bacteria, actinomycetes, arbuscular

mycorrhizae, saprophytes, and microbial biomass compared to all other treatments (Table 2.5). Interestingly, the same trend was not seen in compost amended treatments in two-year rotations, except in the case of the percent of the total composed of gram positive bacteria. This could be the result of significantly higher precents of undifferentiated microbes, which were observed in all two-year rotation treatments compared to those in three-year rotations. When comparing compost amended treatments to standard practice treatments only, ratios of saturated to unsaturated fatty acids were higher in plots which were amended one time or not at all, and lower in twice amended plots. Ratios of monounsaturated to polyunsaturated fatty acids were highest in once amended plots, and not significantly different between twice amended and unamended plots.

In plots which were fumigated, the only PLFA metric which was significant was that unfumigated plots compared to standard practices had significantly lower ratios of saturated to unsaturated fatty acids, originating in the membranes of bacteria. With respect to PLFA, two-year rotations had significantly higher protozoan percent of the total, protozoan biomass, and higher ratios of saturated to unsaturated fatty acids.

When linear regressions were constructed to explore the relationships of soil health properties to total yields in all treatments in the final year of the study, significant relationships ($p < 0.001$) of soil properties to yield were zinc ($R^2 = 2.14 \pm 0.43$), copper ($R^2 = 0.38 \pm 0.08$), Olsen phosphorus ($R^2 = 0.04 \pm 0.01$), ACE protein ($R^2 = 0.37 \pm 0.1$), percent total organic carbon ($R^2 = 1.91 \pm 0.43$), and organic matter ($R^2 = 0.95 \pm 0.20$). While treatments resulted in significantly different metrics within the PLFA data, none of these data had significant relationships to yield according to linear regressions.

2.3.5 Correlation relationships of different management factors to yields

The most important factors which were both significantly different in the final year by the treatments and had significant relationships to yield were zinc, copper, Olsen phosphorus, percent total organic carbon, and organic matter (Tables 2.6 and 2.7). Within the treatments, specific management factors were significantly correlated with these soil properties. Of these factors, zinc and total organic carbon were significantly positively correlated with compost amendment (0.84 and 0.64, respectively). Organic matter was positively correlated with compost amendment (0.42) and negatively correlated with rotation length (-0.59). Copper and Olsen phosphorus were significantly negatively correlated with rotation length (-0.56 and 0.59, respectively). Correlation analysis results can be found in Figure 2.1.

When subset and compared to standard practice treatments, treatments which had mustard green manures or were planted with non-grain rotation crops were not significantly correlated with any physiochemical factor in the final sampling timepoint. Similarly, of the two varieties used, subset standard practice treatments planted with ‘Russet Burbank’ and ‘Caribou Russet’ were not significantly correlated to any soil properties. The fumigated treatment was significantly positively correlated with pH, calcium, Solvita, and CEC compared to standard practice, although these plots initially were higher in calcium and cation exchange capacity.

A PCA was constructed using correlation coefficients to indicate the amount of variance that is explained by the five soil properties which were significantly different both by the treatments and which had significant relationships with yield, total yield as a z-score, and the two management factors which were resulted in significantly different yields in 2022 (compost amendment and rotation length). This more clearly indicated that compost was highly positively correlated with zinc, and somewhat positively related to percent total organic carbon, organic

matter, Olsen phosphorus, and copper. Rotation length was highly negatively correlated with organic matter, percent total organic carbon, Olsen phosphorus, and copper (Figure 2.2).

Table 2.1a Three-year rotation treatments carried out over four years from 2019 to 2022 at the Aroostook Research Farm in a study of management impacts to soil health in potato producing fields in Maine. All three-year rotations had potatoes planted in 2019 and 2022, and rotations planted in 2020 and 2021.

Rotation Length	Treatment	Potato Variety	Rotation Sequence[1]	Goal	Management factors[2]
3 years	1	Russet Burbank	Year1: Potatoes Year 2: Barley / Red Clover Year 3: Canola Year 4: Potatoes	Standard practice	
	2	Caribou Russet	Year1: Potatoes Year 2: Barley / Ryegrass Year 3: Canola – Winter Rye Year 4: Potatoes	Standard practice	
	3	Russet Burbank	Year1: Potatoes Year 2: Barley / Ryegrass Year 3: Canola – Winter Rye (Compost) Year 4: Potatoes	Improved rotation with compost preceding potato	Compost
	4	Caribou Russet	Year1: Potatoes Year 2: Peas / Buckwheat Year 3: Mustard Green Manure – Rapeseed Year 4: Potatoes	Disease suppression	Green manure, non-grain rotation crops
	5	Russet Burbank	Year1: Potatoes Year 2: Soybean Year 3: Corn Year 4: Potatoes	Midwest Standard	Non-grain rotation crops
	6	Caribou Russet	Year1: Potatoes – Oats / Clover Year 2: Late Mustard – Buckwheat - Garlic Year 3: Sudangrass Year 4: Potatoes	Intensive potato, vegetable, cover crop rotation	Non-grain rotation crops

[1] / indicates a crop was underseeded; – indicates a crop followed the previous
[2] Not including rotation length and variety selection

Table 2.1b Two-year rotation treatments carried out over four years from 2019 to 2022 at the Aroostook Research Farm in a study of management impacts to soil health in potato producing fields in Maine. All two-year rotations had potatoes planted in 2020 and 2022, and rotations planted in 2019 and 2021.

Rotation Length	Treatment	Potato Variety	Rotation Sequence[1]	Goal	Management factors[2]
2 years	7	Russet Burbank	Year 1: Barley / Red Clover Year 2: Potatoes Year 3: Barley / Red Clover Year 4: Potatoes	Standard practice	
	8	Caribou Russet	Year 1: Barley / Ryegrass Year 2: Potatoes Year 3: Barley / Ryegrass Year 4: Potatoes	Standard practice	
	9	Russet Burbank	Year 1: Barley / Ryegrass (Compost) Year 2: Potatoes Year 3: Canola / Winter Rye (Compost Year 4: Potatoes	Improved rotation with compost preceding potato	Compost
	10	Caribou Russet	Year 1: Barley / Ryegrass Year 2: Potatoes – Winter Rye Year 3: Mustard Green Manure – Rapeseed Year 4: Potatoes	Disease suppression	Green manure
	11	Caribou Russet	Year 1: Barley / Ryegrass (Compost) Year 2: Potatoes – Winter Rye Year 3: Peas – Buckwheat Year 4: Potatoes – Winter rye	Rotation with pulse and cover crops with compost preceding every other potato planting	Compost, non-grain rotation crops
	12	Russet Burbank	Year 1: Barley / Red Clover (Fumigation) Year 2: Potatoes Year 3: Barley / Red Clover (Fumigation) Year 4: Potatoes	Standard with fumigation	Fumigation

[1] / indicates a crop was underseeded; – indicates a crop followed the previous
[2] Not including rotation length and variety selection

Table 2.2 Average soil health metrics measured from soil samples collected in the initial sampling timepoint in the first year of the study in 2019 (spring at planting for 3-year rotations and fall at harvest in 2-year rotations) in a study of management impacts to soil health in potato producing fields in Maine.

Chemical Soil Metrics													
Initial Year Sampling Timepoint Averages: Spring Potatoes for 3-year and Fall Rotations for 2-year													
Rotation Length	ACE Protein (mg/gm)	Ammonium (ppm)	Boron (ppm)	Calcium (ppm)	Cation Exchange Capacity (meg/100g)	Copper (ppm)	Iron (ppm)	Magnesium (ppm)	Manganese (ppm)	Nitrate-N (ppm)	Olsen Phosphorus (ppm)	pH	Potassium (ppm)
3-year	8.34	15.78	0.26	596.2	8.67	5.81	76.67	140.63	8.46	54.7	62.03	5.21	194.4
2-year	8.2	3.88	0.27	773.8	9.27	6.99	70.47	114	3.39	8.07	49.4	6.13	104.6

	Chemical Soil Metrics (Continued)				**Biological Soil Metrics**					**Physical Soil Metrics**			**Pathogen Testing**	
	Initial Year Sampling Timepoint: Spring Potatoes for 3-year and Fall Rotations for 2-year				**Initial Year Sampling Timepoint: Spring Potatoes for 3-year and Fall Rotations for 2-year**					**Year 1 Summer 2019 (2-year in rotations; 3-year in potatoes)**			**Study Year One (2-year Fall in Rotations; 3-year Spring in potatoes)**	
Rotation Length	Salts (mmhos/ cm)	Sodium (ppm)	Sulfur (ppm)	Zinc (ppm)	POXC (ppm)	Solvita (ppm)	Organic Matter (Percent)	Total Carbon (Percent)	Total Organic Carbon (Percent)	Large Macro-aggregate Percent	Macro-aggregate Percent	Micro-aggregate Percent	Root Lesion Nematodes (per 100cc of soil)	Verticillium Propagules per gram of soil
3-year	0.37	23.47	5.63	0.62	444.83	152.74	4.18	1.81	1.77	18.76	42.41	24.19	369.93	0
2-year	0.09	15.3	4.43	0.31	438.33	234.76	4.03	1.74	1.72	15.86	31.78	36.92	191.6	0.07

Table 2.3 Yields and disease incidence (inc.) and severity (sev.) from the first and second years of potato production in a study of management impacts to soil health in potato producing fields in Maine. The first year of potato production was 2019 for three-year rotations and 2020 for two-year rotations. All plots were planted in potatoes in 2022. Potato early dying (PED) severity was not collected in 2019 or 2020. ANOVA p-values are listed below each category, either separated by rotation length in the first year of production to account for the crop being harvested in different years or together for the second year.

Treatment	First Year of Potato Production							
	Total Yield (Mg/ha)	Marketable Yield (Mg/ha)	Common Scab Inc.	Black Scurf Inc.	PED Inc.	Hollow Heart Inc.	Common Scab Sev.	Black Scurf Sev.
1[bc]	21.43	15.82	17.60	27.20	0.00	0.00	3.55	16.53
2[bd]	26.70	23.13	16.94	25.20	0.80	0.00	3.44	16.15
3[bce]	25.18	20.13	16.59	40.45	0.41	0.00	3.47	15.55
4[bdg]	29.41	25.05	14.40	31.20	0.00	0.00	3.42	9.85
5[bcf]	21.23	16.10	21.63	22.00	0.00	0.00	3.54	11.94
6[bdf]	26.07	22.36	15.24	22.87	0.00	0.00	2.57	13.89
p =	0.599	0.44	0.998	0.603	0.362	1	0.071	0.421
7[ac]	16.76	11.19	8.80	30.80	41.60	0.00	9.45	10.86
8[ad]	13.87	11.71	14.00	21.60	14.80	0.00	8.77	8.42
9[ace]	19.75	14.57	18.80	18.40	42.00	0.00	10.27	6.87
10[adg]	14.00	12.43	12.80	24.40	23.60	0.00	7.99	8.53
11[adef]	18.68	16.40	8.80	12.40	30.80	0.00	7.42	4.43
12[ach]	16.63	11.28	15.35	33.20	45.20	0.00	15.13	14.54
p =	0.386	0.203	0.757	0.863	0.416	1	0.257	0.636

[a] Two-year rotation
[b] Three-year rotation
[c] Russet Burbank
[d] Caribou Russet
[e] Compost
[f] Non-grain Rotation Crops
[g] Green manure
[h] Fumigation

Table 2.3 (continued) Yields and disease incidence (inc.) and severity (sev.) from the first and second years of potato production in a study of management impacts to soil health in potato producing fields in Maine. The first year of potato production was 2019 for three-year rotations and 2020 for two-year rotations. All plots were planted in potatoes in 2022. Potato early dying (PED) severity was not collected in 2019 or 2020. ANOVA p-values are listed below each category, either separated by rotation length in the first year of production to account for the crop being harvested in different years or together for the second year. Bold values indicate significant differences.

Treatment	Second Year of Potato Production								
	Total Yield (Mg/ha)	Marketable Yield (Mg/ha)	Common Scab Inc.	Black Scurf Inc.	PED Inc.	Hollow Heart Inc.	Common Scab Sev.	Black Scurf Sev.	PED Sev.
1[bc]	23.69 ef	19.5 bcd	14.8	9.2 bc	40.4 bc	0.80	4.97	5.4 bcd	5.72 cd
2[bd]	26.5 cdef	12.42 f	22	2 d	48.4 ab	2.00	6.89	6.00 bc	5.31 d
3[bce]	30.23 cd	25.34 a	25.6	2.8 d	40.8 bc	6.00	8.90	4.13 bcd	7.33 abc
4[bdg]	29.02 cde	12.58 f	42	0.4 d	40.8 bc	1.60	6.97	1 cd	6.01 bcd
5[bcf]	20.54 f	16.2 def	20.8	4.8 cd	39.2 bc	4.00	12.59	8.17 ab	8.03 a
6[bdf]	26.13 def	13.33 ef	34.4	0.8 d	41.6 bc	3.20	8.20	1 cd	5.36 d
7[ac]	32.6 bc	13.6 def	26	0 d	53.2 ab	1.60	6.72	0 d	5.42 d
8[ad]	28.11 cde	21.92 abc	15.6	15.2 a	51.01 ab	0.40	7.09	8.65 ab	8.38 a
9[ace]	42.13 a	17.99 cde	36	2.8 d	33.6 c	1.60	11.40	4.4 bcd	5.8 cd
10[adg]	29.38 cde	22.33 abc	27.2	13.6 ab	48.8 ab	0.40	7.11	12.14 a	7.58 ab
11[adef]	29.18 cde	23.62 ab	24.4	2 d	47.6 bc	0.80	6.49	3.33 bcd	6.94 abcd
12[ach]	36.76 ab	16.16 def	48.8	0 d	62.4 a	0.80	7.44	0 d	5.94 bcd
p =	**< 0.001**	**< 0.001**	0.434	**< 0.001**	**0.026**	0.087	0.229	**< 0.001**	**0.001**

[a] Two-year rotation
[b] Three-year rotation
[c] Russet Burbank
[d] Caribou Russet
[e] Compost
[f] Non-grain Rotation Crops
[g] Green manure
[h] Fumigation

Table 2.4 Soil health chemical metrics measured from soil samples collected in the final sampling timepoint in the last year of the study in 2022 from soil sampled collected in the summer, approximately 60 days after planting in a study of management impacts to soil health in potato producing fields in Maine. All plots were planted in potatoes. Bold values indicate significant differences.

Treat-ment	**Chemical Soil Metrics**												
	Final Year 60 Days after Planting: All potatoes												
	Ammonium (ppm)	Calcium (ppm)	Cation Exchange Capacity (meg/100g)	Copper (ppm)	Iron (ppm)	Magnesium (ppm)	Manganese (ppm)	Nitrate-N (ppm)	Olsen Phosphorus (ppm)	pH	Potassium (ppm)	Sulfur (ppm)	Zinc (ppm)
1[bc]	8.44	1028	7.12 abc	5.04 c	48.12	191.2	4.37 c	45.2	44.6 bc	6.02 ab	129.2	9.8	0.23 e
2[bd]	6.5	963.6	6.64 abcd	5.33 bc	47.3	171	4.16 c	22.3	43.6 c	6.16 ab	130.4	7.8	0.23 e
3[bce]	6.1	1193.4	7.94 a	5.13 c	49.14	179.8	4.71 bc	33.2	52.6 abc	6.28 a	151.6	11.8	0.75 b
4[bdg]	7.08	1106.4	7.46 ab	5.24 c	52.36	187	4.55 c	29.8	45.2 bc	6.08 ab	121.6	9.2	0.26 de
5[bcf]	8.44	1088.6	7.5 ab	5.09 c	39.92	195.2	4.47 c	33.3	48.6 bc	6.1 ab	140.4	9.4	0.2 e
6[bdf]	5.76	984.6	6.82 abcd	4.97 c	57.94	178.4	4.77 bc	29.7	45.6 bc	5.96 bc	137.2	9	0.25 de
7[ac]	8.78	756.8	5.42 d	6.68 ab	64.7	146	6.41 abc	44.8	56.4 ab	5.4 d	130.2	9.8	0.32 de
8[ad]	10.38	807.2	5.64 cd	6.32 abc	56.18	142.4	7.68 a	46.6	54.8 abc	5.48 d	134.6	12.8	0.31 de
9[ace]	5.58	904	6.14 bcd	7.15 a	52.22	148.2	7.44 ab	33.1	61.4 a	5.66 cd	135	10.2	0.94 a
10[adg]	7.76	970.8	6.62 abcd	7.28 a	76.42	163	8.69 a	51.7	53 abc	5.4 d	139	19.8	0.41 cd
11[adef]	7.24	781.4	5.38 d	7.10 a	61.18	131.8	9.22 a	46.2	62.2 a	5.44 d	127	13.2	0.50 c
12[ach]	5.34	1021.8	6.98 abcd	6.23 abc	53.52	168.2	6.45 abc	32.7	55.2 abc	5.96 bc	153.4	10.8	0.29 de
p =	0.682	0.051	**0.029**	**0.001**	0.448	0.057	**0.002**	0.446	**0.040**	**< 0.001**	0.867	0.266	**< 0.001**

[a] Two-year rotation
[b] Three-year rotation
[c] Russet Burbank
[d] Caribou Russet
[e] Compost
[f] Non-grain Rotation Crops
[g] Green manure
[h] Fumigation

Table 2.4 (continued) Soil health physical, pathogen, and biological metrics measured from soil collected in the final sampling timepoint in the last year of the study in 2022 from soil collected in the summer, approximately 60 days after planting in a study of management impacts to soil health in potato producing fields in Maine. Soil biological metrics were measured at planting only and included for thoroughness. All plots were planted in potatoes. Bold values indicate significant differences.

Treat-ment	**Physical Soil Metrics**			**Pathogen Testing**		**Biological Soil Metrics**					
	Final Year 60 Days after Planting: All potatoes								**At Planting: All Potatoes**		
	Large Macro-aggregate Percent	Macro-aggregate Percent	Micro-aggregate Percent	Root Lesion Nematodes (per 100cc of soil)	Verticillium Propagules per gram of soil	POXC (ppm)	Solvita (ppm)	ACE Protein (mg/gm)	Organic Matter (Percent)	Total Carbon (Percent)	Total Organic Carbon (Percent)
1[bc]	14.124	32.792	11.792	74.2	0	307.2	91.96 abcd	6.52	3.48 d	1.46 cd	1.42 cd
2[bd]	14.928	32.208	11.644	15.8	0	335	86.56 bcd	6.24	3.42 d	1.44 cd	1.4 cd
3[bce]	14.032	30.728	11.212	6	0	365.6	106.62 a	7.6	4.2 abc	1.86 a	1.84 a
4[bdg]	16.068	33.408	10.772	33	0	333	86.92 bcd	6.54	3.5 d	1.36 d	1.36 d
5[bcf]	14.628	37.48	11.056	83.2	0.4	354.4	104.56 ab	6.9	3.74 cd	1.52 cd	1.52 bcd
6[bdf]	14.48	33.444	11.044	32.4	0	289.4	84.4 cd	6.76	3.52 d	1.44 cd	1.4 cd
7[ac]	16.368	35.104	11.432	96.8	0	314	75.38 d	7.14	4.24 abc	1.54 cd	1.52 bcd
8[ad]	16.064	34.452	10.56	34	0	332.8	79.36 d	6.76	3.9 bcd	1.48 cd	1.48 bcd
9[ace]	15.716	34.74	11.436	21.2	0.4	337.4	98.78 abc	7.7	4.5 a	1.84 ab	1.84 a
10[adg]	15.98	35.24	11.252	83.6	0	314	78.54 d	7.82	4.18 abc	1.56 bcd	1.56 abcd
11[adef]	14.604	42.352	11.204	22.6	0.4	283.6	80.32 d	7.3	4.26 abc	1.66 abc	1.66 abc
12[ach]	15.216	38.64	10.928	15.2	0	396.8	100.74 abc	7.9	4.38 ab	1.72 abc	1.72 ab
p =	0.912	0.436	0.989	0.068	0.622	0.727	**0.006**	0.371	**< 0.001**	**0.018**	**0.008**

[a] Two-year rotation
[b] Three-year rotation
[c] Russet Burbank
[d] Caribou Russet
[e] Compost
[f] Non-grain Rotation Crops
[g] Green manure
[h] Fumigation

Table 2.5 Results of Phospholipid Fatty Acid Analysis (PLFA) biological metrics measured from soil sampled in the summer, 60 days after planting in the final year of the study of of management impacts to soil health in potato producing fields in Maine. All plots were planted in potatoes. Bold values indicate significant differences.

Treatment	Total Biomass (ng/g)	Total Bacteria	Total Fungi	Diversity Index	Fungi to Bacteria	Mono to Poly	Sat to Unsat	Predator to Prey
		Percent			Ratio			
1[bc]	3844.61 bcd	37.95 b	7.88 ab	1.39 abc	0.21 ab	18.65	2.4 de	0.003 bc
2[bd]	3987.44 b	37.63 b	6.64 bc	1.36 abcd	0.18 bc	43.33	2.54 cd	0 c
3[bce]	5511.41 a	42.36 a	10.53 a	1.45 a	0.25 a	10.32	2 e	0.002 bc
4[bdg]	3760.17 bcd	37.42 b	6.28 bcd	1.35 abcd	0.17 bcd	46.52	2.6 cd	0 c
5[bcf]	3882.13 bc	37.47 b	7.63 b	1.4 ab	0.2 ab	62.10	2.47 cd	0.004 abc
6[bdf]	3794.36 bcd	37.97 b	7.02 bc	1.37 abcd	0.18 abc	23.93	2.45 d	0 c
7[ac]	3388.43 cd	32.39 c	1.85 e	0.96 g	0.05 e	21.60	3.58 a	0.007 ab
8[ad]	3380.97 cd	32.76 c	2.57 e	1.02 efg	0.07 e	15.32	3.42 a	0.01 a
9[ace]	4104.5 b	35.58 bc	3.87 de	1.19 cdef	0.11 de	33.28	2.9 bc	0.005 abc
10[adg]	3616.85 bcd	32.98 c	2.26 e	0.99 fg	0.06 e	19.42	3.43 a	0.008 ab
11[adef]	3343.51 d	34.61 bc	3.5 e	1.17 def	0.1 de	95.17	3.23 ab	0.004 abc
12[ach]	3662.72 bcd	34.84 bc	4.52 cde	1.21 bcde	0.12 cde	14.09	2.76 cd	0.004 abc
p =	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	0.12	**< 0.001**	**0.02**

[a] Two-year rotation
[b] Three-year rotation
[c] Russet Burbank
[d] Caribou Russet
[e] Compost
[f] Non-grain Rotation Crops
[g] Green manure
[h] Fumigation

Table 2.5 (continued) Results of Phospholipid Fatty Acid Analysis (PLFA) biological metrics measured from soil sampled in the summer, 60 days after planting in the final year of the study of management impacts to soil health in potato producing fields in Maine. All plots were planted in potatoes. Bold values indicate significant differences.

Treatment	Percent of Total						
	Gram Positive	Gram Negative	Actinomycete	Arbuscular Mycorrhizae	Saprophytes	Protozoa	Undifferentiated Microorganisms
1[bc]	28.64 bc	9.3 b	8.44	1.64 b	6.24 ab	0.23	54.07 d
2[bd]	28.51 bc	9.12 b	8.34	1.57 b	5.08 bc	0.32	55.73 cd
3[bce]	29.45 ab	12.91 a	8.13	3.17 a	7.37 a	0.19	47 e
4[bdg]	28.79 bc	8.63 bc	8.38	1.52 b	4.77 bcd	0.26	56.3 bcd
5[bcf]	28.31 c	9.17 b	8.32	1.77 b	5.86 ab	0.14	54.74 cd
6[bdf]	28.58 bc	9.39 b	8.38	1.67 b	5.36 abc	0.13	55.01 cd
7[ac]	30.3 a	2.08 e	8.29	0.38 d	1.47 e	0.11	65.53 a
8[ad]	29.84 a	2.92 e	8.08	0.53 d	2.04 e	0	64.36 a
9[ace]	30.36 a	5.22 cde	7.95	1.19 bc	2.68 de	0.11	60.37 abc
10[adg]	30.48 a	2.5 e	8.35	0.44 d	1.79 e	0	64.53 a
11[adef]	30.34 a	4.27 de	8.57	0.77 cd	2.73 de	0.16	61.76 ab
12[ach]	28.31 c	6.53 bcd	7.89	1.25 bc	3.27 cde	0	60.51 abc
p =	**< 0.001**	**< 0.001**	0.47	**< 0.001**	**< 0.001**	0.059	**< 0.001**

[a] Two-year rotation
[b] Three-year rotation
[c] Russet Burbank
[d] Caribou Russet
[e] Compost
[f] Non-grain Rotation Crops
[g] Green manure
[h] Fumigation

Table 2.6 Linear regression results indicating the significance of relationships of total yield of potatoes and soil properties in a study of management impacts to soil health in potato producing fields in Maine. These tests were performed iteratively on the entire dataset using data from soil collected in the summer, approximately 60 days after planting. Italicized metrics were collected in the spring at planting only and included in the analysis for thoroughness. All plots were planted in potatoes. Bold values indicate significant differences.

Soil property metric	*p*	Soil nutrient metric	*p*	Soil biology metric	*p*
pH	0.083	Potassium	0.064	*Organic matter*	**< 0.001**
Microaggregates	0.288	Calcium	0.228	Pratylenchus	0.274
Macroaggregates	0.090	Boron	0.334	*Verticillium* spp. (p/g)[1]	0.441
Large Macroaggregates	0.345	Zinc	**< 0.001**	Solvita	0.291
Moisture Content	**0.019**	Iron	0.425	POXC	**0.023**
		Manganese	**0.020**	ACE protein	**< 0.001**
		Copper	**< 0.001**		
		Nitrate-nitrogen	0.220		
		Phosphorus (Olsen)	**< 0.001**		
		Cation exchange capacity	0.951		
		Salinity	0.494		
		Magnesium	**0.037**		
		Sulfur	0.550		
		Sodium	0.735		
		Total Organic Carbon %	**< 0.001**		
		NH4	0.275		

[1] Propagules per gram of soil

Table 2.6 (continued) ANOVA results indicating the significance of individual soil properties by the management factors in a study of management impacts to soil health in potato producing fields in Maine. These tests were performed iteratively on the entire dataset using data from soil collected in the summer, approximately 60 days after planting. Italicized metrics were collected in the spring at planting only and included in the analysis for thoroughness. All plots were planted in potatoes. Bold values indicate significant differences.

Soil property metric	*p*	Soil nutrient metric	*p*	Soil biology metric	*p*
pH	**< 0.001**	Potassium	0.867	*Organic matter*	**< 0.001**
Microaggregates	0.989	Calcium	0.051	Pratylenchus	0.068
Macroaggregates	0.436	Boron	0.501	*Verticillium* spp. (p/g)[1]	0.622
Large Macroaggregates	0.912	Zinc	**< 0.001**	Solvita	**0.006**
Moisture Content	0.123	Iron	0.448	POXC	0.727
		Manganese	**0.003**	ACE protein	0.371
		Copper	**0.001**		
		Nitrate-nitrogen	0.446		
		Phosphorus (Olsen)	**0.040**		
		Cation exchange Capacity	**0.029**		
		Salinity	0.685		
		Magnesium	0.057		
		Sulfur	0.266		
		Sodium	0.425		
		Total Organic Carbon %	**0.008**		
		NH4	0.682		

[1] Propagules per gram of soil

Table 2.7 Results of ANOVA tests performed to determine differences in yield and disease incidences when subset to compare management factors to standard practices in a study of management impacts to soil health in potato producing fields in Maine. Data was collected in the final year of the study when all plots were planted in potatoes at the same time. These data were subset to compare management factors individually with standard practice treatments. Bold values indicate significant differences.

Management factor	Yield	Potato Early Dying (*Verticillium* spp.)	Black Scurf (*Rhizoctonia solani)*	Common Scab (*Streptomyces scabies)*	Hollow Heart
Treatment	**< 0.001**	**0.026**	**< 0.001**	0.434	0.087
Rotation Length	**0.04**	0.213	0.594	0.725	0.749
Variety	0.756	0.28	0.638	0.815	0.749
Green manure usage	0.444	0.283	0.673	0.138	0.626
Compost integration	**0.018**	0.118	0.090	0.35	**0.038**
Fumigation	0.074	0.111	None	0.15	0.397
Non-grain rotations	0.716	0.405	**0.009**	0.147	0.184

Figure 2.1 Correlations of soil properties and potato yield affected by management factors in a study of management impacts to soil health in potato producing fields in Maine. Only significant correlations displayed, blue coloring indicates a negative correlation and red coloring indicates a positive correlation.

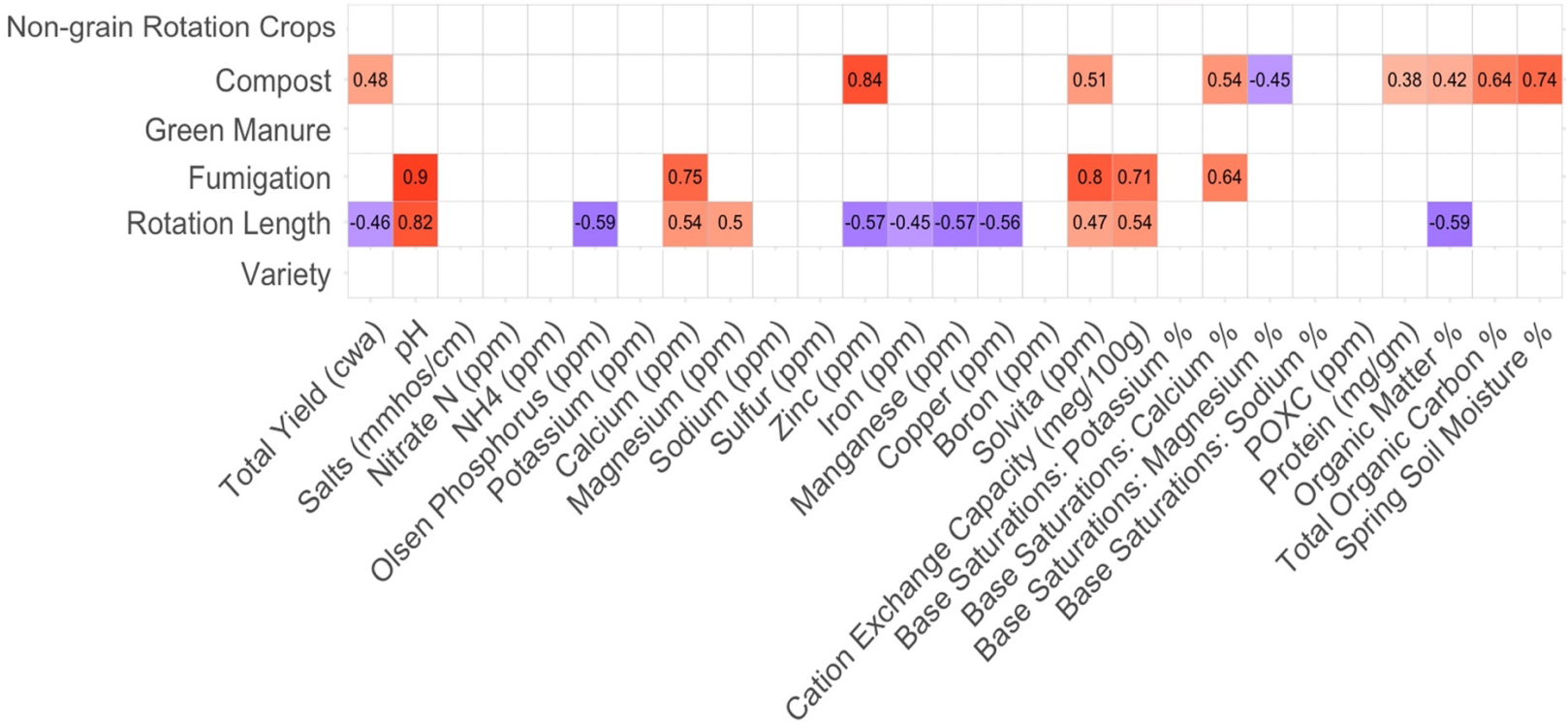


Figure 2.2 Principal component analysis (PCA) of potato yield and significant soil properties in a study of management impacts to soil health in potato producing fields in Maine. Compost and rotation length were included as these treatments resulted in yields which were significantly different from standard practices.

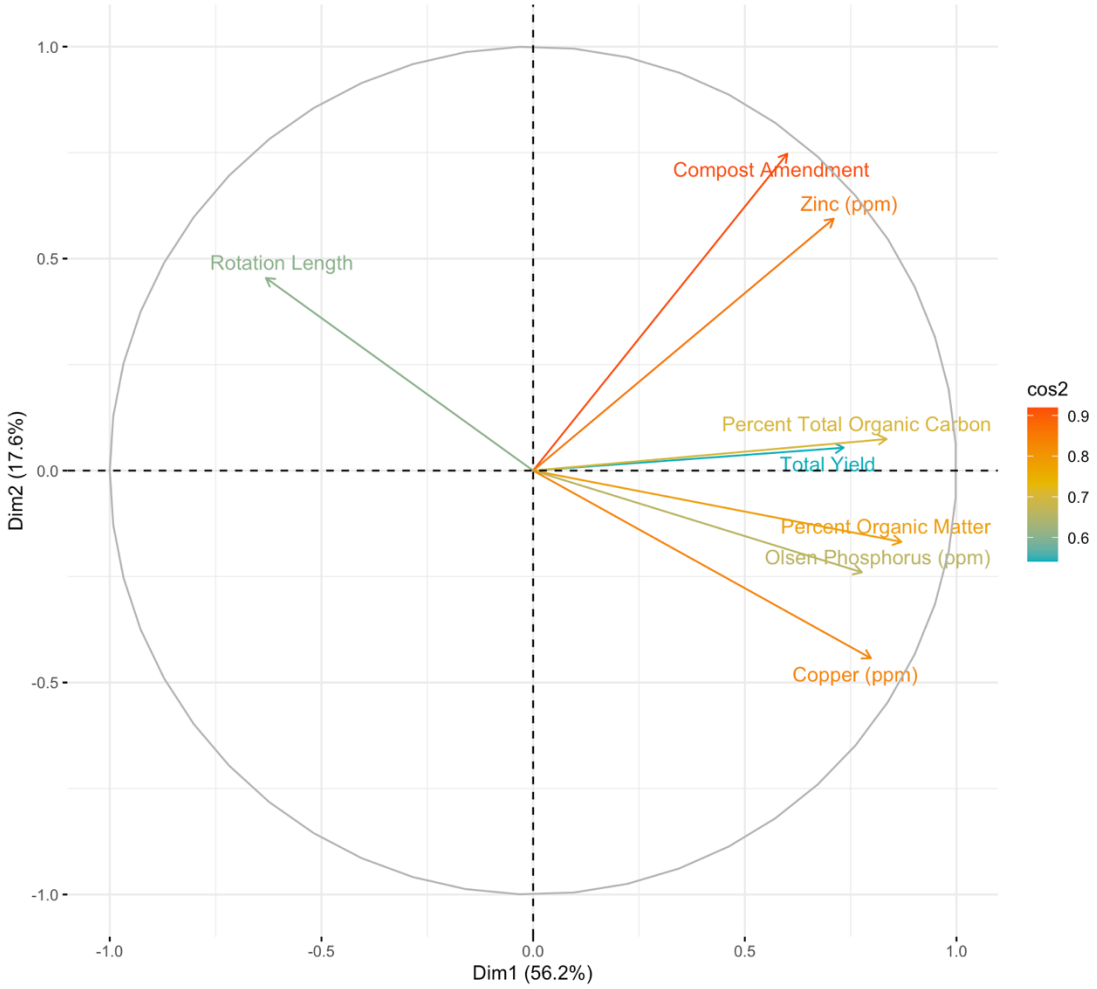
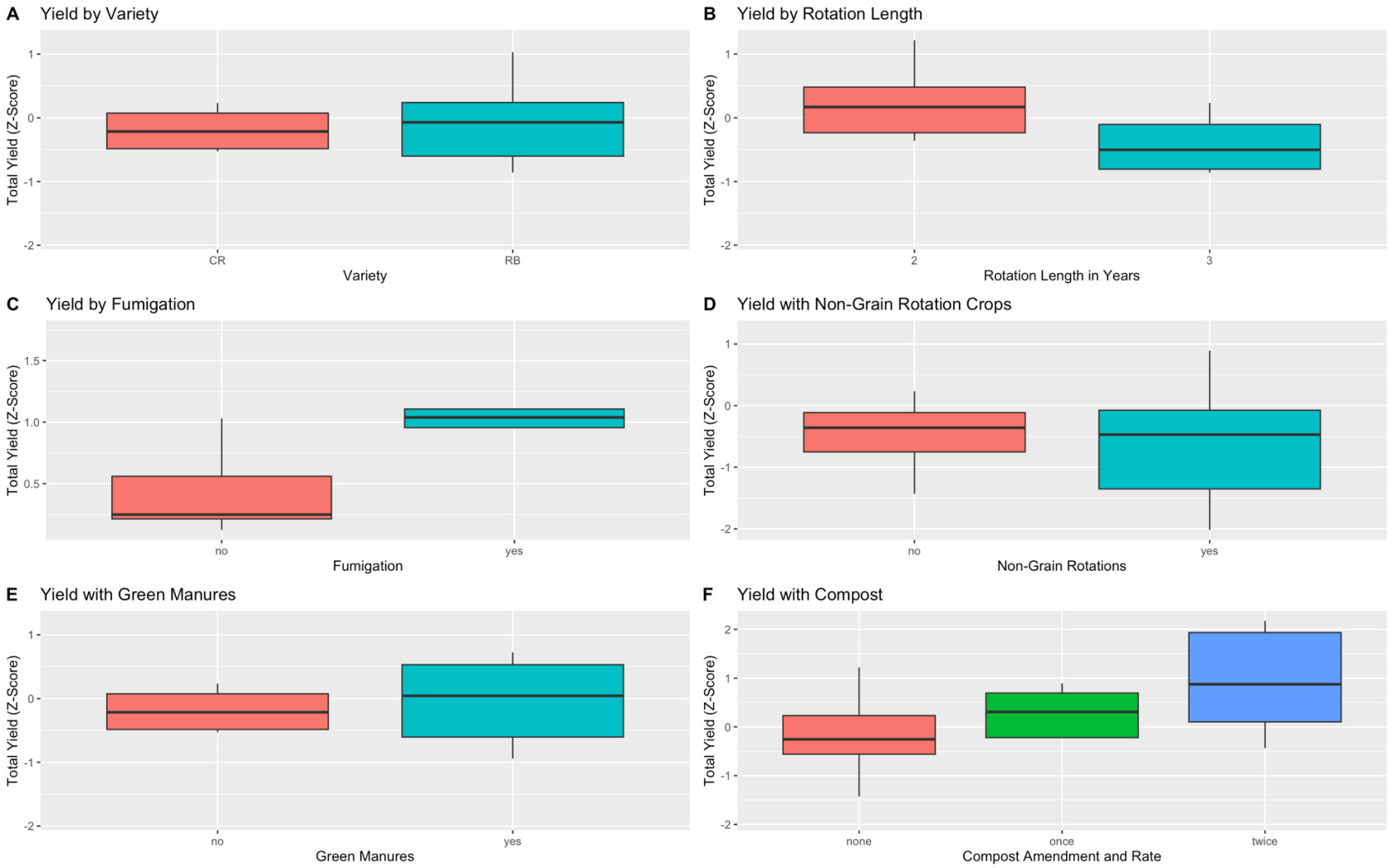


Figure 2.3 Effects of management factors on potato yields (as z-scores) in 2022 in a study of management impacts to soil health in potato producing fields in Maine.



2.4 Discussion

Overall, when looking at soil chemical properties in the final year of the study, rotation lengths of three-years resulted in significantly higher pH and cation exchange capacity, while rotation lengths of two-years resulted in significantly higher copper, manganese, and Olsen phosphorus. However, soil physical properties were not significantly different based on the individual treatments. Soil biological properties were most clearly driven by the treatments, as compost amended treatments consistently increased levels of organic matter, soil organic carbon, total organic carbon, and soil respiration. Within soil community groups detected by PLFA, a similar trend was observed, and the compost amended treatment in the three-year rotations had the most significantly increased measurements of microbial biomasses and percentages of soil microbial groups.

When subset to gain more resolution, the soil properties which were most affected by both the treatments and had significant relationships with yields were zinc, copper, Olsen phosphorus, percent total organic carbon, and organic matter. The use of compost amendments was the management factor that was most positively related to zinc, percent total organic carbon, and organic matter, and could be seen as the most influential management factor with respect to increasing yield and soil health improvements. Olsen phosphorus was most positively related with compost when compared to all other treatments, but not when compared to standard practices only. On the other hand, rotation length was negatively related to zinc, Olsen phosphorus, and copper. Overall, compost amendment was positively correlated with yield and rotation length was negatively correlated with yield, potentially as a result of these alterations to soil properties.

Zinc is an essential plant nutrient, primarily produced from the erosion of zinc-containing rocks, and is the most commonly deficient plant nutrient in agricultural soils (Broadley et al. 2007). Zinc aids plants in a number of enzymatic activities and deficiencies can lead to stunted shoot development or in severe cases, shoot dieback, chlorosis, and leaf curling (Broadley et al. 2007; Jones 2012). Compost amendment was found to be strongly positively correlated with soil zinc content. In a compost analysis report, zinc was found to be present at 0.005g/kg. The compost which was procured was made of a blend of plant matter with some animal manures. Zinc is one of many heavy metals known to be found in manures (van der Meer et al. 2012). Accumulation of excess zinc from soil amendments can occur, and cause phytotoxicity, which is rare, or risks to the health of humans or other animals (McGrath et al. 1994; Broadley et al. 2007). For this reason, many countries have imposed regulation on zinc application. In the United States, per EPA part 503 regulations for sewage sludge applied to land, is a concentration of 7500g/kg (McGrath et al. 1994). The impacts of zinc on potato yields found in this study are similar to those found in a study performed in India in which potato yields increased with zinc fertilization, up to a threshold of 6.0 kg/ha (Banerjee et al. 2016). Additionally, zinc is understood to aid a plant in defense from pathogens, although this was not observed in this study (Cabot et al. 2019).

Copper was negatively correlated with rotation length, although it is not clear why this was the case. Copper can form complexes with organic matter and result in less detectible copper in soil samples (Zang et al. 2015). However, with the exception of the three-year compost amended treatment, organic matter was higher overall in two-year rotations, so this was likely not the cause of higher copper concentrations in two-year rotations. Copper is a micronutrient which aids plants in performing photosynthesis, enzyme activation, and cellular maintenance

(Troeh and Thompson 2005; Jones 2012). Copper ions in soils are tightly bound within organic matter by cation exchange capacity, and are thus not very mobile (Troeh and Thompson 2005). Copper deficiencies are not a common occurrence, as plant nutritional requirements are relatively low, and thus toxicity is more common resulting in leaf chlorosis, iron deficiency, and reduced root formation and elongation (Jones 2012). However, copper is also a well-known foliar fungicide, and toxicity is not likely to develop through the use of this product (Jones 2012).

In this study, Olsen phosphorus was one of the soil properties which were most affected by the treatments and had significant impacts on yields. Specifically, Olsen phosphorus was most negatively correlated with rotation length when subset and compared within standard practices. Phosphorus is essential for forming amino acids, nucleic acids, chlorophyll, alkaloids, proteins, and other biological molecules (Jones 2012). As essential as phosphorus is, it is often insoluble and thus unavailable to plants (Troeh and Thompson 2005). It exists as either organic forms, which are derived from organic matter, or inorganic forms, which are derived from minerals within rocks (Troeh and Thompson 2005). Phosphorus content of soils in this study were measured using Olsen bicarbonate P (Agvise Laboratories, Northwood, ND), which is best performed with calcareous soils, but is considered suitable on a wide range of acidic and alkaline soils (Pierzynski 2000). In Maine, the Modified Morgan phosphorus is the mostly widely used test, however there is no exact conversion to Modified Morgan from Olsen because of the impact of soil series on extractants (Herlihy and McCarthy 2006).

Similarly to phosphorus, carbon is also present in soils as organic and inorganic forms, however, organic carbon is present in all agricultural soils, unlike inorganic carbon which may or may not be present (Bisutti et al. 2004; Nelson and Sommers 1983). Soil organic carbon is directly related to soil organic matter, but only contains the carbon fraction in a ratio which

ranges from 1-2 (Nelson and Sommers 1983). Tillage is known to reduce soil organic carbon through the disturbance of aggregates which are subject to rapid degradation by soil microorganisms (Rice et al. 2021). On the other hand, agricultural practices which involve less soil disturbance, such as the use of cover crops, reduced tillage, and crop rotations tend to increase soil organic carbon (Rice et al. 2021). Organic amendment addition is also a major driver of increasing soil organic carbon, which is similar to the trend seen in this study with respect to compost amendment being significantly positively correlated with soil organic carbon (Rice et al. 2021).

Soil organic matter was significantly increased with the use of compost amendments and was significantly lower in three-year rotations compared to two-year rotations. Soil organic matter is composed of plants and animals that are living or at different stages of decomposition (Magdoff and Van Es 2021). Fractions of organic matter are easily utilized by organisms in the soil and converted to forms which can be taken up by plants, while other fractions, known as humus, are very difficult to decompose (Magdoff and Van Es 2021). Soil organic matter is essential for healthy crops, supporting soil ecology, and maintaining soil fertility and structure (Magdoff and Van Es 2021). Because of the inherent utility of soil organic matter, it can be easily depleted quickly and should be replenished with new organic materials (Troeh and Thompson 2005). Organic matter also drives microbial activity, including the decomposition of pesticides and contaminants, and nitrogen release (Troeh and Thompson 2005). While organic matter is subject to a number of natural factors including temperature, precipitation, soil texture, and landscape location, human impacts can also alter organic matter content in soils, particularly agricultural soil (Magdoff and Van Es 2021). Many common agricultural management techniques can reduce soil organic matter especially through the loss of topsoil, such as tillage

and the use of synthetic nitrogen fertilizer which can lead to enhanced decomposition of soil organic matter (Magdoff and Van Es 2021). Alternatively, soil organic matter can increase through the use of specific rotation and cover crops which produce residues which will remain in the soil or through the use of organic soil amendments such as manure or compost (Magdoff and Van Es 2021). Organic matter can also aid in the management of micronutrients, such as zinc and copper, as it can form chelates and maintain these metallic micronutrients in bioavailable forms (Magdoff and Van Es 2021).

In general longer and more diversified crop rotations have been shown to increase soil organic carbon and soil organic matter (King and Blesh 2018; Liu et al. 2022). In this study, the opposite effect was found, and a potential reason that soil organic carbon and total organic matter were negatively related to rotation length could be the climactic conditions which occurred during years when rotation crops were grown. For two-year rotations, rotation crops were grown in the first and third years of the study (2019 and 2021), but three-year rotations were grown in the second and third years of the study (2020 and 2021). The first, third, and final years of the study had similar amounts of rainfall and average high temperatures, 3.29-3.71 inches average per month and 90-92ºF. The second year of the study, 2020, was both drier and hotter, as the average amount of rainfall was 2.82 inches per month with a high temperature of 96ºF (NOAA National Weather Service n.d.). The field was also not irrigated, which is common practice in Maine potato production, and consequently the crops were highly subject the climactic conditions. While data on the yields and vigor of rotation crops were not collected, this reduced vigor as a result of climactic extremes could explain poorer performance of crops and consequently less effective use as rotation crops. In a study of rotation crop performance under climate change, it was found that even adapting rotation crop planting date and variety was not

sufficient to overcome the effects of climate change and resulted in decreases in aboveground biomass production (Teixeira et al. 2018).

While the majority of PLFA metrics (sixteen out of twenty-two metrics) were significantly related to treatments, none were significantly related to yield. Compost amended plots were significantly different from unamended plots with respect to ten out of the twenty-two PLFA metrics. Except for percent bacteria relative to total microbial biomass, and ratios of saturated to unsaturated fats and monounsaturated to polyunsaturated fats, plots which were amended twice had significantly higher total and individual microbial group biomasses and percentages of the total biomass, while unamended plots and plots amended once were lower but not significantly different from each other. In general, more frequent compost amendments increased microbial biomasses more significantly than the percent of the total of different microbial groups such as bacteria overall, actinomycetes, total fungi, arbuscular mycorrhizae, and saprophytes. This is not surprising, as compost is well known to be rich in microorganisms and organic matter which can promote indigenous microbial communities (Mehta et al. 2014; Singh and Nain 2014).

In general, with respect to potato yields and disease incidences, none of the treatments were consistently the best performing, which could point to the short duration of this study and the complexity of overlapping management factors within the treatments. For this reason, the data were subset and reanalyzed in a series of balanced comparisons to limit the number of management factors being analyzed. When the data were subset, the addition of compost and two-year rotations compared to three-year rotations significantly increased yields. As rotation length was a major factor which impacted yields, within two-year rotations the compost amended and fumigated treatments had the highest yields and in the three-year rotations, the compost

amended treatment had the highest yields. While this approach resulted in less statistical power in the analysis, it also improved resolution, and ensured that interactions with specific soil properties were attributed to the appropriate management factor where possible. With respect to disease incidences and severities, two treatments had the worst outcomes of black scurf and PED compared to all other treatments in the final potato growing season, two-year rotations with 'Caribou Russet', both as the standard practice and with the green manure addition. Common scab incidences and severities were not impacted by any of the treatments and were not significantly different within treatments in the final year of the study.

Fumigation reduced the ratios of saturated to unsaturated fatty acids recognized by the PLFA analysis. These changes have been previously attributed to disturbance chemically or physically, nutrient availability stress, and microbial community flux (McKinley et al. 2005; Zelles et al. 1992). In Maine, methyl isothiocyanate (MITC) generating soil fumigants, such as Vapam HL, are commonly used (Aaron Buzza, personal communication). These chemical fumigants rapidly degrade into MITC which is not very mobile in soil and careful application with respect to weather conditions such as temperature and amount of rainfall are critical to ensure successful application (Duniway 2002). Soil chemical fumigation, which can be costly and hazardous, is commonly performed in the United States and generally is thought to reduce soilborne diseases, increase beneficial microbial communities, and enhance yield (Neilson et al. 2020). However, often results are conflicting and may indicate bacterial diversity declines, increases, or changes annually (Neilson et al. 2020). This similar response was seen in this study, as fumigation when compared to standard unfumigated plots was not found to significantly increase yields, reduce disease incidences, nor enhance microbial groups detected in PLFA. In fact, in this study, fumigation merely enhanced a PLFA metric indicative of bacterial community

stress and reduced the populations of male *Pratylenchus* sp. The results of this study do not support the hypothesis that fumigated plots would reduce disease pressure and increase yields.

Surprisingly, no diseases nor soil properties were significantly affected through the usage of green manure crops in this study. This could be potentially due to environmental conditions or ineffective incorporation of green manures, which can have varying efficacies based on growing conditions and incorporation technique. In a study comparing biofumigant green manure incorporation techniques, it was found that fresh plant matter had the lowest suppressive abilities to *Pythium* sp. (Lazzeri et al. 2004). This lower suppressiveness and potential for the biofumigant green manures not having been incorporated quickly enough could have reduced their effectivity. Alternatives to fresh biofumigant plant matter, such as seed meals or frozen biofumigant plant matter, indicate that in order for biofumigants to be successful, their incorporation and application technique must be optimized (Lazzeri et al. 2004).

Compost production, through the action of microorganisms, transforms plant residues and other agricultural wastes into nutrient-rich and stable substances (Agnew and Leonard 2003). While composting of plant residues is a natural process which occurs constantly in forests, fields, and other ecosystems, successful compost production commercially relies on temperatures above 40°C, high moisture, and adequate airflow to ensure the proper environment for microbial activity (Agnew and Leonard 2003; Magdoff and Van Es 2021). Fungi and bacteria capable of degrading cellulose and lignin make up this microbial community, including many microbes which are described as beneficial and useful in plant nutrient uptake and biological control (Singh and Nain 2014; Mehta et al. 2014). In general, the compost dwelling microorganisms which benefit plants through disease suppression are saprophytes which consume dead plant material, but which are also capable of biological control (Mehta et al. 2014). Properly prepared

compost is capable of killing weed seeds and pathogenic propagules, but it is critical to note that compost quality and composition is highly subject to its inputs, and can range in nutrient quantity and microbial activity (Magdoff and Van Es 2021; Fuchs 2010). In addition to compost amendment reducing pathogen populations, it can also add organic matter which can improve soil structure and health (Mehta et al. 2014). In the present study, compost amendment significantly increased organic matter and total organic carbon, both of which were found to be drivers of potato yield. No differences were seen with respect to disease levels and compost amendment overall, but when subset and compared to non-composted treatments, hollow heart, an abiotic disorder, and common scab severity were enhanced with compost amendment. However, it should be noted that disease incidence of hollow heart was low overall.

Additionally, when compost amended treatments were separated by variety, this significant difference between compost amendments was no longer seen, indicating that this could have been a result of the varieties having varied hollow heart susceptibilities. Interestingly, this difference between varieties was not seen when comparing only standard treatments. Compost amendment and the resulting increase in organic matter is likely impacting the water holding capacity of the soil, a major factor in hollow heart development (Zemánek 2014; McCann and Stark 1989). The influx of nutrients from compost amendment coupled with higher water holding capacity could result in more susceptible varieties of potatoes exhibiting hollow heart symptoms, as was seen in this study.

Variety development and selection is an important tool for increasing yields and reducing susceptibility to plant pathogens (Tessema et al. 2020). ‘Russet Burbank’ was originally released in 1914 and is a variety of global importance and is moderately resistant to common scab, but susceptible to PED (Bethke et al. 2014). ‘Russet Burbank’ was included in this study as an

industry standard. The other variety which was tested in this study is 'Caribou Russet', a variety released by the University of Maine and the Maine Potato Board, which has shown moderate resistance to both common scab and PED (Sutherland 2017). This variety is mid-season maturing and has shown improved yield potentials over 'Russet Burbank', and has particularly demonstrated yields of the largest size class (USDA number 1) to be 1.3 times higher in comparison (Sutherland 2017). However, in this study, no significant differences were seen between these two varieties tested. The only difference observed between these varieties when compared in a subset dataset was the amount of root lesion nematode males present, as significantly more were detected in soil samples collected from plots planted with 'Russet Burbank'. While 'Caribou Russet' is not known to be resistant to root lesion nematodes, which form a disease complex with *V. dahliae* and exacerbate PED, this variety is known to be resistant to golden nematode race Ro1 (Sutherland 2017). However, further research would be required to determine if the moderate resistance to PED expressed by 'Caribou Russet' could be associated with resistance to root lesion nematodes, as there were no significant differences observed in PED incidence or severity by variety.

In potatoes, rotation crops are typically utilized because of the intensive nature of producing this crop. Potato production requires tillage of the soil and potatoes require large amounts of nutrients in order to produce high yields and quality tubers (Harris 1992). The industry standard of a rotation length is 2 years, although benefits have been seen in increasing the length of crop rotation (Larkin et al. 2021b, 2021a). In this study, rotation length was negatively correlated with yield, a finding which did not support the initial hypothesis. This may be a misleading result due to the short-term duration of this study, as longer rotation lengths in potato cropping systems have been shown to significantly increase yields compared to shorter

rotations, especially once rotations are established (Mohr et al. 2011). Prior to this field being planted with potatoes, it was in oats for at least the last 25 years and was not heavily managed, and as the treatments were only planted in potatoes twice over the course of this study, it could be argued that the rotations were not yet established. Other studies have shown that not only do longer crop rotation in potatoes increase yields after a few years in a rotation sequence, but tuber size can be reduced with shorter rotation, another finding which was not supported in this study (Carter and Sanderson 2001; Mohr et al. 2011; Wright et al. 2017). Though this study incorporated both a two-year and three-year rotation length scheme, in practice, the fields were only planted with potatoes twice over the course of the study, and the first year of potatoes was implemented in different years in the 2-yr and 3-yr fields. Thus, plots which were in two-year rotations had a more recent addition of fertilizer, which could have carryover effects and result in higher yields.

Within the plots planted with non-grain rotation crops, overall, non-grain rotation crops appeared to reduce the incidence of black scurf. Higher black scurf incidences were seen in plots planted with grain rotations in both two-year and three-year rotation lengths compared to those planted with non-grain rotation crops. However, when considering individual rotation crops, results were inconsistent, with some rotation crops resulting in potatoes with slightly higher or lower incidences of black scurf symptoms. There may be many factors which are reducing the ability to discern the impact of different rotation crop selection, or this could be the result of the short duration of this study. It has also been found that the use of most rotation crops may temporarily suppress soilborne pathogens within one rotation cycle, this effect may not continue for more than two rotation cycles, and may require intentional selection of rotation crops (Larkin et al. 2017; Larkin and Lynch 2018; Larkin et al. 2010). For example, the use of *Brassica* and

mustard green manures, sudangrass, fall cover crops, longer rotation lengths, and diversity in rotation crop selection resulted in significant reductions in black scurf and common scab (Larkin et al. 2011, 2017). However, when selecting rotation crops, it is essential to select crops which are not reservoirs for disease because of their ability to be infected by pathogens which also infect potatoes, thus enhancing disease pressure. An example of this is white mold, which can infect potatoes, and rotation crops which may be used such as beans or canola (Bohl and Johnson 2010a). Another consideration when planting rotation crops is the type of equipment and skills needed to grow non-grain alternative crops which may be less familiar, the need for highly organized farms and record keeping, and the potential economic loss of producing a less profitable crop (Zegada-Lizarazu and Monti 2011). However, this familiarity with non-grain alternative crops can lead to another potential benefit of crop rotations in that farmers can diversify production for more economic opportunities and climate resiliency as a result of diversified plantings (Zegada-Lizarazu and Monti 2011).

2.5 Conclusion

In this study, five soil properties were found to be related to both management factors and yield: percent organic matter, percent total organic carbon, copper, Olsen phosphorus, and zinc. Of the management factors, compost application and rotation length had the strongest relationships with potato yield and these five soil properties. Unsurprisingly, compost amendment was positively correlated with organic matter and total organic carbon, as compost is well known for these contributions. However, compost amendment also increased zinc levels in soils, which could be directly traced back to initial compost chemical analyses. Longer rotation length resulted in reduced correlated with organic matter, copper, phosphorus, and zinc, which

did not support the hypothesis. This could have been the result of the short study period, potential fertilizer carryover, or a lack of crop residues and resulting organic matter as the result of a year of drought and high temperatures during the rotation crop year. Fumigation was not positively correlated with yield nor a reduction in disease incidences compared to standard practices, and PLFA results indicated microbial stress. Green manure usage did not result in any significant differences. The use of non-grain rotation crops reduced the incidences of black scurf compared to standard practice with grain rotation crops, but soil properties were not altered by their usage. Variety selection also did not impact any soil health metrics, but a significantly reduced number of *P. penetrans* males were found to be associated with 'Caribou Russet'. Thus, of the management factors examined here, compost amendment had the greatest positive effects on soil properties, microbial properties, and potato yield, but did not affect soilborne diseases, whereas all other management factors, including potato variety, rotation length, addition of green manure, type of rotation crop, and fumigation, had lesser overall effects but still may be important factors to potato production, especially under more long-term conditions. Potato production requires many management decisions, which can impact soil health and consequently potato yield. It is critical for the longevity of soil health, disease suppression, and productive crops to make informed decisions.

CHAPTER 3

MANAGEMENT IMPACTS TO SOIL MICROBIAL COMMUNITIES IN MAINE POTATO PRODUCTION

Abstract: Healthy soil can provide crops with protection from diseases, improve nutritional fertility, prevent erosion through improved physical structure, and consequently result in higher crop yields. Soil health can be significantly impacts by agricultural management. Potato production can be particularly taxing on soil health because of intensive tillage, short crop rotations, and numerous economically significant soilborne diseases. To better understand the impacts of different management strategies on soil health, cropping systems incorporating different management factors, including rotation length, non-grain rotation crops, green manures, compost amendments, and a soil fumigant were studied using two potato varieties 'Caribou Russet' and 'Russet Burbank'. Soil from each treatment was sampled at various times corresponding to potato planting, sixty days after planting, and at harvest, to quantify soil bacterial and fungal communities using amplicon sequencing of the 16S rRNA region and ITS rDNA region, respectively. Sequencing results from the first two years of the study did not indicate significant differences between bacterial and fungal community populations. However, in the final year of the study after four years, in which all plots were planted in potatoes, significant differences were found. In general, fungi were more sensitive and variable to the treatments overall compared to bacteria, specifically with respect to rotation length, compost amendment, fumigation, and the use of non-grain rotation crops. Within management practices, fumigation and compost had the largest impacts to microbial communities. In general, fungi which were less differentially abundant by fumigation were saprotrophs or had unidentified

functions, while the two largest groups of fungi which were more abundant by fumigation were saprotrophs or plant pathogens. Of differentially abundant fungi by compost amendment, over 3.5 times more fungal amplicon sequencing variants (ASVs) were abundant with compost than without. Within these fungal ASVs which were more abundant with compost, plant pathogens made up 4% of the differentially abundant ASVs, while in soils without compost, plant pathogens made up 25%. In general, compost appeared to positively impact soil microbial communities and fumigation appeared to negatively impact them. The results of this study could aid farmers in developing crop management plans which can improve soil health, reduce disease, and increase yields.

3.1 Introduction

Potato production in Maine is incredibly important to the state's economy, and in 2022, nearly 2 billion pounds of potatoes were produced, resulting in a production value of nearly one quarter of a billion dollars (USDA: NASS 2022b). However, the production of potatoes is well known to be impactful to soil health. Production of the potato crop can result in deterioration of soil structure, depletion or excess of residual soil nutrients, crop preparation and harvest involve the use of heavy machinery causing compaction, and typically high inputs of pesticides are required (Davenport et al. 2005; Auerswald et al. 2006; Powell et al. 2020). Although potato production can be impactful to soil quality, in a survey of potato farmers, 91% stated that soil health is either extremely or very important (Maas et al. 2023). Additionally, a number of potato management practices that can improve soil health have been well documented, notably reducing tillage, altering crop rotations and rotation crop type, reducing fumigation, using cover crops and green manures, and using organic amendments (Hills et al. 2020). It is consequently critical to better understand how management practices in potato production impact soil health. This study was established as part of the Potato Soil Health Project, a national initiative to determine how soil management practices are impacting soil health in potato production (Potato Soil Health Project n.d.).

Soil health is a term used to describe the efficacy of soil in supporting healthy plants, environments, animals, and humans, all in a sustainable fashion which can lead to longevity of agricultural production (USDA NRCS 2023). Soil health can be characterized through the use of a large number of metrics, however, soil biological metrics are thought to be the most sensitive to management (Nelson et al. 2009). Biological metrics associated with soil health include characteristics of microorganism populations, including bacteria, fungi, and protozoa. Together,

soil organisms are thought to constitute 25% of diversity on the planet (Magdoff and Van Es 2021). Soil biological metrics are critical for crop production, as properly functioning soil microbial communities can offer many benefits and potentially ameliorate pests and pathogens, aid in nutrient availability, and promote plant growth (Ray et al. 2020). While soil communities can be complex and the understanding is still very limited, higher abundances of biological communities can also be seen as evidence of available nutrients and organic matter which are necessary to support organisms within soil environments (Powell et al. 2020). Historically, soil biological metrics relied on culturing and substrate utilization, although it has been estimated that culturing techniques only resolve about 1% of all microorganisms within a single sample, leading to drastic underestimations of diversity (Fierer 2017; Youseif et al. 2021).

As molecular genetic approaches have become less cost prohibitive and readily available to more deeply investigate soil microbial communities, the identification and exploration of functions of soil dwelling microorganisms remains an important area of research, especially as it relates to agricultural management. The use of amplicon sequencing has become a common technique to taxonomically describe and analyze microbial communities in various environments, including soil. This targeted approach, also known as metabarcoding, utilizes highly conserved genes, such as the 16S rRNA or internal transcribed spacer (ITS) to detect bacterial or eukaryotic communities, respectively (Lundberg et al. 2013; Alteio et al. 2021).

However, there are challenges associated with amplicon sequencing, particularly when performed from soil samples, as humic and fulvic acids can impact the DNA extraction processes and later downstream in PCR, and these ubiquitous substances are present in all soil environments as the result of crop residues and other organic matters (Daniel 2005; Baar et al. 2011). These investigations into soil microbial communities are warranted, as microbial

communities play a critical role in the soil environment and consequently in agricultural production. While there has been more research on the diversity of soil microbial communities, soil is still very poorly understood, highly diverse, and functionally redundant (Maron et al. 2011; Fierer 2017).

As soil health is essential for soil conservation, nutrition, and crop productivity, and is known to be sensitive to management techniques, it is hypothesized that crop management will impact soil microbial communities (Westermann 2005a; Nelson et al. 2009; Magdoff and Van Es 2021). However, it is worth noting that there is a massive amount of microbial diversity in soils and it is still not clear what exactly this diversity can be attributed to, either environmental, management, or other factors (Janssen 2006). In previous studies, management impacts to soil microbial communities have been assessed using a number of culture-based and substrate utilization assays. The use of compost and green manures enhanced the number of cultured bacteria and altered the communities of microbes determined through the use of fatty acid methyl ester assays (Bernard et al. 2012).

Other Maine-based studies have indicated that management practices can improve soil health, including compost amendment, longer rotation lengths, the use of green manures, diverse rotation crops, and cover crops (Larkin et al. 2010, 2021b, 2021a). It is hypothesized that these management techniques will promote not only more abundant microbial communities, but also those which can be described as beneficial microorganisms. Higher abundance and diversity of soil microorganisms could contribute to utilization of resources within the soil environment through higher metabolic activity, and contribute to more ecosystem services (Ferris and Tuomisto 2015). As many microorganisms perform more than one ecosystem service, and often in tandem with other soil microorganisms, higher soil microbial diversity could also contribute to

resiliency following disturbance (Kibblewhite et al. 2008). As soil microbial communities play critical roles and provide various ecosystem services within the soil environment, a better understanding of these communities in Maine potato producing fields was explored. In addition to exploring the general make-up of these communities, the impacts of various management factors over the course of four years were explored. As management factors have been demonstrated to impact soil physical, chemical, and biological metrics, soil microbial communities are expected to be altered as the result of management, including variety selection, rotation length, the use of non-grain rotation crops, the use of green manures, compost amendment, and chemical fumigation.

3.2 Methods

3.2.1 Field design and plot layout

This study utilized the same field experimental design described in Chapter 2, investigating six different management factors. Over the course of four years from 2019 to 2022, twelve treatments were designed to investigate common potato production practices: rotation length (2 or 3 years), variety of potato used ('Caribou Russet' or 'Russet Burbank'), green manure crops in rotations, compost integration (once, twice, or not amended), fumigation usage, and rotations which included non-grain cash crops (broccoli, garlic, legumes, or corn). The 12 individual treatments are described in Table 2.1a and 2.1b (Chapter 2). This study was carried out at the Aroostook Research Farm in Presque Isle, Maine (46.653704, -68.020309). The plots were 40 feet long and 18 feet wide to allow space for six rows of potatoes during potato production years. The plots were arranged in a randomized complete block design with five replications. The northern half of the field was arranged with a rotation length of three years, and

the southern half of the field was arranged with a two-year rotation length. All plots were managed by standard techniques for weed and insect management. Potato plots were fertilized at planting using a 14-14-14 fertilizer at a rate of 1.23 Mg ha^{-1} with no additional micronutrients, produced by McCain Fertilizers (Presque Isle, Maine). All plots were tilled, planted, prepared, and harvested using standard practices for northern Maine potato production.

3.2.2 Bulk field soil collections and processing for microbial community analyses

Field soil was sampled from plots with approximately 40 cores per plot collected to a depth of approximately six inches using one inch soil probes. Soil was homogenized by mixing in individual Ziploc bags labeled with plot number and transported on ice to the lab where it was stored at 4°C and processed within 72 hours. Soil samples for microbial community analyses were collected and assessed during spring (at planting) and summer (60 days after planting in 2022, the fourth growing season, when all plots were planted to potato). Dates of soil collection varied based on weather and soil conditions. Soil samples in the spring and summer were only collected during potato growing years and fall soil samples were only collected in the fall of rotation crop in the year prior to potato cropping. Consequently, soil sampling timepoints in which every plot was sampled at the same time were performed in the fall of 2021, and the spring and summer of 2022.

To explore Maine soil microbial communities overall, sequencing results from all sampling timepoints over all four-years were combined to be analyzed together. To determine the short-term impacts of management on soil microbial communities, soil samples collected during the spring at planting and the summer 60 days after planting of the fourth growing season were used for analysis. To process soil for analysis of soil microbial communities, homogenized bulk soil was processed through a 2-mm sieve, and 15 grams of soil were collected and stored at

-80°C until DNA extraction. Additional soil was stored as an archive. DNA was extracted from 0.25 grams of soil using the DNEasy PowerSoil Pro Kit according to the provided protocol (Qiagen, Düsseldorf, Germany). Extracted DNA was quantified using a NanoDrop which was blanked with ultrapure water between each sample before shipping to The University of Minnesota Genomics Center (Minneapolis, Minnesota) for sequencing. For the detection of bacterial community members, 16S rRNA amplicons were produced from the V3V4 region using the 341F primer (CCTACGGGAGGCAGCAG) and the 806R primer (GGACTACHVGGGTWTCTAAT). For the ITS2 rDNA region to detect fungal community members, the ITS3 forward primer (TCGATGAAGAACGCAGCG) found within the 5.8S subunit, and the ITS4 reverse primer (TCCTCCGCTTATTGATATGC) within the large 28S subunit were used (Bokulich and Mills 2013; Klasek et al. 2023).

3.2.3 Microbial community analysis sequence preprocessing and quality assurance

To maintain consistency within analysis across all states included in this study, bacterial 16S rRNA and fungal ITS rDNA amplicon sequencing data, processing and quality assurance was performed with all sequences processed together and returned to each state as both finished phyloseq objects and as fastq.gz raw files for further analysis. To maintain consistency within this study and allow for comparisons with other states, the phyloseq objects were used to carry out the statistical analysis. Specifics of the quality assurance, sequencing trimming, and taxonomy assignment are described by Klasek et. al (2023).

3.2.4 Statistical analysis

Many management factors overlapped within individual treatments. To resolve treatment effects and reduce noise, treatments were subset according to the analysis being performed. All treatments were compared to a control treatment which shared the same rotation length, variety,

and rotation crops if applicable. To compare variety and rotation length, only standard practice treatments were investigated to isolate only rotation length and variety as management factors.

To determine alpha and beta diversity, bacterial and fungal richness and evenness were calculated to determine the number of different amplicon sequence variants (ASVs) and the distribution within samples. Alpha diversity is the diversity within individual samples, while beta diversity is the variation between samples, and both were calculated using the R package Phyloseq version 1.46.0 (McMurdie and Holmes 2013). Phyloseq was used to calculate alpha diversity statistics, including the Shannon diversity index, evenness, richness (using Abundance-based Coverage Estimators (ACE) and Chao1), and observed ASV diversity (Chao 1984; McMurdie and Holmes 2013; Shannon and Weaver 1964). Normality and kurtosis within the dataset were determined using Shapiro tests in the R package PerformanceAnalytics version 2.0.4 (Peterson et al. 2018). For normally distributed data, analysis of variance (ANOVA) tests were performed or for non-normal data, Kruskal-Wallis tests were performed using the R package Vegan version 2.6-4 (Dixon 2003). To corroborate these results, a series of permutational multivariate analysis of variance (PERMANOVA) tests were performed using the R package Vegan using 10,000 permutations and seed set to 999 (Dixon 2003).

In order to visualize dissimilarly within the dataset based on treatments, principal coordinate analysis (PCoA) ordinations of Jaccard and Bray-Curtis dissimilarities were constructed using the R package ggplot2 version 3.4.4 (Wickham 2011). To determine if clusters were overlapping or significantly different, PERMANOVAs were performed using 10,000 permutations with beta dispersion values calculated using Vegan (Dixon 2003).

Core community members were identified using the R package microbiome version 1.24.0 (Lahti and Shetty 2018). A detection rate of 0.001 and prevalence of 70% were selected to

ensure that core members were both common within and among groups of samples. Core community members were then compared to the alternative group using Venn Diagrams drawn using a tool created by the Universiteit Gent Department of Bioinformatics and Evolutionary Genomics (Venn Diagram n.d.). ASVs which were shared between both management factors and were core to all samples were considered as highly common soil microorganisms and were not investigated further as core members specific to either management factor level, and were referred to as "common core". Core members which remained after removing the common core members were referred to as "conditional core," and represented those individuals which were core to a particular management factor level. Finally, ASVs which were unique to only one management factor were considered as "uniquely core" and represented an ASV which was common across one individual treatment level only.

Differential abundance of ASVs was determined using the R package DESeq2 version 1.42.0 (Love et al. 2014). ASVs were separated by treatment levels, and only those with an adjusted p-value less than 0.01 were considered significantly differentially abundant. Values for log2foldchange were used to determine in which treatment an individual ASV was more or less abundant. To better understand the ecological role that fungal ASVs played in the soil environment, ASVs which could be identified to at least genus were assigned to a primary lifestyle using the dataset compiled by FungalTraits version 0.0.3 (Põlme et al. 2020).

ASVs which were both more abundant with a management factor and were core to a specific management factor were described as highly associated.

3.3 Results

3.3.1 Overview of Maine soil microbial communities

The processing of these sequences resulted in all samples remaining in the dataset following quality assurance steps. Overall, over the course of the four years and 360 samples collected while this study was carried out, there were 54,581 bacterial taxa and 4,750 fungal taxa detected in these Maine potato producing soils. Within the bacterial ASVs detected, 32.4% belonged to the phylum Proteobacteria, followed by Actinobacteriota and Bacteroidota constituting 14.7% and 11.3%, respectively (Figure 3.1). Of the fungal ASVs, 46.7% belonged to the phylum Ascomycota, followed by Basidiomycota, and Chytridiomycota making up 27.0% and 8.5%, respectively (Figure 3.1). Over all sampling timepoints and treatments, the most abundant bacterial ASV was a *Bradyrhizobium* sp. and the most abundant fungal ASV was *Mortierella minutissima.*

In the final year of the study in which all plots were planted in potatoes, Maine soil microbial communities associated with potato production there were 25,477 bacterial taxa detected and 2,784 fungal taxa detected. Of the bacteria detected in the final year, a similar trend of taxonomic make up was observed, and Proteobacteria, Actinobacteriota and Bacteroidota were the top three phyla observed (30.3%, 14.5%, and 9.75%, respectively). Of the fungi detected in the final year of the study, 2,697 were detected and similarly, the top three phyla were Ascomycota, Basidiomycota, and Chytridiomycota (51.9%, 25.1%, and 10.3%, respectively).

The top twenty most abundant bacterial taxa in the final year were very similar to what was found when analyzing the full dataset. The most abundant bacterial taxa were in the phylum Proteobacteria, followed by Actinobacteria, and Acidobacteriota (50%, 15%, and 15%,

respectively). All of the top twenty most abundant fungal ASVs in the final year were represented by only three phyla, Ascomycota, Basidiomycota, and Mortierellomycota (65%, 20%, 15%, respectively). When these twenty fungal ASVs were analyzed using FungalTraits, 70% of these fungi were described as saprotrophs, 15% were plant pathogens, 5% were animal parasites, and 10% had unknown roles.

There were 66 bacterial members which were core to all treatments, and referred to as common core members, and belonged to the phyla Proteobacteria, Actinobacteria, Acidobacteria, Verrucomicrobiota, Gemmatimonadota, Chloroflexi, and Firmicutes (47.0%, 18.2%, 15.2%, 5.6%, 6.1%, 3.0%, and 3.0%, respectively). There were 74 fungal ASVs which were common core members, and belonged to the phyla Ascomycota, Basidiomyctoa, Mortierellomycota, Mucoromycota, and Monoblepharomycota (74.3%, 12.2%, 9.5%, 2.7%, and 1.4%, respectively). When primary lifestyles were assigned by FungalTraits, the majority were various types of saprotrophs (58.1%) followed by plant pathogens, mycoparasites, animal parasites, and those with unidentified roles (17.6%, 9.5%, 2.7%, and 12.2%, respectively).

A baseline understanding of the microbial communities present in Maine potato growing soils was used to understand and differentiate those ASVs which were impacted by the treatments. The abundances of the top twenty ASVs in the whole dataset in the final year of the study were visualized using heatmaps to determine preliminary trends (Figure 3.2). The heatmaps clearly indicated of the top twenty most abundant ASVs over the entire dataset, two ASVs in the family *Micrococcaceae* were more abundant with fumigation and two ASVs were less abundant in the family *Xanthobacteraceae*. With compost amendment, a different ASV in the family *Xanthobacteraceae* appeared to be less abundant with compost amendment performed twice in the three-year rotations (Figure 3.2). For the top 20 most abundant fungi in the final year

of the study, one ASV in the family *Mortierellaceae* was less abundant with compost amendment performed twice in three-year rotations, and one ASV in the family *Plectosphaerellaceae* was less abundant with green manure usage in three-year rotations (Figure 3.2). However, because of the overlapping management factors within the dataset, the data was subset in the same way as performed for the physiochemical analysis by management factor to improve clarity (see Chapter 2).

3.3.2 Variety impacts to soil microbial communities

When bacterial and fungal alpha diversity was analyzed using observed diversity, evenness, and the Shannon, ACE, or Chao diversity indices using PERMANOVAs, no significant differences were found between the two varieties (Table 1a, Table 1b). In PCoA ordinations of both Jaccard and Bray-Curtis dissimilarity, there was no differentiation in the clustering of different varieties in neither bacterial nor fungal communities (Figure 3.3a and 3.3b).

When bacterial core community members were identified, 65 members were found to be core to Caribou Russet, and 67 were found to be core to Russet Burbank (Table 3.2). However, because many core members may be highly common in soil environments, when common core members which were shared between these two varieties and all other treatments were removed, two bacterial ASVs were conditionally core to Caribou Russet, and five were conditionally core to Russet Burbank. Of these, only one bacterial ASV was uniquely core to the variety ‘Caribou Russet’. Similarly, while 73 fungal ASVs were initially described as core to Caribou Russet and 76 were considered core to Russet Burbank, when fungal common core members were removed, two were conditionally core to Caribou Russet and five were conditionally core to Russet

Burbank. Of these, none were uniquely core to either variety as all were found to be core with other management factors.

No bacterial ASVs were differentially abundant by variety. However, seven fungal ASVs were differentially abundant by variety. Within Caribou Russet, four fungal ASVs were significantly more abundant, and all were identified to species (two subspecies of *Rhizopus arrhizus, Cephalotrichum lignatile,* and *Pseudeurotium ovale*). Within Russet Burbank, three fungal ASVs were significantly more abundant, but only two were named to species (*Oliveonia olivonia, Plectophaerella cucumerina*, and a *Hypocreales* sp.).

3.3.3 Rotation length impacts to soil microbial communities

Within rotation lengths of two and three years, bacterial diversity factors were not significantly different (Table 1a). However, all fungal diversity metrics were significantly different by rotation length, with Shannon alpha diversity being the most significantly different ($p < 0.001$; Table 1b). When PCoA ordinations of Jaccard and Bray-Curtis dissimilarity were constructed, bacterial clusters of ASVs by rotation length did not significantly differentiate (Figure 3.3a). However, fungal clusters of ASVs indicated that with both Bray-Curtis and Jaccard dissimilarity, clusters had differentiated ($p < 0.001$ and 0.001, respectively; Figure 3.3b).

When bacterial core community members were identified, 72 were found to be core to two-year rotations and 58 were found to be core to three-year rotations (Table 3.2). When common core community members were removed, 9 were conditionally core to two-year rotations and none were conditionally core to three-year rotations. Of these core members, only one was uniquely core to two-year rotations and could not be attributed to any other factor group (an unidentified ASV in the order *Burkholderiales* and family *A21b*). When fungal core community members were identified, 77 were found to be core to two-year rotations and 76 were

found to be core to three-year rotations. When common core members were removed, six were found to be conditionally core to two-year rotations and six were found to be conditionally core to three-year rotations. Of these, none of the two-year and five of the three-year rotation core members were uniquely core to three-year rotations and no other treatment group (*Pseudogymnoascus pannorum, Oidiodendron truncatum, Mortierella gamsii, Solicoccozyma terricola*, and *Exophiala equina*).

When comparing differentially abundant bacterial species, there were sixteen ASVs by rotation length. The two-year rotations had nine more abundant bacterial ASVs, none of which were named to species, but six of which were identified to genus (*Dyella* sp., *Actinospica* sp., *Mucilaginibacter* sp., *Gemmatimonas* sp., *Scopulibacillus* sp., and *Streptosporangium* sp.). The three-year rotations had seven more abundant bacterial ASVs, one of which was named to species (*Glycomyces lechevalierae*), of the remaining six, four were identified to genus (*Flavisolibacter* sp., *Glycomyces* sp., *Streptomyces* sp., and *Caenimonas* sp.). Within fungal ASVs, there were 69 differentially abundant by rotation length. Two-year rotations had 40 more abundant fungi, 24 of which were named to species and three-year rotations had 29 more abundant fungal ASVs, 20 of which are named to species. Of the 40 more abundant fungal ASVs within the rotation length of two-years, 31 belonged to the phylum Ascomycota. The primary lifestyle of these fungi described by FungalTraits which were more abundant in the two-year rotations were mostly different types of saprotrophs (52.5%), plant pathogens (10%), arbuscular mycorrhizae (7.5%), animal parasites (7.5%), or were unidentified functionally (22.5%). Within the three-year rotation, a similar trend was seen, and 58.6% of fungal ASVs which were more abundant were various types of saprotrophs. The remaining ASVs were described as plant

pathogens, algal parasites, lichen parasites, animal parasites, or functionally unidentified (10.3%, 3.4%, 3.4%, 6.9%, and 17.2%, respectively).

3.3.4 Green manure impacts to soil microbial communities

Within treatments which either had green manures included in crop rotations or not, no bacterial nor fungal diversity metrics were significantly different (Table 1a and 1b). When PCoA ordinations of both Jaccard and Bray-Curtis dissimilarity were constructed, bacterial communities were not significantly different, but fungal communities were significantly different (p = 0.011 and 0.014, respectively; Figure 3.3a and 3.3b).

When bacterial core communities were identified, 59 ASVs were identified as core with green manure and 62 were identified as core without green manure (Table 3.2). When all common core community members were excluded, two bacterial ASVs remained conditionally core to green manure, both of which were uniquely core members to green manure treatments and no other management factors (*Puia* sp. and *Strepomyces* sp.). For treatments without green manure, when all other common core members were excluded, two were conditionally core to treatments without green manures and only one was uniquely core to treatments without green manure and no other management factor (*Streptomyces* sp.). For fungal communities, 70 were core to green manure treatments and 74 were core without green manure. When common core communities were excluded, two core members were shared between treatments with and without green manures (*Mortierella gamsii* and *Monocillium griseo-ochraceum).* Within treatments with green manure, four were conditionally core and of those, one was uniquely core member to only green manure treatments and no other management factors (*Mortierella amoeboidea*). Of treatments without green manure, two were conditionally core, and both were

uniquely core members to treatments without green manure and no other management factors (*Gyoerffyella* sp. and *Sanchytriales* sp.).

When comparing differentially abundant microbial communities, no bacterial ASVs were differentially abundant with green manure use, and 10 fungal ASVs were differentially abundant. There were six more abundant fungi with green manure, three of which were identified to species (*Plectophaerella cucumerina, Albifimbria verrucaria,* and *Pseudogymnascus appendiculatus*). There were four more abundant fungal ASVs without green manure, one of which identified to species (*Rhizopus arrhizus*).

3.3.5 Non-grain rotation crops impacts to soil microbial communities

With non-grain rotation crops, no bacterial diversity metrics were found to be significant, but found that for fungal communities, Shannon diversity, Observed taxa, ACE Richness, and Chao1 Richness were all significant (p = 0.014, 0.002, 0.005, and 0.01, respectively; Table 1a and 1b). When PCoA ordinations of Bray-Curtis and Jaccard dissimilarity were drawn, bacterial communities were not significantly different, but fungal communities were significantly different (p = 0.014 and 0.011, respectively; Figure 3.3a and 3.3b).

When bacterial core communities were investigated, 57 were found to be core to treatments with non-grain rotation crops and 64 were found to be core to treatments without non-grain rotation crops (Table 3.2). When common core members were excluded, none were shared, three were conditionally core to non-grain rotation crops and one was conditionally core to treatments without non-grain rotation crops. Of those conditionally core bacterial community members, none were uniquely core by these management factors only. When the fungal core community members were investigated, 65 were found to be core to non-grain rotation crop treatments, and 64 were found to be core to treatments without non-grain rotation crops. When

fungal common core fungal members overall were excluded, two were conditionally core to treatments with non-grain rotation crops, and none were conditionally core to treatments without non-grain rotation crops. However, none of these core fungal ASVs were uniquely core members to only this factor.

When comparing differentially abundant bacterial ASVs, one was significantly increased when non-grain rotation crops were planted (*Pseudarthrobacter* sp.). Within fungal ASVs, 23 were differentially abundant with non-grain rotation crop usage, 15 of which were in the phylum Ascomycota. Of those, 17 ASVs were more abundant with non-grain rotation crops and of those, 13 were identified to species. Of all ASVs identified to be in increased abundance with non-grain rotation crops, approximately 65% were saprotrophs, 17% were plant pathogens, and 17% were unidentified functionally. There were six fungal ASVs which were more abundant without non-grain rotation crops, and of those, three were identified to species. Of those fungal ASVs which were more abundant without non-grain rotation crops, FungalTraits identified that one was a saprotroph, one was a plant pathogen, and one was an animal parasite.

3.3.6 Compost impacts to soil microbial communities

Of all bacterial diversity metrics, Shannon alpha diversity and evenness were significant with compost amendment (p = 0.008 and 0.015, respectively; Table 1a). Of all fungal diversity metrics, only Chao richness was significant (p = 0.01; Table 1b). When PCoA ordinations were constructed to display Jaccard and Bray-Curtis bacterial and fungal dissimilarity, all were significantly differentiated (Figure 3.3a and 3.3b).

When core bacterial communities were investigated by compost amendment, 54 were found to be core to compost amended plots, and 71 were found to be core without compost, and 52 were shared between then (Table 3.2). When common core members were excluded, none

were shared, one was conditionally core with compost amendment and 7 were conditionally core without compost. Only one core member was uniquely core to compost amendment (*Sphaerobacter thermophilus*), none were uniquely core to treatments without compost. For fungal core community members, 67 were core to compost amendments and 78 were core to without compost, with 62 shared by both treatments. When common core members were excluded, three were shared, five were conditionally core to compost and two were conditionally core without compost. Of those, four were uniquely core members to the compost management factor only (two subspecies of *Acrodontium hydnicola*, *Wardomyces inflatus*, and an ASV in the *Extremaceae* family).

There were 183 differentially abundant bacterial ASVs with compost amendment. Of these, six were more abundant without compost, none of which were named to species, and there were 177 bacterial ASVs which were more abundant with compost amendment, twelve of which were identified to species. With compost amendment, the top three phyla in which ASVs increased in abundance as a result of the treatments were Actinobacteriota, Firmicutes, and Chloroflexi, with ASV counts of 54, 47, and 25, respectively (Table 3). Without compost amendment, the top three phyla with increased ASVs were Actinobacteriota, Chloroflexi, and Firmicutes, however, much fewer ASVs were enhanced compared to with compost amendment (2, 1, and 1, respectively; Table 3). Of fungal ASVs, there were 91 which were differentially abundant with compost. Of these, 20 were more abundant without compost amendment, 7 of which were identified to species and there were 71 more abundant fungi with compost amendment, 39 of which were identified to species. Of the 71 fungal ASVs which increased in abundance with compost amendment, the majority, 55%, were identified by Fungal Traits as saprotrophs of various kinds. Of the remaining ASVs, 38% were unidentified, 4% were plant

pathogens, and 3% were animal parasites. Many of the ASVs associated with compost amendment were not able to be identified regarding functionality, however, of those which were, the majority were saprotrophic. Of the fungal ASVs which were more abundant without compost, the largest group was again various saprotrophs representing 40%, although it is important to note that this consisted of only 8 ASVs total in comparison to the 39 saprotrophic ASVs which were more abundant with compost. Without compost, the next most abundant functional groups were not identified (30%), plant pathogens (25%), and algal parasites (5%).

3.3.7 Fumigation impacts to soil microbial communities

Bacterial diversity metrics were not significant except for evenness (p = 0.021; Table 1a). Observed fungal diversity, fungal evenness, and Chao1 richness were all significant (Table 1b). When PCoA ordinations of Jaccard and Bray-Curtis dissimilarity by fungal and bacterial communities were constructed, all four were significantly different between fumigated and non-fumigated treatments (Figure 3.3a and 3.3b).

When core bacterial communities were described, 63 ASVs were found to be core to fumigation and 70 were core without fumigation, 45 were shared by both treatments. When bacterial common core members were excluded, 13 were conditionally core to fumigation, 12 were conditionally core without fumigation, and one was shared between both fumigated and unfumigated treatments (Table 3.2). Of the 13 uniquely core bacterial ASVs identified, all 13 were uniquely core to fumigated treatments, and of the 12 conditionally core to non-fumigated treatments, four of those were uniquely core. For fungal ASVs, 56 were identified to be core to fumigated treatments and 79 were core without fumigation, of these, 38 were shared between both. When common core members were excluded, one was shared, 15 were unique to fumigation (14 being uniquely core to fumigation), and 10 were conditionally core to non-

fumigated plots (with 4 uniquely core members to non-fumigated plots). Of the 13 uniquely core bacterial ASVs to fumigation, none were identified to species, but 8 were identified to genus (*Candidatus Udaeobacter* sp., *Mesorhizobium* sp., *Gemmatimonas* sp., *Sphingomonas* sp., *Pseudarthrobacter* sp., *Nocardioides* sp., *Roseimicrobium* sp., and *Tychonema CCAP 1459-11B* sp.). Of the 14 uniquely core fungal ASVs to fumigation, 11 were assigned functions by FungalTraits, these included 7 saprotrophs and 4 plant pathogens.

There were 190 differentially abundant bacteria with fumigation, of these 116 were less abundant with fumigation, and 74 were more abundant with fumigation. Of the 116 less abundant with fumigation, none were identified to species. The top three phyla which were significantly less abundant with fumigation were Proteobacteria, Acidobacteriota, and Actinobacteriota, with 33, 23, and 15 bacterial ASVs, respectively (Table 3.3). Of the 74 more abundant with fumigation, 8 were identified to species. The top three phyla which were significantly more abundant following fumigation belonged to the Actinobacteriota, Proteobacteria, and Acidobacteriota phyla with 21, 21, and 8 ASVs belonging to each, respectively (Table 3.3). For fungal ASVs, there were 128 differentially abundant with fumigation, which was the most of any other management factor. Of these, 67 were less abundant with fumigation, 33 of which were identified to species. There were 61 more abundant fungi with fumigation, and of these 30 were identified to species. ASVs in the Ascomycota phylum were approximately evenly enhanced and suppressed (40 and 37, respectively). The next largest group to have increased abundances were in the phylum Chytridiomycota, and 12 ASVs were enhanced with fumigation. Of the fungi which were more abundant with fumigation, Fungal Traits indicated that 46% were saprotrophs and 28% were plant pathogens, the remaining ASVs were either lichen parasites or unidentified (2% and 24%, respectively). Of the fungal

ASVs which were reduced by fumigation, 37 ASVs were identified to be members of the Ascomycota phylum. The next most reduced phylum were those ASVs in Mortierellomycota, with 10 significantly less abundant after fumigation. Overall ASVs which were less abundant with fumigation, 49% were saprotrophs, 4% were plant pathogens, and the remaining were either unidentified, mycoparasites, root endophytes, or algal or animal parasites (36%, 6%, 1%, 2%, and 2%, respectively).

3.3.8 Results summary

Overall, bacterial community diversity, evenness, and richness were less influenced by the management factors compared to fungal communities. Bacterial community diversity metrics were only significantly different with compost amendments and fumigation. On the other hand, fungal community diversity metrics were significantly different by rotation length, fumigation, compost, and with non-grain rotation crops. Similarly, bacterial PCoA ordinations of Bray-Curtis and Jaccard dissimilarity clusters were significantly different by rotation length, compost, and fumigation. For fungal PCoA ordinations, Bray-Curtis and Jaccard dissimilarity were significantly different by all management factors except variety. The most bacterial and fungal uniquely core members were identified by fumigation, indicating that specific species were more able to proliferate overall following fumigation, including saprotrophs and plant pathogens. For differential abundances, it was found that bacterial species overall had the most variation as the result of the management factors, however, the bulk of the differentiation within bacterial species was as the result of fumigation (55.2%) and compost amendment (41%), with the remaining differentiation as the result of rotation length (3.6%) and green manure usage (0.2%). Fungal differentiation was more influenced by the management factors and differentiation was seen

across all treatments. Fumigation (39%) and compost amendment (27.7%) were once again responsible for the bulk of fungal differentiation.

Table 3.1a Results of bacterial diversity metrics as *p*-values analyzed using PERMANOVAs with 10,000 permutations of soils collected in a study of management impacts to soil health in potato producing fields in Maine. Significant numbers indicate that management factors were significantly different either from or within standard practices. Bold values indicate that these diversity statistics were significantly different.

Management Factor	Alpha Diversity		Evenness	Richness	
	Shannon	Observed		ACE	Chao
Variety	0.616	0.925	0.724	0.960	0.973
Rotation length	0.555	0.845	0.179	0.648	0.665
Green Manure	0.597	0.994	0.478	0.884	0.823
Compost	**0.008**	0.295	**0.015**	0.360	0.306
Fumigation	0.429	0.070	**0.021**	0.672	0.067
Non-grain Rotation Crops	0.501	0.709	0.963	0.478	0.466

Table 3.1b Results of fungal diversity metrics as *p*-values analyzed using PERMANOVAs with 10,000 permutations. Significant numbers indicate that management factors were significantly different either from or within standard practices. Bold values indicate that these diversity statistics were significantly different.

Management Factor	Alpha Diversity		Evenness	Richness	
	Shannon	Observed		ACE	Chao1
Variety	0.618	0.466	0.809	0.742	0.861
Rotation length	**< 0.001**	**0.004**	**0.007**	**0.014**	**0.020**
Green Manure	0.203	0.791	0.17	0.811	0.818
Compost	0.156	0.475	0.261	**0.035**	0.061
Fumigation	0.056	**0.013**	0.385	**0.010**	**0.016**
Non-grain Rotation Crops	**0.014**	**0.002**	0.130	**0.005**	**0.010**

Table 3.2 Core community members by different management factors from soils collected in a study of management impacts to soil health in potato producing fields in Maine. Total with common core members indicated core members including those which were core to all other treatments. Conditionally core members were core to that management factor level. Uniquely core members were not core to any other management factor or level, but only core to that specific management factor level.

Management factor	Management factor level	Total 16S including common core	16S conditionally core	16S uniquely core	Total ITS including common core	ITS conditionally core	ITS uniquely core
Variety	Caribou Russet	65	2	1	73	2	0
	Russet Burbank	67	5	0	76	5	0
Rotation length	Two-year	72	9	1	77	6	0
	Three-year	58	0	0	76	6	5
Green manure	With green manures	59	2	2	70	4	1
	Without green manures	62	2	1	74	2	2
Fumigation	With fumigation	63	13	13	56	15	14
	Without fumigation	70	12	4	79	10	4
Compost	With compost	54	1	1	67	5	4
	Without compost	71	7	0	78	2	0
Non-grain rotations	With non-grain rotations	57	3	0	65	2	0
	Without non-grain rotations	64	1	0	64	0	0

Table 3.3 Differential abundance counts by bacterial or fungal phylum separated by management factors and levels of microbial communities isolated from soils collected in a study of management impacts to soil health in potato producing fields in Maine.

Differentially Abundant Phyla					
Factor		**Bacteria**		**Fungi**	
		Phyla	Count	Phyla	Count
Variety	Caribou Russet			Mucoromycota	2
	Caribou Russet			Ascomycota	2
	Russet Burbank			Basidiomycota	1
	Russet Burbank			Ascomycota	2
Rotation Length	Two-year	Actinobacteriota	2	Ascomycota	31
	Two-year	Firmicutes	2	Glomeromycota	3
	Two-year	Bacteroidota	1	Mortierellomycota	3
	Two-year	Chloroflexi	1	Chytridiomycota	2
	Two-year	Gemmatimonadota	1	NA	1
	Two-year	Proteobacteria	1		
	Two-year	Verrucomicrobiota	1		
	Three-year	Actinobacteriota	3	Ascomycota	14
	Three-year	Proteobacteria	2	Basidiomycota	9
	Three-year	Bacteroidota	1	NA	2
	Three-year	Gemmatimonadota	1	Chytridiomycota	1
	Three-year			Monoblepharomycota	1
	Three-year			Mortierellomycota	1
	Three-year			Mucoromycota	1
Green Manure	With			Ascomycota	6
	Without			Chytridiomycota	1
	Without			Ascomycota	2
	Without			Mucoromycota	1

Table 3.3 (continued) Differential abundance counts by bacterial or fungal phylum separated by management factors and levels of microbial communities isolated from soils collected in a study of management impacts to soil health in potato producing fields in Maine.

Differentially Abundant Phyla					
Factor		**Bacteria**		**Fungi**	
		Phyla	Count	Phyla	Count
Fumigation	With	Actinobacteriota	21	Ascomycota	40
	With	Proteobacteria	21	Chytridiomycota	12
	With	Acidobacteriota	8	Mortierellomycota	4
	With	Verrucomicrobiota	7	Basidiomycota	3
	With	Bacteroidota	5	Monoblepharomycota	1
	With	Chloroflexi	4	Mucoromycota	1
	With	Myxococcota	3		
	With	Cyanobacteria	2		
	With	Armatimonadota	1		
	With	Bdellovibrionota	1		
	With	Gemmatimonadota	1		
	Without	Proteobacteria	33	Ascomycota	37
	Without	Acidobacteriota	23	Mortierellomycota	10
	Without	Actinobacteriota	15	Basidiomycota	6
	Without	Chloroflexi	15	Chytridiomycota	4
	Without	Verrucomicrobiota	12	Mucoromycota	4
	Without	Gemmatimonadota	7	NA	2
	Without	Bacteroidota	5	Kickxellomycota	1
	Without	Myxococcota	2	Monoblepharomycota	1
	Without	Nitrospirota	2	Rozellomycota	1
	Without	Planctomycetota	2	Zoopagomycota	1
Compost	With	Actinobacteriota	54	Ascomycota	48
	With	Firmicutes	47	Basidiomycota	13
	With	Chloroflexi	25	Chytridiomycota	3
	With	Proteobacteria	24	Mucoromycota	2
	With	Myxococcota	7	Mortierellomycota	2
	With	Acidobacteriota	4	NA	2
	With	Bacteroidota	4	Monoblepharomycota	1
	With	Gemmatimonadota	3		
	With	Deinococcota	2		
	With	Halanaerobiaeota	1		

Table 3.3 (continued) Differential abundance counts by bacterial or fungal phylum separated by management factors and levels of microbial communities isolated from soils collected in a study of management impacts to soil health in potato producing fields in Maine.

Differentially Abundant Phyla					
Factor		**Bacteria**		**Fungi**	
		Phyla	Count	Phyla	Count
Compost	Without	Actinobacteriota	2	Ascomycota	13
	Without	Chloroflexi	1	Basidiomycota	3
	Without	Firmicutes	1	Chytridiomycota	3
	Without	Gemmatimonadota	1	Mucoromycota	1
	Without	Verrucomicrobiota	1		
Non-Grain Rotations	With	Actinobacteriota	1	Ascomycota	13
	With			Basidiomycota	2
	With			Mucoromycota	1
	With			NA	1
	Without			Ascomycota	2
	Without			Basidiomycota	1
	Without			Chytridiomycota	2
	Without			Mucoromycota	1

Figure 3.1 Distribution of all bacterial (A) and fungal (B) ASV phyla over all treatments over all sampling timepoints isolated from soils collected in a study of management impacts to soil health in potato producing fields in Maine. Bacteria which were described as "other" were present in amounts of less than 5% of the total, fungi described as "other" were present in amounts less than 4% of the total.

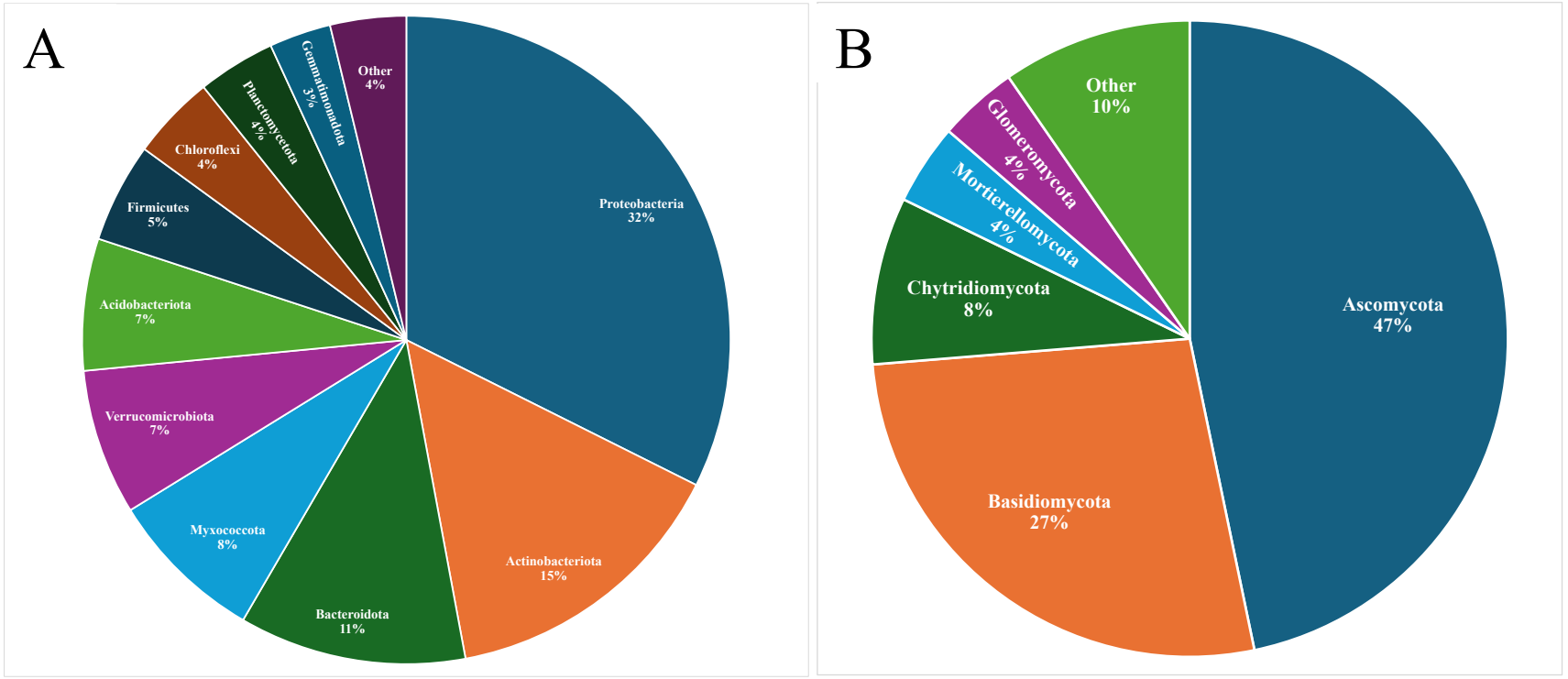


Figure 3.2a Heatmap indicating the abundances by treatments of the top twenty most abundant bacterial ASVs over the entire dataset in the final year of the study of management impacts to soil health in potato producing fields in Maine.

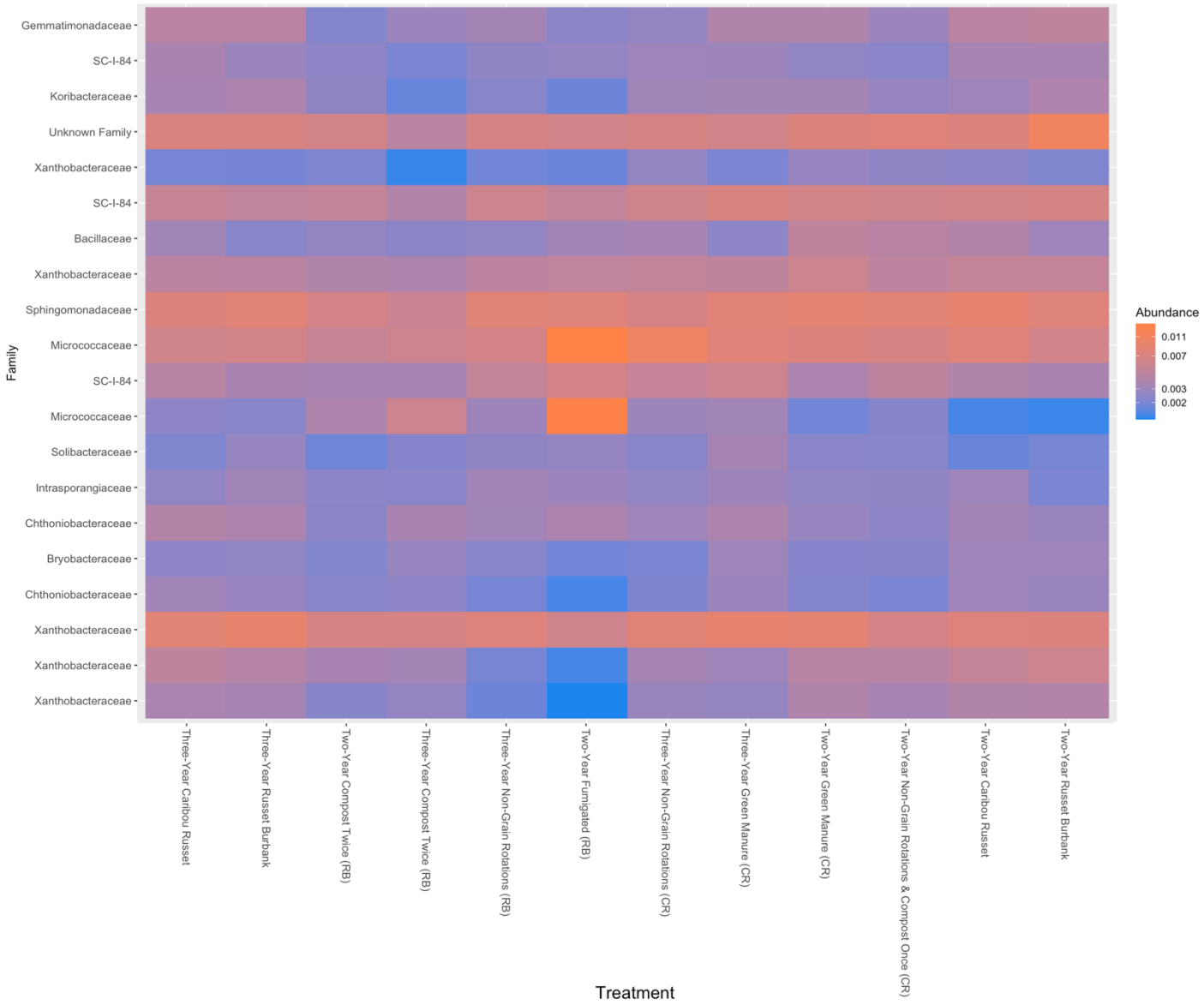


Figure 3.2b Heatmap indicating the abundances by treatments of the top twenty most abundant fungal ASVs over the entire dataset in the final year of the study of management impacts to soil health in potato producing fields in Maine.

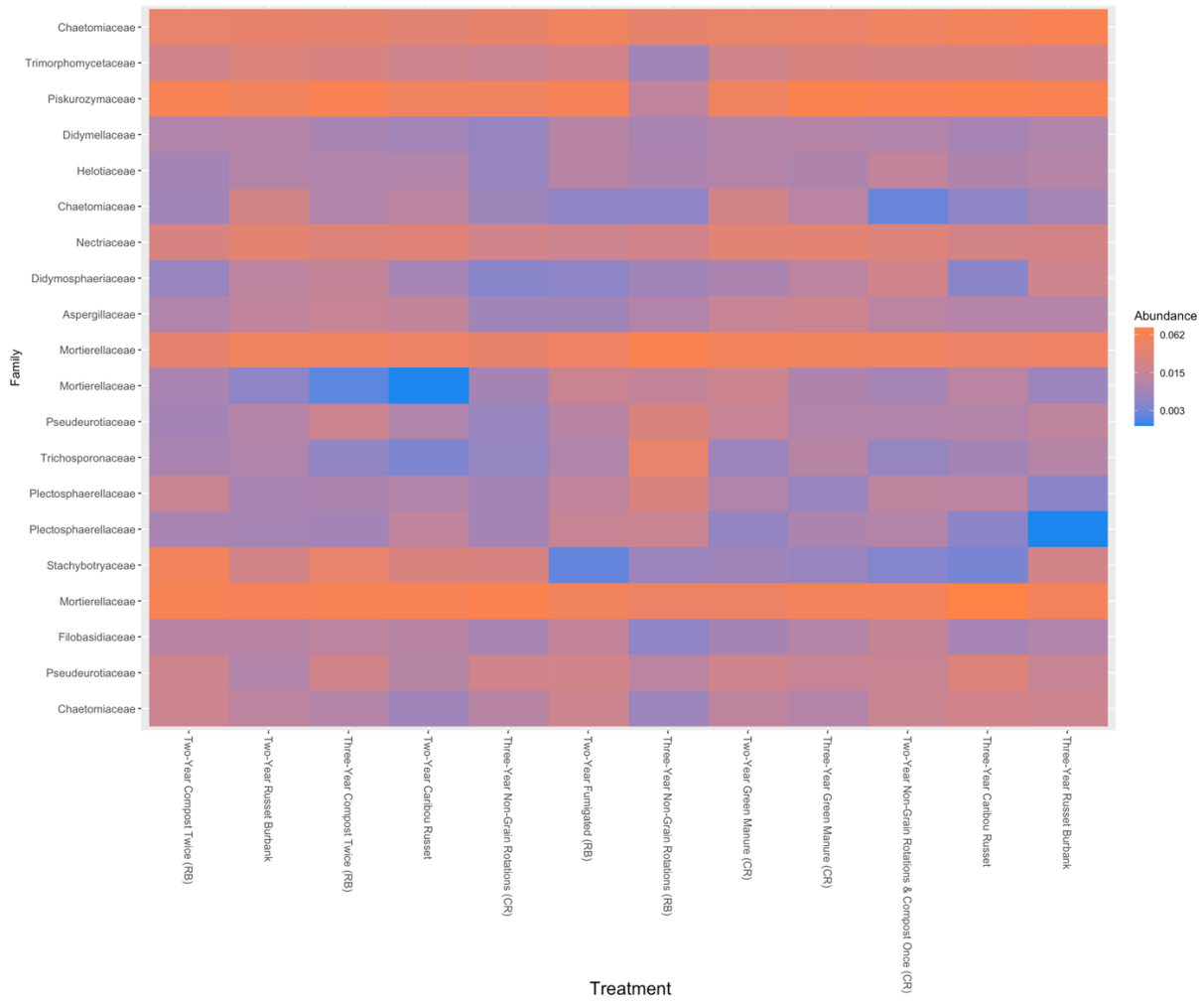


Figure 3.3a Principal Coordinate Analysis (PCoA) of bacterial Bray-Curtis Dissimilarity by different management factors subset and compared either to or within only standard practice treatments. Bacterial communities were isolated from soils collected in a study of management impacts to soil health in potato producing fields in Maine.

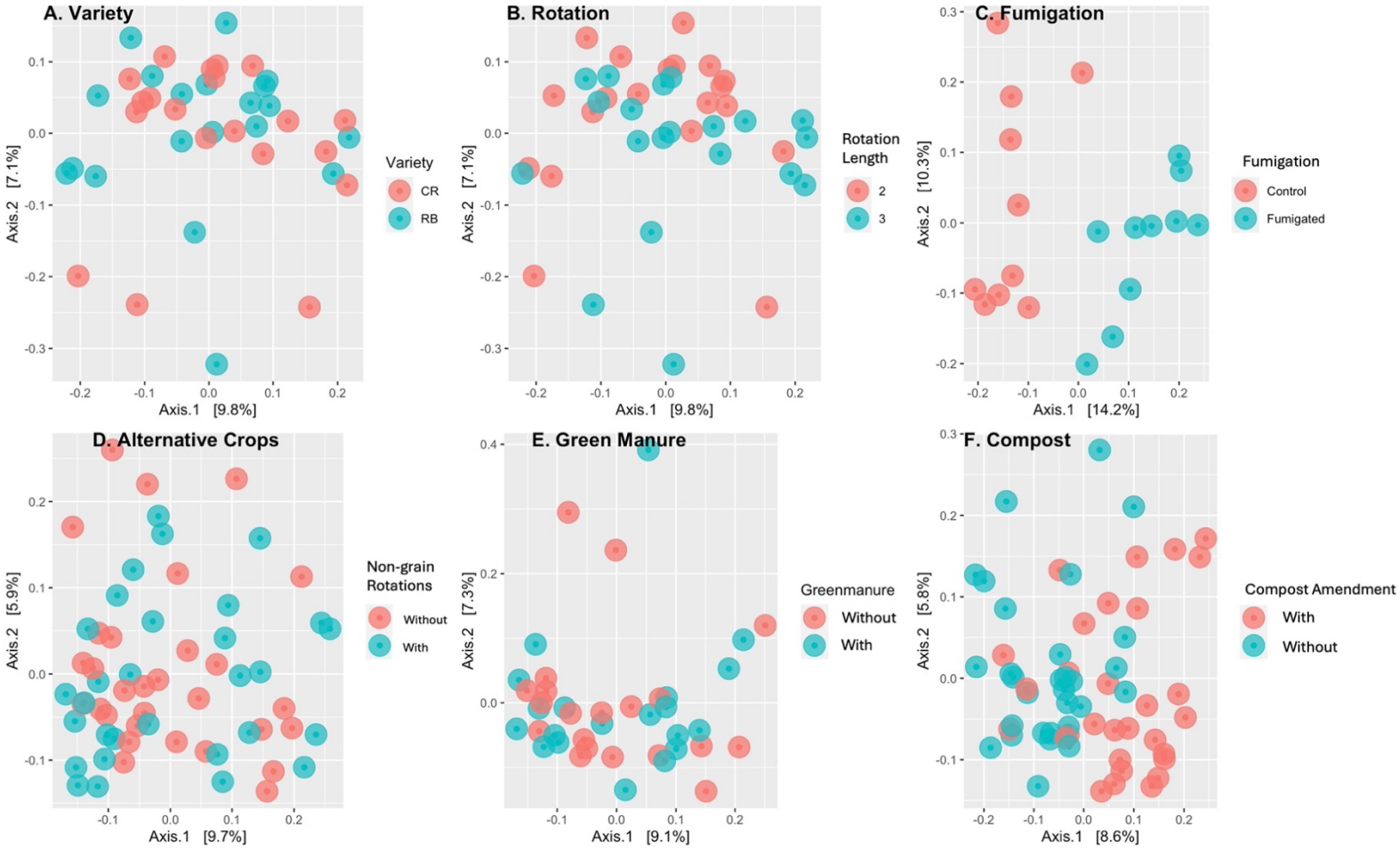
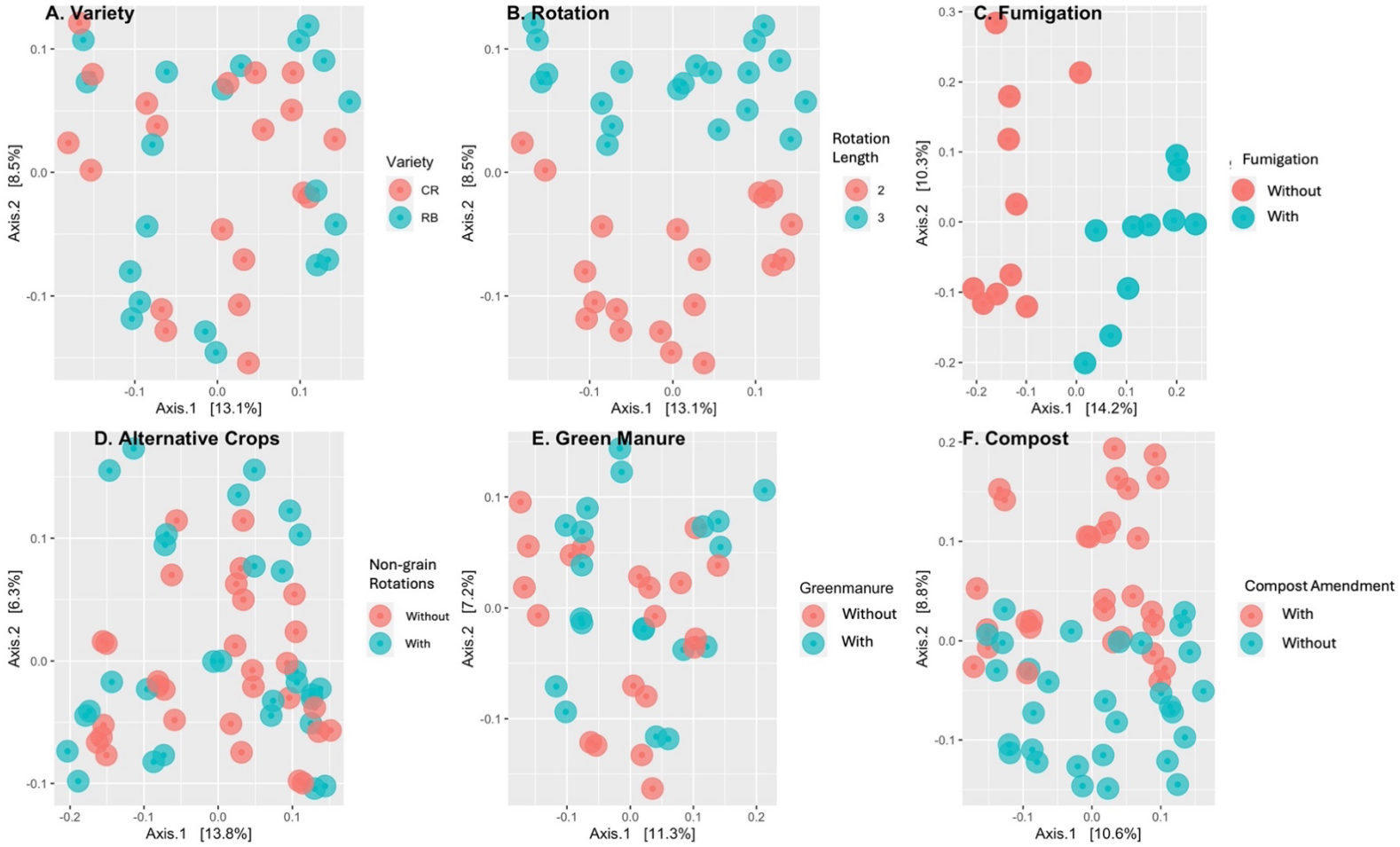


Figure 3.3b Principal Coordinate Analysis (PCoA) of fungal Bray-Curtis Dissimilarity by different management factors subset and compared either to or within only standard practice treatments. Fungal communities were isolated from soils collected in a study of management impacts to soil health in potato producing fields in Maine.



3.4 Discussion

3.4.1 Maine soil microbial communities overall

Maine potato production is distinct from potato production in other states, and this can be partially attributed to the differences in soil types, as Maine soils contains more organic matter and total carbon comparatively, and lack of irrigation (Klasek et al. 2023). Additionally, regional differences in the soil microbial communities in potato producing states are critical, as Bray-Curtis Dissimilarity of both fungi and bacterial communities has been shown to vary by 54-60% due to field site (Klasek et al. 2023).

Of bacterial ASVs identified over all sampling timepoints in this study, the top three phyla present were Proteobacteria, Actinobacteriota, and Bacteroidota. These groups were found to be present in similar proportions across seven other potato producing states (Klasek et al. 2023). The most abundant bacterial ASV was a *Bradyrhizobium* sp. over all sampling timepoints and treatments. It was demonstrated that Maine soils are dominated by bacteria in the phylum Proteobacteria, as this phylum constituted the largest percentages of ASVs overall and within core communities.

Fungal ASVs of the top ten phyla over seven different potato producing states indicated that a smaller proportion of the most abundant fungi in potato producing soils belong to the phylum Ascomycota in Maine. Compared to other states, ASVs in the phylum Ascomycota made up 50%-75% of the most abundant ASVs detected (Klasek et al. 2023). In this study, when the top twenty most abundant fungal ASVs were analyzed, only 48% were in the phylum Ascomycota. In potato soil microbial communities at a national scale, three species of *Mortierella* were detected to be core to Maine potato producing soils, although *M. minutissima* has been found to be present in higher amounts in Maine compared to other potato producing

states (Klasek et al. 2023). This fungal taxa was also found to Michigan in slightly lower levels than Maine, but was much less abundant compared to western potato producing states, albeit still present (Klasek et al. 2023). *M. minutissima* was the most abundant fungal ASV over all sampling timepoints and treatments. The largest proportion of fungal ASVs in Maine were described by FungalTraits as various kinds of saprotrophs, when considering all ASVs detected at all sampling time points or within core communities. While the majority of fungi were described as saprophytes through the use of amplicon sequencing and FungalTraits, a similar proportion of fungi were identified as saprotrophs when a phospholipid fatty acid (PLFA) analysis performed on the same soil samples (Chapter 2). Using sequencing, it was determined that 70% of the top twenty most abundant ASVs were identified as saprophytes, compared to the average of 75% which were identified as saprophytes using a PLFA analysis. In a study of global fungal community structure, it was found that of fungal ecological groups, soil nitrogen content and mean annual precipitation were positive predictors and soil pH was a negative predictor of richness of saprophytes, among other factors (Mikryukov et al. 2023). The authors explain that temperate rainforest regions, such as the Appalachian Mountains, which pass through Maine, including the potato producing regions, were described as diversity hotspots for saprotrophs and molds (Mikryukov et al. 2023). Although there were trends overall in Maine potato producing soils, in this study it was demonstrated that within these soil communities, management factors can significantly impact microbial communities.

3.4.2 Microbial differentiation by management factors

Bacterial communities were less differentially abundant and impacted overall than fungal communities as a result of the treatments. However, the use of fumigation, compost, and non-grain rotation crops all resulted in differentially abundant bacterial ASVs. For treatments with

non-grain rotation crops, only one bacterial ASV was more abundant, an unidentified *Pseudarthrobacter* sp. in the Actinobacteriota phylum. Potato variety had minimal impact on the soil microbial communities, with no significant differences observed for any measured parameter and only minor changes in differential abundance. However, these microbial communities were based solely on bulk soil samples. Potential variety effects are expected to be greatest in the rhizosphere and on the root surfaces, as this has been seen in other studies analyzing potato rhizosphere microbial communities (İnceoğlu et al. 2012). Thus, if rhizosphere communities had been sampled, it is possible more varietal effects would have been observed.

The top three phyla which were significantly more abundant following fumigation belonged to the Actinobacteriota, Proteobacteria, and Acidobacteriota phyla, and in the absence of fumigation, ASVs in the phyla Proteobacteria, Acidobacteriota, and Actinobacteriota were significantly less abundant. Proteobacteria, Acidobacteriota, and Actinobacteria, in that order, are considered the top three most common bacterial phyla which are detected through 16S rRNA sequencing of soil samples (Janssen 2006). Actinobacteria are filamentous gram positive bacteria, which produce mycelia, and are common to both terrestrial and aquatic environments (Hazarika and Thakur 2020). These bacteria are well known to produce antibiotics, enzymes, and are capable of decomposing various organic substances (Hazarika and Thakur 2020). This bacterial phyla, which was more suppressed than enhanced with fumigation (21 ASVs compared to 15 ASVs), contains species which are considered beneficial bacteria which are capable of plant growth promotion and antagonism of pathogens (Hazarika and Thakur 2020). ASVs in the phylum *Proteobacteria* were also both enhanced and suppressed by fumigation, although more were suppressed than enhanced (33 ASVs compared to 21 ASVs). Of the ASVs which were either more or less abundant with fumigation in the phylum *Proteobacteria*, all of them were

either in the classes Alphaproteobacteria or Gammaproteobacteria. Proteobacteria are common in soils, regardless of soil type, and especially Alphaproteobacteria are common (Zhang and Xu 2008). Bacteria in the Acidobacteria phylum are also highly common in soil environments, but are not well studied as they are difficult to culture (Thrash and Coates 2015; Kalam et al. 2020). Acidobacteria possess genes to perform a large range of soil ecosystem services, such as nitrogen, carbon, and sulfur cycling, plant growth promotion, influencing soil structure, and producing secondary metabolites (Kalam et al. 2020). Bacteria in the phylum Acidobacteria are also capable of producing biofilms, possess mobile genetic elements, and are highly tolerant to extreme environmental conditions ranging from extremes in pH to starvation (Kalam et al. 2020). As such, Acidobacteria are also considered keystone microbial species in agricultural soil environments, meaning that they have influence over the microbial community composition and function regardless of their abundance (Banerjee et al. 2018). Bacterial ASVs in the phylum Acidobacteria were found to be significantly less abundant as the result of fumigation, although some ASVs were more abundant (23 ASVs compared to 8 ASVs). In summary, fumigation altered bacteria in the three most abundant phyla in soil environments. However, fumigation suppressed more ASVs belonging to the phyla Actinobacteria, Proteobacteria, and Acidobacteria than it enhanced, which clearly impacted the bacterial community structure as also indicated by bacterial diversity ordinations of Bray-Curtis and Jaccard dissimilarity, which were significantly different under fumigation. These highly common phyla also contain many species known to be beneficial and keystone microbial species. Fumigation in general reduced bacterial diversity, although not significantly, but significantly increased bacterial evenness, indicating that ASVs were less varied in their abundances (Hill et al. 2003). This could indicate a reduction in highly

abundant and common bacterial phyla members, reduction in diversity overall, and a homogenizing of ASV abundance distribution.

With compost amendment, the top three phyla in which ASVs increased in abundance as a result of the treatments were Actinobacteriota, Firmicutes, and Chloroflexi. Without compost amendment, the top three phyla with increased ASVs were Actinobacteriota, Chloroflexi, and Firmicutes, however, many fewer ASVs were enhanced compared to with compost amendment. Bacteria in the phylum Firmicutes are considered beneficial bacteria due to their capacity for plant growth promotion, biological control, and their extensive relationship with plant roots (Hashmi et al. 2020). Additionally, Firmicutes are capable of bioremediation of heavy metal contaminated soils and even promoting the growth of plants being grown in metal contaminated soils (Hashmi et al. 2020). Firmicutes are also capable of suppressing various fungal, bacterial, and oomycete pathogens through the production of antibiotics and antifungal agents (Hashmi et al. 2020). While these beneficial bacteria have been seen to suppress pathogens across a diverse range of plant hosts, bacteria in the Firmicutes and Actinobacteria phyla have also been shown to be more abundant in potato fields with decreased bacterial soft rot incidences, caused by *Pectobacterium carotovorum*, compared to fields with higher disease incidences (Kurm et al. 2024). Bacteria in the phylum Chloroflexi are not yet fully validated, but in general, members are mostly filamentous and gram negative but with atypical cell envelopes (Hanada 2014). Many uncultured strains are speculated to exist in a many different environments (Speirs et al. 2019). Chloroflexi bacteria are known to be capable of remediating toxic contaminants, wastewater, and sludge, but are still relatively understudied (Hanada 2014; Speirs et al. 2019; West-Roberts et al. 2021). In summary, with compost amendment two phyla containing beneficial bacteria, Actinobacteria and Firmicutes, and a phylum containing bacteria capable of bioremediation,

Chloroflexi, were enhanced significantly compared to unamended treatments. Compost amendment resulted in differential abundances of phyla which are known to be capable of bioremediation, plant growth promotion, and plant pathogen suppression, although the addition of compost increased these three groups in much greater quantities with compost compared to without (126 ASVs increased with compost compared to 4 increased without compost). A similar trend could also be seen in the change in diversity metrics by compost amendment, in which Shannon diversity and evenness were both significantly higher with compost amendment. This is in contrast to fumigation, which decreased diversity, but enhanced evenness.

Fungal ASV abundances were more sensitive to the treatments and significantly different abundances were seen in all of the management factors. However, three treatments, variety, green manure, and the use of non-grain rotation crops, all resulted in smaller amounts of differentially abundant fungal ASVs, 7, 10 and 23, respectively. Variety did not result in any other significant differences, nor did the use of green manure except when Bray-Curtis and Jaccard dissimilarity PCoA ordinations were constructed. This could be the result of improper green manure incorporation. Green manures, such as mustards which were used in this study, produce glucosinolates as the active biofumigant, but up to 75% of this compound can be lost after grinding fresh plant matter within three minutes (Lazzeri et al. 2004). Consequently, the successful application of green manures is highly sensitive to the incorporation technique, which could have impacted the results of this study.

The use of non-grain rotation crops resulted in more abundant fungal ASVs, the majority of which belonged to the phylum Ascomycota, a large group which ranges from plant pathogens to mutualists to yeasts (Berbee 2001). Fungi in the Ascomycete phylum have been found to be dominant in soil environments, particularly the members of the subdivision Pezizomycotina

(Egidi et al. 2019). Many fungi in the Pezizomycotina subdivision can be classified as plant pathogens, and are thought to be dominant because they are wind dispersed, and have a large number of genes for stress tolerance and the uptake of nutrients (Berbee 2001; Egidi et al. 2019). Of these common classes, three specifically are known to contain a large number of plant pathogens: Sordariomycetes, Dothideomycetes, and Leotiomycetes (Berbee 2001). However, the majority of all ASVs with increased abundance following the use of non-grain rotation crops were identified as saprotrophs. This is unsurprising that the use of non-grain rotation crops in a rotation series could enhance saprotrophs, as there can be more organic matter left over when various crops are grown, especially compared to potatoes, which are known to produce small amounts of crop resides post-harvest (Wright et al. 2017; Larkin 2021). Additionally, while not highly abundant, the increased abundance of three plant pathogen species is also not surprising, as one of the most critical decisions in selecting a rotation crop is to ensure that it is not an alternative host of the major crop being grown, as all plants are susceptible to various and sometimes nonspecific plant pathogens (Larkin 2015). In general, non-grain rotation crops could be seen to be very slightly altering fungal communities, however, as this study took place over the course of only four years with a wide variety of non-grain rotation crops tested, it is difficult to determine how specific non-grain rotation crops could be promoting or suppressing different microbial community members in the long term. In other studies investigating the impacts of non-grain rotation crops on microbial communities, it has been noted that these differences could be expected to increase over many growing seasons as some carryover effect could be seen when potatoes were planted following non-grain rotation crops (Larkin 2003).

Fungal ASV abundances were most differentiated within management factors of rotation length, fumigation, and compost amendment. While there were few distinctions in differential

abundances, all fungal diversity measures were still significantly different, with three-year rotations consistently having significantly lower richness, diversity, and evenness. This is in contrary to what is commonly found in the literature, as longer rotations have been found to significantly increase substrate utilization patterns by microorganisms, reduce soilborne disease pressure, and improve soil health metrics (Bucher and Lanyon 2005; Larkin et al. 2010; Wright et al. 2017). Before this study was established, this field had been planted in oats for at least the last 20 years. These inverse results could be the result of these trials being recently established, and with only two growing seasons of potatoes over the four-year study period. Additionally, lower yields were also found in three-year rotations compared to two-year rotations (Chapter 2).

The second most abundant fungal phylum after Ascomycota following fumigation was the phylum Chytridiomycota. Fungi in the phylum Chytridiomycota reproduce by producing motile zoospores, and are considered by some as an intermediate group between Fungi and protists due to their locomotion (James et al. 2006). While fungi in the Chytridiomycota phylum require water for zoospore dispersal, these organisms occupy a variety of locations ranging from aquatic environments, soil, and the rumen and digestive tract of livestock (Powell and Letcher 2014). This phylum also contains plant pathogens, such as *Synchytrium endobioticum*, the causal agent of potato wart, which was not identified in these soil samples. According to FungalTraits, the majority of fungal ASVs which were more abundant after fumigation were mostly saprotrophs, plant pathogens, lichen parasites, or had undetermined lifestyles.

Of the fungal ASVs which were reduced by fumigation, after the phylum Ascomycota, the phylum Mortierellomycota contained the most ASVs which were significantly less abundant following fumigation. Fungi in the phylum Mortierellomycota occur in a wide variety of environments, produce filamentous growth in soil, and are known as root endophytes

(Rungjindamai and Jones 2024). These fungi also aid in decomposition of organic matter and the carbon cycle (Muneer et al. 2021). Within this phylum, all ASVs were identified to be in the *Mortierella* genus, which contains species which are capable of plant growth promotion, improve plant nutrient bioavailability, and aid in protection from plant pathogens (Ozimek and Hanaka 2020). Overall, fumigation enhanced more ASV genera which were identified as plant pathogens than it reduced, and the amounts of saprotrophs reduced and enhanced were very similar (33 ASVs without fumigation and 28 ASVs with fumigation). Additionally, 10 ASVs which represented the beneficial genus *Mortierella* were reduced with fumigation compared to only 4 which were enhanced with fumigation. Fumigation has been shown to result in varied effects on microbial communities (Dangi et al. 2017). In a long-term study of the impacts of metam sodium, there was determined to be a lasting effect on the microbial communities, but with repeated fumigation, diversity changes stabilized over time (Li et al. 2022). In the present study, treatment plots were exposed to metam sodium twice over the course of four years after a long history of 20 or more years of being planted with grain rotation crops. Fungal communities were found to be significantly less rich and diverse following fumigation. Interestingly, it was found that 17 ASVs which were described as plant pathogens were more abundant following fumigation, and only 3 plant pathogen ASVs were less abundant following fumigation. This could eventually result in impacts to crops, however in this study, there were no significant differences between treatments which were fumigated or not with respect to yields, or disease incidences or severities (Chapter 2). It was expected that fumigation would suppress pathogens and consequently enhance yields, however, that was not the case in this study. On the other hand, more ASVs which were described as saprotrophs, mycoparasites, or root endophytes were less abundant with fumigation, indicating that fumigation could be impacting communities of fungi

which could be considered beneficial in the soil environment. This indicates the need for additional research concerning fumigation and the impacts to beneficial microbial communities. Soil fumigants can impact non-target microorganisms, and beneficial or neutral microbial community members could be negatively and inadvertently impacted (Dangi et al. 2017; Li et al. 2022).

Compost was another important driver of fungal differential abundance, with 91 total ASVs either enhanced or reduced as a result of compost amendment. The majority of these ASVs were more abundant following compost amendment, as 71 ASVs were more abundant compared to 20 which were less abundant. The majority of these more abundant ASVs were identified to be mostly saprophytes or unidentified functionally. Compost enhances soil microbial communities through inputs of organic matter, which drive microbial communities (Pérez-Piqueres et al. 2006). However, compost is also rich in microorganisms which can be suppressive to plant pathogens and promote plant growth (Gong et al. 2005; Mehta et al. 2014; Singh and Nain 2014). In this study, the promotion of beneficial bacteria and fungi with compost amendment was found. Additionally, compost amendment was associated with increased yield (Chapter 2). The compost which was used was composed of manures and plant residues, including potato residues. With the use of compost, it is always important to consider the source of inputs, as this can result in a wide range of compost quality, and potential risks associated with antibiotic resistance genes which could arise as a result of manure derived from livestock treated with antibiotics (Straathof and Comans 2015; Dincă et al. 2022). Additionally, there are concerns of accumulation of contaminants such as heavy metals as a result of compost amendment (Dincă et al. 2022). Interestingly, two phyla of bacteria, Chloroflexi and Firmicutes,

which are capable of bioremediation of heavy metals, were enhanced as the result of compost amendment, which could offer a unique solution to this challenge.

3.4.3 Microbes which were both differentially abundant and core members to treatments

There were eight bacterial ASVs and twelve fungal ASVs which were both uniquely core to a particular treatment and were significantly differentially abundant as a result of the treatments. Thus, these ASVs were interpreted to be "highly associated" with a particular management factor. Of the management factors, there were 10 ASVs which were highly associated with fumigation, seven highly associated with the absence of fumigation, and three which were highly associated with compost amendment.

For fumigation, four bacterial ASVs and six fungal ASVs were highly associated. Of the bacterial ASVs, two were identified only to family: one in *Roseiflexaceae*, and the other in *Blastocatellaceae*. None of the bacterial ASVs were identified to species. The family *Roseiflexaceae* is in the phylum Chloroflexi and is known to contain members which have habitats in activated sludge systems and are filamentous anoxygenic phototrophic bacteria (Thiel and Hanada 2021). The family *Blastocatellaceae* is in the Acidobacteriota phylum and have been described as gram negative bacteria which are strictly aerobic and chemoorganotrophic (Pascual et al. 2015). However, it is difficult to glean function or ecological role without a clearer understanding of the specific species detected, although often this does not provide full resolution either. The other two ASVs which were highly associated with fumigation were identified to the genera *Roseimicrobium* and *Tychonema CCAP 1459-11B*. *Roseimicrobium* only has one known species, but it is impossible to determine if this is the species detected (Otsuka 2019). The other ASVs was in the genus *Tychonema CCAP 1459-11B*, a freshwater cyanobacterium which has been found previously in rainwater samples (Suda et al. 2002; Dillon

et al. 2020). With respect to fungi which were highly associated with fumigation, six different ASVs were detected and all but one were identified to species. The unidentified ASV which was highly associated with fumigation was in the order *Lobulomycetales*, one of four currently identified orders in the phylum Chytridiomycota (Simmons et al. 2009). Members of this order are challenging to isolate and it has been speculated that new members will be identified as a result of sequencing from various environments (Simmons et al. 2009). Of the remaining five fungal ASVs which were highly associated with fumigation, three were identified as plant pathogens by FungalTraits. The three plant pathogens are *Gibberella tricincta, Fusarium culmorum,* and *Paraphoma radicina. G. tricincta* is a fungal pathogen of wheat which was previously identified to be a core member in a study investigating the impacts of glyphosate on soil microbial communities (Lupwayi et al. 2022). *F. culmorum* is another pathogen of wheat, but is considered a major pathogen which can cause wheat seedling blight, foot rot, and head blight (Wagacha and Muthomi 2007). This pathogen is also a major concern because it is capable of producing mycotoxins which can contaminate wheat and are a health hazard to both humans and other animals (Wagacha and Muthomi 2007). There are inconsistent results of the efficacy of fungicides against this pathogen, but in general are only 70% effective in field trials (Wagacha and Muthomi 2007). The final plant pathogenic ASV which was found to be highly associated with fumigation was *P. radicina*, the causal agent of alfalfa root rot (Dang et al. 2021). This pathogen is estimated to reduce the annual production of alfalfa worldwide by up to 20% (Dang et al. 2021). This pathogen has been only recently discovered, and as such there are very limited disease management techniques (Dang and Li 2022). The two other ASVs which were highly associated with fumigation were *Candida sake* and *Mucor hiemalis*. *C. sake* is a yeast which has been used as a biological control agent to control post-harvest diseases (Viñas et al. 1998). This

yeast species was also found to be highly tolerant of a wide range of temperatures and ranges of pH (Teixidó et al. 1998). *M. hiemalis* is a fungi known to be capable of biosorption of various metals, including nickel and chromium (Tewari et al. 2005; Shroff and Vaidya 2011). Biosorption is technique which employs either living or non-living biomass of various organism for removal of contaminants such as heavy metal ions (Tewari et al. 2005). In summary, of the six fungal ASVs which were highly associated with fumigation, three were plant pathogens, two were capable of living in stressful or contaminated environments, and one was an unidentified ASV in the Chytridiomycota phylum.

Of the ASVs which were highly associated with unfumigated treatments, three were bacterial ASVs and four were fungal ASVs. All three bacterial ASVs were not identified to species, however, one was identified to the family *Micropepsaceae,* and two were identified to the genera *Bradyrhizobium* and *Candidatus Udaeobacter*. Members of the family *Micropepsaceae* are found in a wide range of habitats ranging from peatland to rice plant roots, are gram negative, and all members are capable of carbon fermentation (Bräuer et al. 2018). *Bradyrhizobium* spp. are able to adapt to a variety of stressful growing environments, are gram negative, do not form spores, and are characteristically known to enter the root hairs of legumes and form root nodules which aid plants via nitrogen fixation (Kuykendall 2015; Ormeño-Orrillo and Martínez-Romero 2019). Bacteria which fall in the genus *Candidatus Udaeobacter* prefer acidic environments and are well known to be ubiquitous to soils and produce antibiotics (Willms et al. 2020, 2021). Of the four fungal ASVs which were highly associated with unfumigated soils, two were soil saprotrophs (*Ramicandelaber longisporus* and *Mortierella minutissima*), one was a mycoparasite (*Classicula sinensis*) and one was a root endophyte (*Acidomelania panicicola*), when assigned a primary lifestyle by Fungal Traits. *R. longisporus* is

a fungal species in the Kickxellales phylum and can be occasionally isolated from soil samples (Ogawa et al. 2001; Benny et al. 2016). *M. minutissima* is a saprotrophic fungi in the Mortierellomycota phylum, which is capable of degrading chitin and starches, and promoting plant growth (Trytek et al. 2009; Ozimek and Hanaka 2020; Klasek et al. 2023). *M. minutissima,* the most abundant fungal ASV found in this study, is one of the core fungal members found in the Potato Soil Health Project, which categorized soil microbial communities across top potato producing states in the United States, of which this study is affiliated (Klasek et al. 2023). *C. sinensis* is a fungi in the phylum Basidiomycota which was isolated from decaying leaves and is an aquatic hyphomycete, described as a mycoparasite by FungalTraits (Qiao et al. 2018). The final fungal ASV which was highly associated with unfumigated soils was *Acidomelania panicicola,* a root-colonizing fungi in the phylum Ascomycota (Walsh et al. 2014). *A. panicicola* is commonly associated with roots of plants growing in acidic and nutrient poor soils, although potential plant growth promoting abilities of this fungus remain unknown (Walsh et al. 2014). In summary, of the seven ASVs which were highly associated with unfumigated soils, the three bacterial ASVs are capable of root nodulation, antibiotic production, and carbon fermentation. Of the fungal ASVs highly associated with unfumigated soils, two were saprotrophs, one of which may promote plant growth, one was a relatively unknown mycoparasite, and the final is capable of plant growth promotion. Fumigation was not found to enhance potato yields relative to unfumigated treatments (Chapter 2).

ASVs were highly associated with compost amendment, but no ASVs were highly associated with the absence of compost amendment. One bacterial ASV and two fungal ASVs were identified to be highly associated with compost amendment. The bacterial ASV was identified to species as *Sphaerobacter thermophilus*, a gram positive, non-spore forming bacteria

which was initially isolated from commercial sewage sludge (Pati et al. 2010). The two fungal ASVs were two subspecies of *Acrodontium hydnicola*, a fungus in the phylum Ascomycota which has been reported to be an indicator of soils which are not disturbed within a forested tourist area (Feng et al. 2023). *A. hydnicola* is widely associated with different habitats, including soils and plant debris (Feng et al. 2023). Compost amendments applied before potato growing years were found to have the highest potato yields compared to all other treatments within the same rotation length (Chapter 2). Additionally, these impacts could be long lasting, as compost amendments decompose at reduced speeds comparatively to conventional fertilizers. As an example, compost has been shown to have residual mineralizable nitrogen and enhanced microbial biomass for four years after amendment, although other studies have shown that organic amendments can have residual effects for up to 17 years (Ginting et al. 2003; Bastida et al. 2007).

3.5 Conclusion

It was found that bacterial communities in Maine are similar in make up at a phylum-level to other potato producing states, however fungal communities may be slightly different comparatively. In general, the majority of the most abundant fungal ASVs were found to be saprotrophs, potentially due to the environmental conditions and soil composition in Maine. Overall, fumigation and compost amendment had the biggest impacts on soil microbial communities in a potato producing field. Fungi were more sensitive and variable over all treatments compared to bacterial communities, specifically with respect to rotation length, compost amendment, fumigation, and the use of non-grain rotation crops. Fumigation impacted many ASVs but resulted in significantly higher abundances of three plant pathogens and fungi

which are known to survive high stress environments or bioremediate contaminated soils. The seven microorganisms which were highly associated with the absence of fumigation, and consequently negatively impacted by fumigation, were associated with plant roots, plant growth promotion, saprotrophic lifestyles, and mycoparasitism. In general, fungi which were less abundant by fumigation were saprotrophic or unidentified, while the two largest groups of fungi which were more abundant with fumigation treatment were saprotrophs or plant pathogens. Of differentially abundant fungi by compost amendment, over 3.5 times more fungal ASVs were more abundant with compost than without. Within these fungal ASVs which were more abundant with compost, plant pathogens made up 4% of the differentially abundant ASVs, while in soils without compost, plant pathogens made up 25%.

CHAPTER 4

EXPLORING LOBSTER SHELL MEAL AS A NOVEL SOIL AMENDMENT IN POTATO PRODUCTION

Abstract: Maine is a unique state with a wealth of agricultural production amidst the lobster industry. The use of lobster processing byproducts as a soil amendment could benefit both industries. Post-processed lobster shells can be finely ground to produce lobster shell meal (LSM), which contains chitin, much like the key components of fungal cells and their overwintering structures. Adding chitin-rich material such as LSM has been shown to increase the populations of some chitin-degrading microorganisms capable of suppressing soilborne pathogens. In greenhouse trials, the efficacy of LSM was determined using potato 'Shepody' inoculated with the soilborne pathogens, *Verticillium dahliae, Streptomyces scabies,* or *Rhizoctonia solani*. In field trials, LSM was tested with potato 'Russet Burbank' by amending the soil in either the fall or spring, inoculating with *V. dahliae*, and the use of soil fumigation. Potato yield, disease intensity, and microbial communities were examined. LSM in a greenhouse study significantly increased the aboveground biomass of potato plants, but this did not translate to the field effects. Disease incidences were also not significantly different in greenhouse nor field trials, but severity of potato early dying was seen to be significantly reduced within fumigated treatments amended with LSM compared to those which were only fumigated and not amended with LSM. Fungal communities were more sensitive to LSM as a soil amendment than bacterial communities. Both fall and spring amendments of LSM reduced fungal diversity, while fungal evenness was only reduced in the fall amended trial. Bacterial communities were not significantly impacted by LSM, but evenness was reduced with the use of fumigation. Core

communities of bacteria with LSM amendment were larger in the fall amended trial compared to the spring LSM-amended trial. The use of LSM presents an opportunity to connect the potato and lobster industries in Maine and transform shellfish industry byproducts into a useful resource.

4.1 Introduction

Potatoes are the most valuable crop in Maine and have been part of the economy since the mid-1700's (Johnston 1972; USDA: NASS 2022a). In 2022, 52,000 acres of potatoes were planted and resulted in a yield of 18,425,000 hundredweight in Maine (USDA: NASS 2022a). However, the potato industry is challenged by the issue of soilborne diseases, as over 40 different soilborne pathogens can infect potatoes (Fiers et al. 2012; Hao and Ashley 2021). Soilborne pathogens include fungi, bacteria, viruses, and nematodes and can cause damages to plants both above the soil and underground (Katan 2017). In general, soilborne pathogens are sensitive to changes in the soil environment, including those occurring as the result of agricultural management practices (Katan 2017). Soilborne pathogens can form overwintering structures in the absence of their plant hosts which can be susceptible to antagonism by other microorganisms or changes in the soil environment (Katan 2017). Soilborne diseases are typically managed through various tools, including using tolerant or resistant varieties, chemical treatments, soil disinfestation, organic amendments, and cultural practices (Katan 2017). The addition of soil amendments can stimulate disease-suppressive beneficial microbial communities in addition to improving soil quality and nutrients (Lazarovits 2001; Larkin 2015). However, the proper use of soil amendments for plant pathogen suppression requires an understanding of modes of action, which can often be complex (Lazarovits 2001). Another important consideration is the source of soil amendments, as there can be different functions and effects on soilborne pathogens and plant hosts (Hao and Ashley 2021).

The state of Maine has 3,478 miles of shoreline, more than all other states except Alaska, Florida, and Louisiana, respectively (NOAA 2017). This unique location of cold coastal waters encourages shellfish production, including bivalves, such as oysters and clams, and crustaceans,

such as crabs and the renowned American Lobster. Shellfish industries produce substantial amounts of byproducts, including the shells of lobsters. Disposing of lobster shell waste is highly costly, for example, a facility processing 15,000 pounds of lobster per day can expect to pay upwards of $4000 per month for disposal services, and while a small portion of lobster shell waste is composted, the majority is sent to landfills (Fulton et al. 2013). However, the post-processed shells of shellfish may also have potential for use in agriculture as soil amendments. Lobster shell meal (LSM) contains a high concentration of chitin, which as a soil amendment, could promote plant growth or provide nutrients for microbes which degrade chitin (Andreo-Jimenez et al. 2021).

Chitin can promote plant growth and defenses, which can be a useful indirect tool in pathogen suppression. As a soil amendment, chitin has been shown to promote beneficial mycorrhizal interactions by up to 20% (Ramírez et al. 2010). An example of this was seen through the production of phytoalexins, antimicrobial molecules which are one of many plant defenses, by rice callus following the introduction of N-acetylchitooligosaccharides, which are oligosaccharides of chitin (Yamada et al. 1993). In a study of cabbage and strawberry plants infected by *Alternaria brassicicola* and *Colletotrichum fructicola*, respectively, the application of chitin nanofibers elicited systemic disease-resistance and had a growth-promoting effect in both hosts (Parada et al. 2018). While in a study of chili plants, chitin in the form of ground prawn shells was added to soil and both reduced the incidence of *Rhizoctonia solani* in inoculated plants and resulted in longer shoot growth when used as a soil amendment (Hussain et al. 2013b).

Another potential benefit of chitin is the promotion of native communities of beneficial soil dwelling microbes, which can be a useful tool in direct suppression of plant pathogens.

These microorganisms may suppress plant pathogens through direct degradation of chitin-containing pathogen cell walls or overwintering structures (Ramírez et al. 2010). In a study of chitin as a soil amendment for fusarium yellows of celery, bacterial populations were higher following the addition of chitin compared to unamended soil and fewer fungi were present following chitin amendments (Bell et al. 1998). It was found that chitin amendments significantly increased actinomycete populations (Bell et al. 1998), which are known to be plant growth promoting rhizobacteria and have many other traits such as nutrient cycling, antibiosis, and potential as biological control agents (Jog et al. 2016; Hazarika and Thakur 2020).

In a greenhouse study, chitin amendments from shrimp and crab shells promoted growth of cucumber seedlings (Jog et al. 2016). However, timing of chitin amendment is also an important consideration, as seedlings in the same study amended then the next day inoculated with *Fusarium oxysporum* f. sp. *radiciscucumerinum*, were more susceptible to disease pressure (Rose et al. 2003). The authors speculated that this could be due to the rapid increase of nitrogen, promoting seedling growth, but without the time to allow antagonistic populations to build and suppress pathogenic populations (Rose et al. 2003). In a separate study of soils amended with chitin from crab shell meal against root rots in ginseng, actinomycete populations exceeded 25 times the amount in the untreated soil and populations of *Cylindrocarpon destructans*, the causal agent of ginseng root rots, were reduced to about half the population compared to untreated soil (Chung and Kim 1978).

Fungal pathogens are not the only organisms which may be inhibited by chitin soil amendments, and in plants inoculated with viable root-knot nematode eggs, *Meloidogyne javanica,* infection was approximately half when amended with chitin compared to unamended soils (Hussain et al. 2013a). Additionally, soybean cyst nematodes and other endo- and

ectoparasitic nematodes were found to be controlled with the use of commercially obtained crustacean chitin amendments (Rodriguez-Kabana et al. 1984).

While chitin is available both commercially and as a raw byproduct post-shellfish processing, in a study of chitin from commercial sources and as an industry byproduct, no difference was seen in between the two as a tool in suppression of *Colletotrichum* spp. of cucumber plants (Dodgson and Dodgson 2017). However, some studies have indicated that certain chitin degrading beneficial bacteria are able to degrade shrimp shells more effectively than colloidal chitin for the production of chitinases (Swiontek Brzezinska et al. 2014). Colloidal chitin requires processing which includes crushing, demineralizing, hydrolyzing, and lipids and pigments are eliminated before suspending in solution (Shimahara and Takiguchi 1988). Alternatively, lobster shell meal requires only drying and crushing, which has potential to be performed at an industrial scale in the supply chain post-processing.

This study aims to utilize post-processed lobster byproducts, otherwise destined for waste streams, as a soil amendment to foster collaboration between these two vital industries. Specifically, the goal is to remedy the dual challenges of managing soilborne pathogens affecting potatoes and reducing the environmental and economic costs associated with lobster shell waste, offering a sustainable solution that benefits both industries.

4.2 Methods

4.2.1 Effects of lobster shell meal on greenhouse grown potatoes

In the University of Maine greenhouse, potato ‘Shepody’ was planted in a mix of Pro-Mix Premium Organic Garden Mix and Premier Tech Pro-Mix BX General Purpose Growing Mix (1:1; Pro-Mix, Quakertown, Pennsylvania) in one-gallon pots in March and July of 2022.

‘Shepody’ was selected because it is susceptible to both *Verticillium dahliae* and *Streptomyces scabies*. The soil was inoculated with either *V. dahliae* or *S. scabies* inocula, the causal agents of potato early dying (PED) and common scab, respectively, and non-inoculated soil was used for a non-diseased control. Inoculum for *S. scabies* was made by growing the bacteria in vermiculite mixed with liquid medium (Say’s solution: 40 g sucrose, 2.4 g asparagine, 1.2 g K2HPO4, 20 g yeast, 1 L sterile water). The bacteria and vermiculite mixture was added to the soil in pots at a rate of 1:10 inoculum to soil and mixed thoroughly to result in a final inoculum concentration of 10^5 colony forming units (CFU) per gram of soil. For *V. dahliae* inoculum preparation, microsclerotia, the rigid overwintering structures, were suspending in sterile water and added to vermiculite before being added at a rate of 20 microsclerotia per gram of soil. Lobster shells were donated by Ready Seafood (Saco, Maine), a local processor, after post-processing once all lobster meat had been removed. These shells were then dried and ground using a grain mill to produce a fine powder, or lobster shell meal (LSM). LSM was blended into the soil at two rates, low and high (2% and 8%, w/w, respectively), as well as an unamended control (0%). These ranges were selected based on rates used in previous studies.

There were five replications of each pathogen inoculation (*V. dahliae, S. scabies,* or uninoculated) and amendment group (no LSM, 2% LSM, and 8% LSM). Two iterations of the entire study were prepared in order to immediately plant potatoes in one iteration once pots were prepared and to time delay the planting of the other and allow amendments and pathogens to remain in the soil for three months without potato tissue present. This gave time for microbial communities to respond to the treatments without the addition of plant tissues and model a fall amendment timeline. Pots were randomly arranged in a greenhouse bench and watered as needed

until the plants began to naturally senesce around 3 months after planting, after which the plants were harvested.

To harvest, plants were cut at the soil line and all plant tissues were placed into paper bags to dry for approximately one week, or until the plant matter was fully desiccated. Desiccated plant matter was weighed and recorded as aboveground biomass. Two weeks after the removal of aboveground plant tissue, tubers were removed from the soil, cleaned of any residual soil, and processed immediately. The total weight of the tubers, tuber numbers, and disease symptoms were recorded. Disease incidence described the presence or absence of symptomology and disease severity was recorded in various ways depending on the disease. To determine PED infection symptom severity, tubers were cut in half longitudinally and the percent of the total vascular ring which was discolored was recorded. To determine the severity of symptoms of infection by *S. scabies,* the percentage of the tuber surface which was covered by scabs was recorded.

A second greenhouse study was performed in March and July of 2023. Six treatments with five replications were established, but with fertilizer added as a management factor and a combined inoculum of *Rhizoctonia solani*, the causal agent of black scurf, and *V. dahliae*, in equal parts. Treatments were again prepared in two iterations as described in the initial greenhouse trial. To best prepare for the forthcoming field trial, rates of inoculum, fertilizer, and LSM were all as similar as possible to those used in the field trial. The rate of inoculum was 20ml/foot, and as the pots were 8” in diameter, a rate of 14ml/pot was used. Virgin grain was added at the same rate to non-inoculated pots. Fertilizer (14-14-14) was applied at a rate of 1100lbs/acre, typically incorporated at a depth of 12”, so each pot received 2.99 g of fertilizer.

The rate of LSM was selected based on recommendations obtained from Coast of Maine, a soil amendment company, to be applied at a rate of 5lbs/100ft2 (Coast of Maine; Portland, Maine). In the field, LSM was incorporated to a depth of approximately 4", resulting in a rate of approximately 1.7% w/w. Plants were maintained and data was collected in the same fashion as in the initial greenhouse study.

4.2.2 Fall and spring lobster shell meal amended field trials

Following the greenhouse studies, two field trials took place at the Aroostook Research Farm in Presque Isle, Maine (46.653704, -68.020309). Potato 'Russet Burbank' was planted in three rows with 12" spacing within 25-foot-long plots with four replicates. The variety of potato used in the field trials was changed to potato 'Russet Burbank' due to variety availability. In the fall of 2022, the first trial was established to represent a fall soil amendment schedule. Soil treatments included unamended soil, unamended soil inoculated at a rate of 20ml/foot with *V. dahliae*, LSM amendment alone, LSM amendment with *V. dahliae* inoculum added, soil fumigation with metam sodium at 50 gal/A amended with LSM and inoculated with *V. dahliae*, and soil fumigation with metam sodium at 50 gal/A inoculated with *V. dahliae* (AMVAC, Newport Beach, California). Soil fumigation was performed in the fall of 2022 under favorable weather conditions and using appropriate safety protocol, and based on treatment descriptions, soil was fumigated approximately one month before soil was amended with LSM. *Verticillium dahliae* was selected as a test pathogen because of the formation of resilient microsclerotia and an integrated relationship with nematodes (Lazarovits 2001). These treatments were selected to compare and explore the integration of LSM as a soil amendment into typical potato agricultural practices. The rate of LSM as an amendment was informed by industry standards provided by

Coast of Maine, a compost and soil amendment company (Portland, Maine). The rate of LSM for the field trial was consequently 5 lb per 100 ft^2 (approximately 1.7% of the soil % w/w). LSM was donated from Coastal Chitin, LLC (Biddeford, Maine), a local processor, which simplified the process of preparing large volumes of LSM, as this product was commercially dried and ground. For the fall amended trial, LSM was applied in the fall of 2022, before planting seed tubers in the spring.

A second, spring amended field trial was established in the spring of 2023. However, because of the challenges associated with fumigation in the spring, this factor was not included in this trial. Consequently, the experimental design was simpler and consisted of four treatments: unamended and not inoculated, unamended and inoculated, amended with LSM only, and amended with LSM and inoculated. Soil was sampled at planting, mid-season, and before harvest.

These trials took place in adjacent fields which have not had exposure to LSM previously and had been planted in oats for at least one year prior to these trials. The fields were maintained during the growing season using standard operational practices, including fertilizer application and pest control. The fields were irrigated with rainwater only. At harvest, potato yield, disease incidence and severity of PED were evaluated to determine the impacts of these treatments. Potato yield was calculated by harvesting one central row from each plot and disease incidence and severity was measured using fifty tubers per plot. Additionally, incidences of hollow heart, a physiological condition, were noted if tubers had hollowing in their core.

4.2.3 Soil DNA extraction, sequencing, and processing

Soil samples were collected from the field trials in the fall-amended trial post-fumigation in the fall, then in both trials at planting, 60 days after planting, and at harvest. Each soil sample consisted of a minimum of twenty cores randomly selected in the plot which were homogenized in a gallon size Ziploc bag to give a representative composite sample of the plot. Soil samples were sieved within one week and stored at -80°C until DNA was extracted. DNA was extracted using 0.25 grams of soil with a Qiagen DNeasy PowerSoil Pro Kit according to the provided instruction (Germantown, Maryland). DNA was quantified and quality ensured using a Nanodrop 2000 before sending for sequencing analysis at The University of Michigan Genomic Sequencing Facility (Thermo Fisher Scientific, Waltman, Massachusetts; University of Michigan Microbiome Core, Ann Arbor, Michigan). Samples were processed using NextGen sequencing on an Illumina MiSeq using a MiSeq Reagent Kit V2 500 cycles to return sequencing reads which were used to identify fungal and bacterial community members (Illumina Inc., San Diego, California). Amplicons of the samples were generated using the V4 region of 16S rRNA gene using the Dual indexing sequencing strategy to determine the presence of bacteria in the soil samples (Kozich et al. 2013). For fungal populations, the ITS2 rDNA region was amplified also using the dual indexing strategy (Taylor et al. 2016).

Raw Illumina sequencing reads were processed to filter low quality sequencing reads, remove chimeras, and clustered into amplicon sequence variants (ASVs) at 97% sequence similarity, which was analyzed using the DADA2 pipeline (Callahan et al. 2016a). Both forward and reverse reads were used for 16S sequences, but only forward reads were used for ITS, as the reverse were very poor quality and consequently few forward and reverse reads could be

successfully merged. Taxonomy was assigned to the sequence reads which passed quality assurance using the Silva taxonomy assignment version 138.1 and UNITE fungal taxonomy assignment version 9.0, for 16S and ITS amplicons, respectively (Quast et al. 2013; Kõljalg et al. 2020; Abarenkov et al. 2024). Metadata were included and contained details on LSM amendment timeline, fumigation, and harvest data. Once the sequences were assigned taxonomy, contaminants were removed, including any ASVs which matched to mitochondria as family or chloroplast as order from the 16S dataset as only bacterial sequences were of interest, and other sequences may have been accidentally identified. The same was true for removing ITS amplicons which did not match to the kingdom Fungi, as other eukaryotes, such as algae, are often identified. Rarefication was performed on both datasets at 7500 and 7800 ASVs per sample for bacterial and fungal communities, respectively. This was performed to avoid incorrectly representing samples with large numbers of ASVs as more diverse, as this is not biologically relevant, but likely an artifact of soil sampling, extracting DNA, or sequencing (Cameron et al. 2021, 2020; Willis 2019).

4.2.4 Statistical analysis

Potato growth, yield, and disease data were analyzed using similar methods for both greenhouse and field studies. Statistical analyses were performed using the R package Agricolae (de Mendiburu 2020). Effects of treatments on disease incidences and severities, yields, and plant biomasses were determined using analysis of variance and protected Fisher's least significant differences to separate means among treatments (de Mendiburu 2020). Z-scores, which aid in normalization of yields to account for differences such as variety and environmental

conditions are calculated by measuring the number of standard deviations a result is from the mean.

To analyze microbial community data, the R packages Phyloseq, DeSeq2, and Microbiome were used to quantify and visualize community differences within and between samples and treatments (McMurdie and Holmes 2013; Love et al. 2014; Callahan et al. 2016b). Microbial diversity was measured by a series of ASV-based analyses of alpha and beta diversity performed in phyloseq, including Shannon diversity index, evenness, richness (using Abundance-based Coverage Estimators (ACE) and Chao1), and observed ASV diversity (Shannon and Weaver 1964; Chao 1984; Rajendhran and Gunasekaran 2011; Rosenzweig et al. 2012; McMurdie and Holmes 2013). Community differences were visualized using principal coordinate ordinations (PCoA) of Bray-Curtis and Jaccard dissimilarity (Callahan et al. 2016b, 2016a; Van Horn et al. 2021). Core members were called and described at a detection rate of 0.001 and prevalence of 70%, should they match a previously described organism to genus and species (Callahan et al. 2016a). In order to identify core community members related to specific treatments and remove those which are simply highly common in Maine soils, community members which were core to the entire dataset were considered common core members and were removed for treatment-based analyses of core communities. The remaining core members were termed “conditionally core,” as they were remained core to a specific management factor level when highly abundant and common ASVs were removed. If a core member was only associated with one specific management factor level and no other management factors, it was termed “uniquely core”. Differential abundance of microbes was performed using DeSeq2 to determine if specific ASVs or phyla were enhanced or reduced following the treatments (Love et al. 2014).

4.3 Results

4.3.1 Effects of lobster shell meal on greenhouse grown potatoes

In the initial greenhouse trial, the aboveground biomass of potato plants increased significantly with increasing rates of LSM, regardless of pathogen inoculation ($p < 0.001$; Figure 4.1). Nominally, the highest aboveground biomass occurred in non-inoculated plants with the highest rate of LSM, but was not significantly reduced when inoculated with *V.dahliae* or *S.scabies*. The number of tubers was not significantly different among treatments ($p = 0.26$; data not shown). The incidences of PED or common scab varied considerably but were not significantly different among treatments ($p = 0.152$ and 0.251, respectively). Severity of PED or common scab also was not significantly different by treatment ($p = 0.967$ and $p = 0.669$; Figure 4.2). Overall growth and disease incidence and severity metrics, except PED incidence, time delaying the treatments was not found to be additive with LSM amendment and were likely due to environmental conditions in the greenhouse. In the case of PED incidence, significantly higher incidences were found in treatments with both high rates of LSM and no LSM which were not time delayed compared to all other treatments ($p = 0.023$).

Figure 4.1 Rate effects of lobster shell meal (LSM) on the aboveground biomass of potatoes in the initial greenhouse trial which explored LSM as a soil amendment. Aboveground biomasses were collected by cutting the plant at the soil surface, and drying before weighing, and measured in grams. Bars topped by the same letter are not significantly different from each other based on Fisher's protected LSD test at $p < 0.05$.

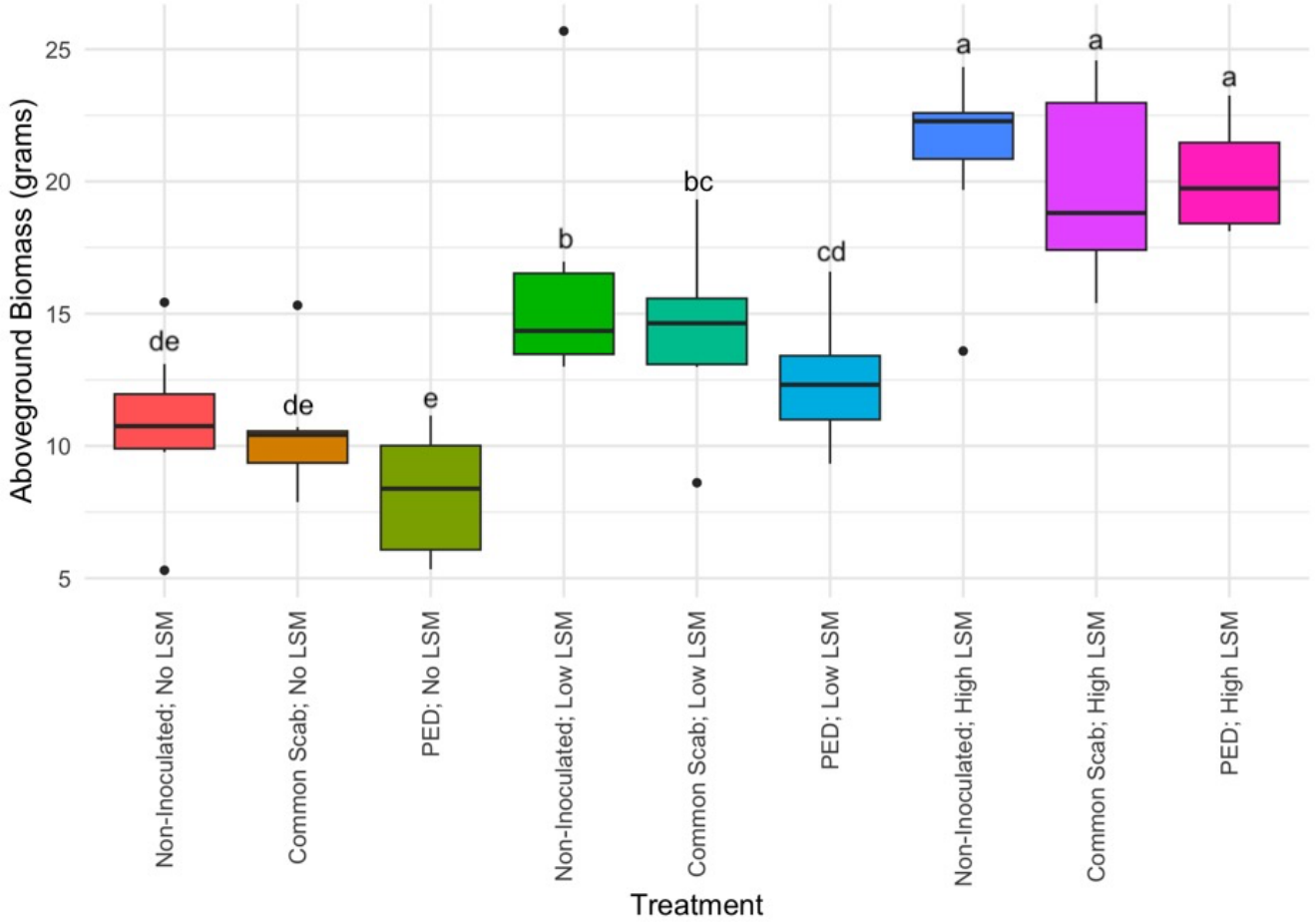
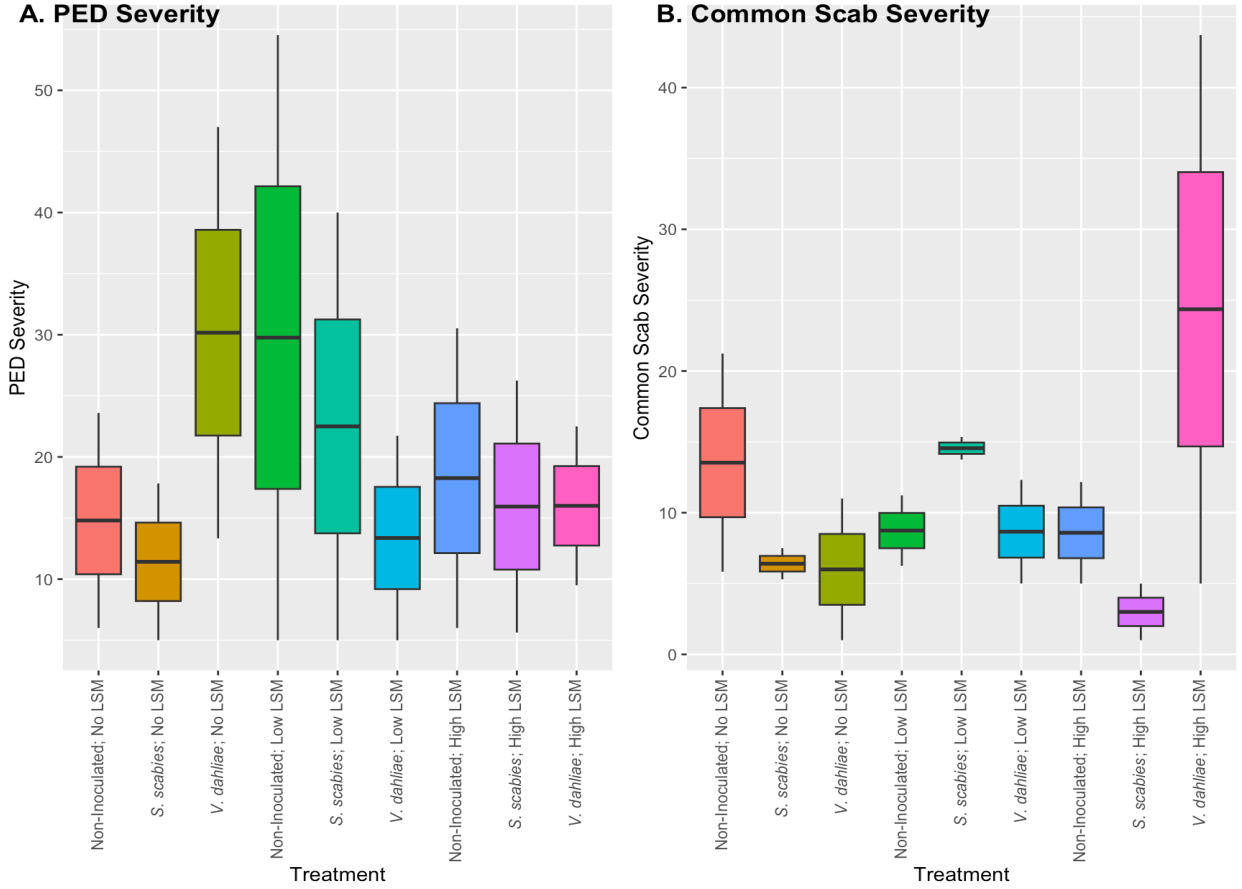


Figure 4.2 Disease symptom severities observed following the initial greenhouse trial exploring LSM as a soil amendment of potato early dying (PED; A) and common scab (B). Severity accounts for the percent of a tuber's vascular ring which is discolored or the percent of a tuber's surface which is covered in scab, caused by either PED or common scab, respectively. Neither PED nor common scab severity was significantly different by treatment (p = 0.837 and 0.669, respectively)



In the second greenhouse trial, aboveground biomass was significantly different among treatments ($p < 0.001$), and the combination of LSM and fertilizer treatment had the largest aboveground biomass (Figure 4.3). Addition of pathogen inoculum reduced aboveground biomass relative to the fertilizer and LSM & fertilizer treatments, but not relative to the LSM only treatment. Total tuber weight and tuber number were not significantly different among treatments ($p = 0.693$ and 0.348, respectively; data not shown). Disease incidence was quite variable among treatments, but there were some significant differences. The incidence of PED was significantly higher in inoculated soils amended with both LSM and fertilizer, with all other treatments not significantly different from each other ($p = 0.008$; Figure 4.4). Incidence of black scurf was highest in treatments which were amended with LSM and fertilizer, however, these pots were not inoculated and indicated that *R. solani* was likely naturally occurring in the soil ($p = 0.003$; Figure 4.4*)*. A similar trend was seen with respect to the incidence of common scab, and highest in the two treatments of amendment with LSM and fertilizer, and inoculum and LSM only had the highest incidences of disease symptoms ($p < 0.001$; Figure 4.4). It is worth noting that these two were the only treatments to express symptoms of common scab, and this could be related to the irregular microbial content of commercially available potting soil or that pots prepared in batches could have played a role in the irregularity. Across all growth and disease incidence and severity metrics, time delaying the treatments was not found to be additive with LSM amendment and could be contributed to environmental conditions in the greenhouse.

Figure 4.3 Effects of soil treatments on the aboveground biomasses of potato plants in the second greenhouse trial which explored LSM as a soil amendment ($p < 0.001$). Blue bars indicate unamended treatments and salmon bars indicate treatments amended with lobster shell meal (LSM). Bars topped by the same letter are not significantly different from each other based on Fisher's protected LSD test at $p < 0.05$.

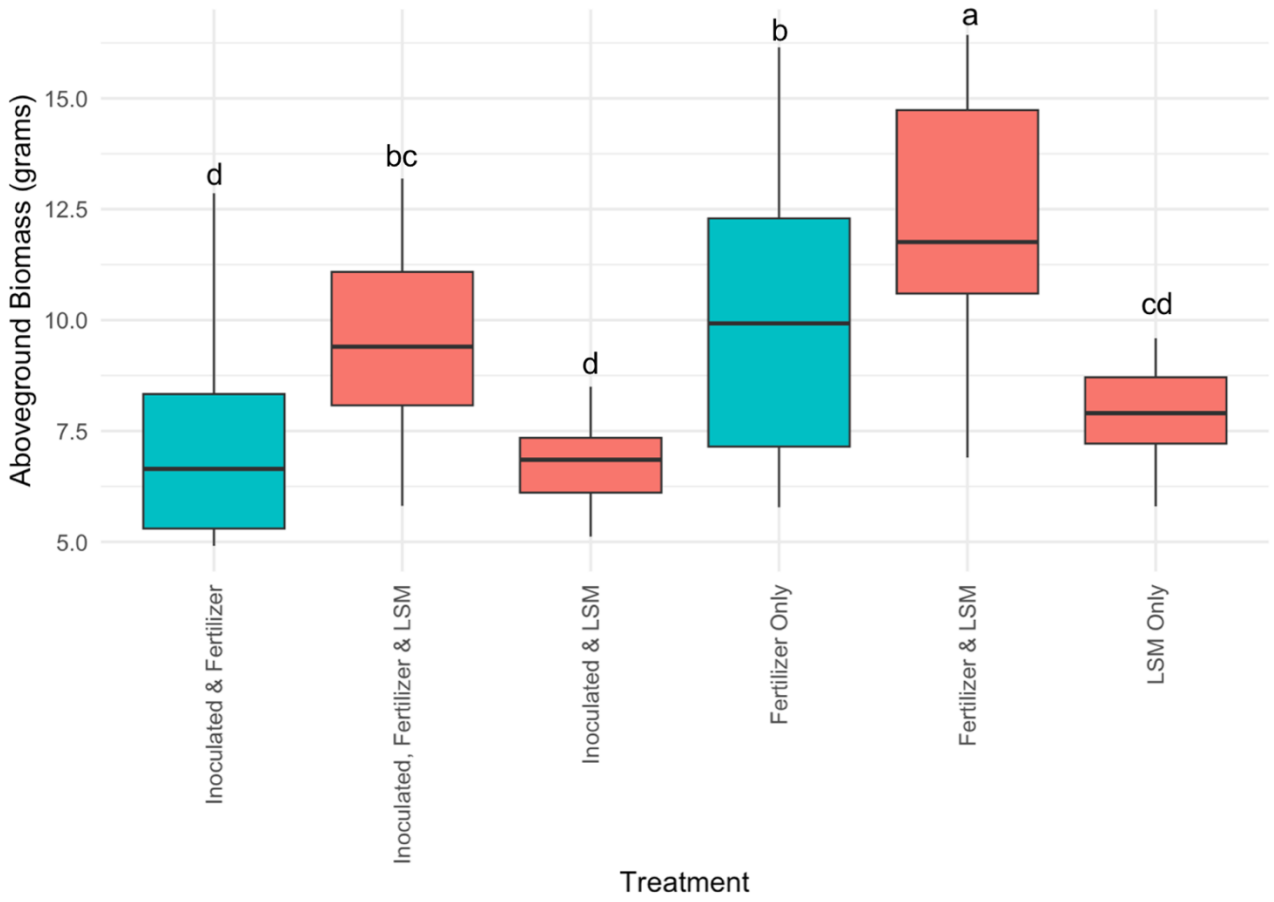
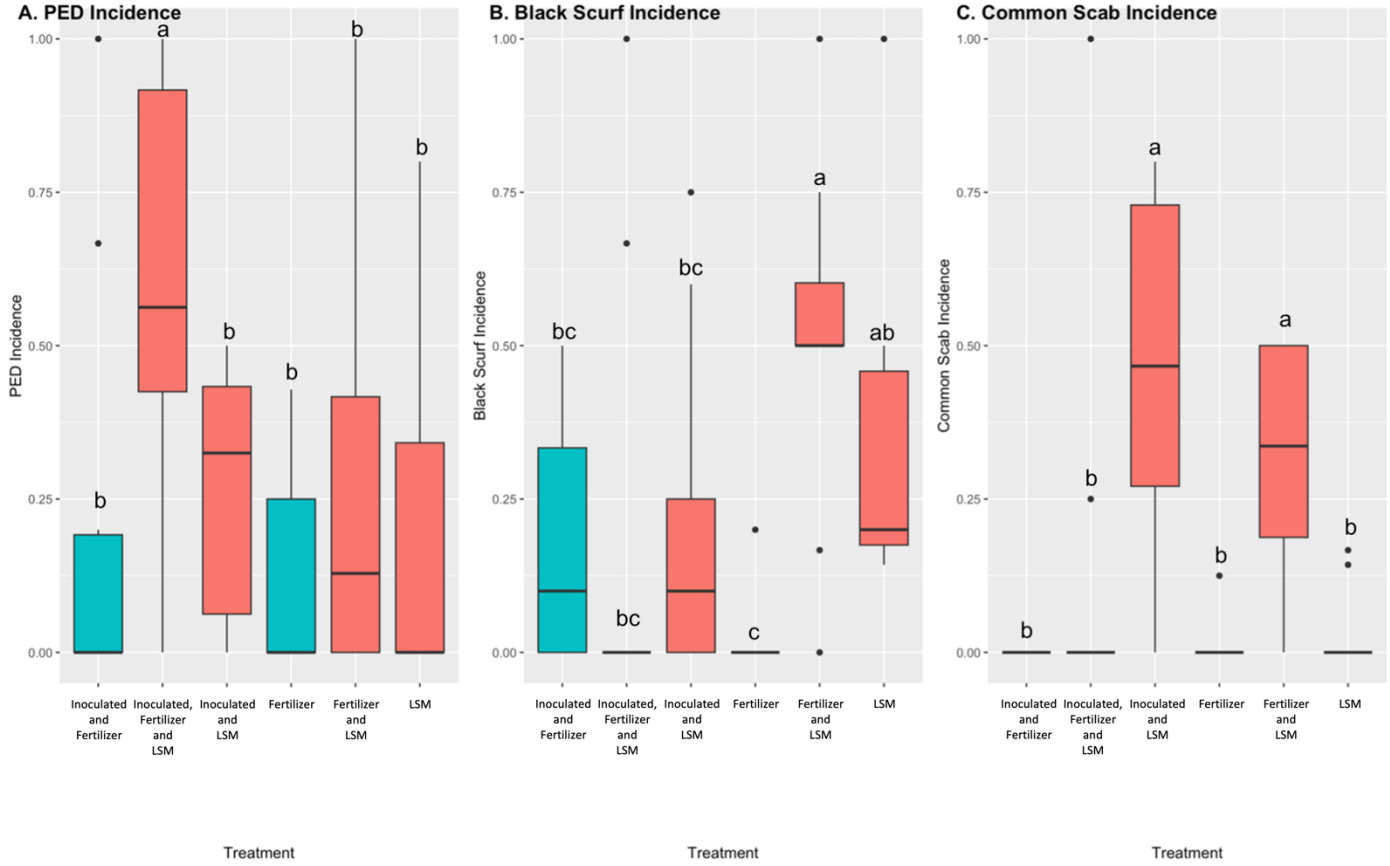


Figure 4.4 Effects of soil treatments on the incidence of potato early dying (PED) in the second greenhouse trial which explored LSM as a soil amendment. Blue bars indicate unamended treatments and salmon bars indicate lobster shell meal (LSM) amended treatments. Disease incidences for potato early dying (PED), black scurf, and common scab were significantly different by the treatments (p = 0.008, 0.003, and < 0.001, respectively). Bars topped by the same letter within each disease group are not significantly different from each other based on Fisher's protected LSD test at $p < 0.05$.



4.3.2 Effects of lobster shell meal amended field trials

In the spring-amended field trials, there were no significant differences among treatments in yield, PED incidence or severity, or incidence of hollow heart (p = 0.59, 0.969, 0.894, and 0.799, respectively; Table 4.1). While PED incidence was not significant, the nominally highest incidence was observed in the unamended and inoculated treatment, and similarly, this treatment also had the most severe PED symptoms.

In the fall-amended field trial, there were no significant differences among treatments in yield (p = 0.059; Figure 4.5, Table 4.1), but the non-inoculated LSM treatment showed somewhat lower yield than the inoculated LSM treatments (at p = 0.06). However, there was a significant blocking effect ($p < 0.001$) which was driven by the replication layout in this trial.. Both PED incidence, and hollow heart incidences were not significantly different among treatments (p = 0.221 and 0.999, respectively). However, the severity of PED was significantly different among treatments, with the most severe symptoms occurring in the fumigated and inoculated treatment, and the least severe symptoms occurring in the treatment which was not inoculated and amended with LSM, and the treatment which was inoculated, fumigated, and amended with LSM (p = 0.048; Figure 4.6; Table 4.1).

Table 4.1 Effects of soil treatments, including amendment of lobster shell meal (LSM), *Verticillium dahliae* inoculation, and fumigation on potato early dying (PED) and yield of potatoes in the fall- and spring-amended field trials. Means were separated by protected Fisher's least significant difference tests.

Amendment Timing	Tmt.	Description	Total Yield (Mg ha^{-1})	Yield (z-Score)	PED Incidence	PED Severity	Hollow Heart Incidence
Spring	1	Unamended; Non-inoculated	33.59	0.07	0.27	0.70	0.29
	2	Unamended; *V. dahliae* Inoculated	35.74	0.43	0.31	0.81	0.36
	3	LSM; Non-inoculated	36.34	0.53	0.30	0.78	0.35
	4	LSM; *V. dahliae* Inoculated	31.63	-0.25	0.30	0.65	0.37
p =			0.59	0.59	0.969	0.894	0.799
Fall	5	Unamended; Non-inoculated	33.24	-0.30 ab	0.39	1.08 ab	0.21
	6	Unamended; *V. dahliae* Inoculated	38.02	0.01 ab	0.31	0.71 bc	0.24
	7	LSM; Non-inoculated	24.98	-0.85 b	0.26	0.59 c	0.22
	8	LSM; *V. dahliae* Inoculated	43.23	0.36 ab	0.36	0.98 abc	0.22
	9	Fumigated; *V. dahliae* Inoculated	47.61	0.65 a	0.35	1.165 a	0.21
	10	LSM & Fumigated; *V. dahliae* Inoculated	39.87	0.14 ab	0.25	0.615 c	0.20
p =			0.059	0.059	0.221	0.048	0.999

Figure 4.5 Total yield as z-scores of tubers collected from soils from a field amended with lobster shell meal (LSM) in the fall, salmon-colored bars indicate LSM amendment. Other treatments were combinations of unamended with LSM, fumigated one month prior to LSM amendment, and inoculated with *Verticillium dahliae*. Yields were not significantly different ($p = 0.059$).

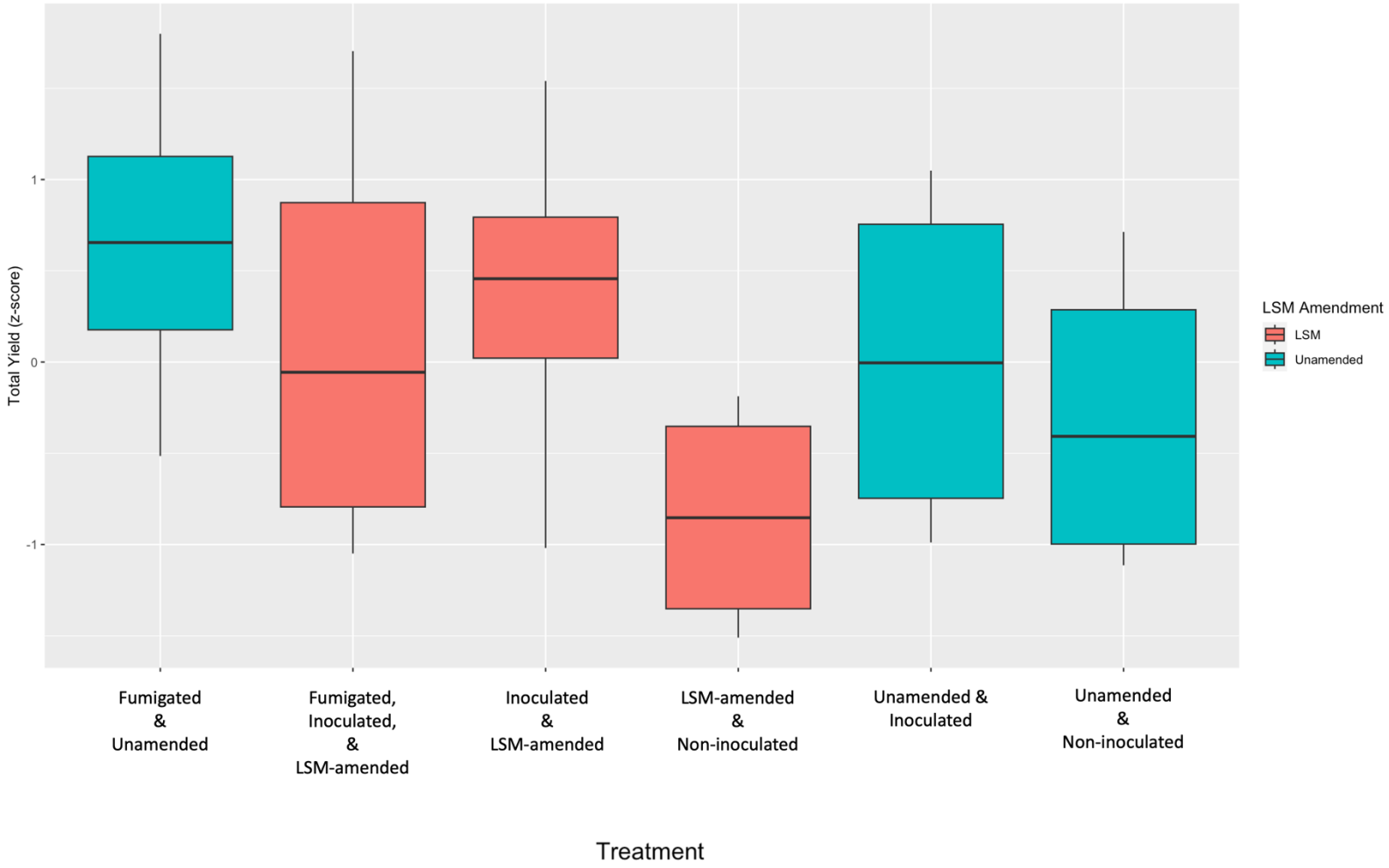
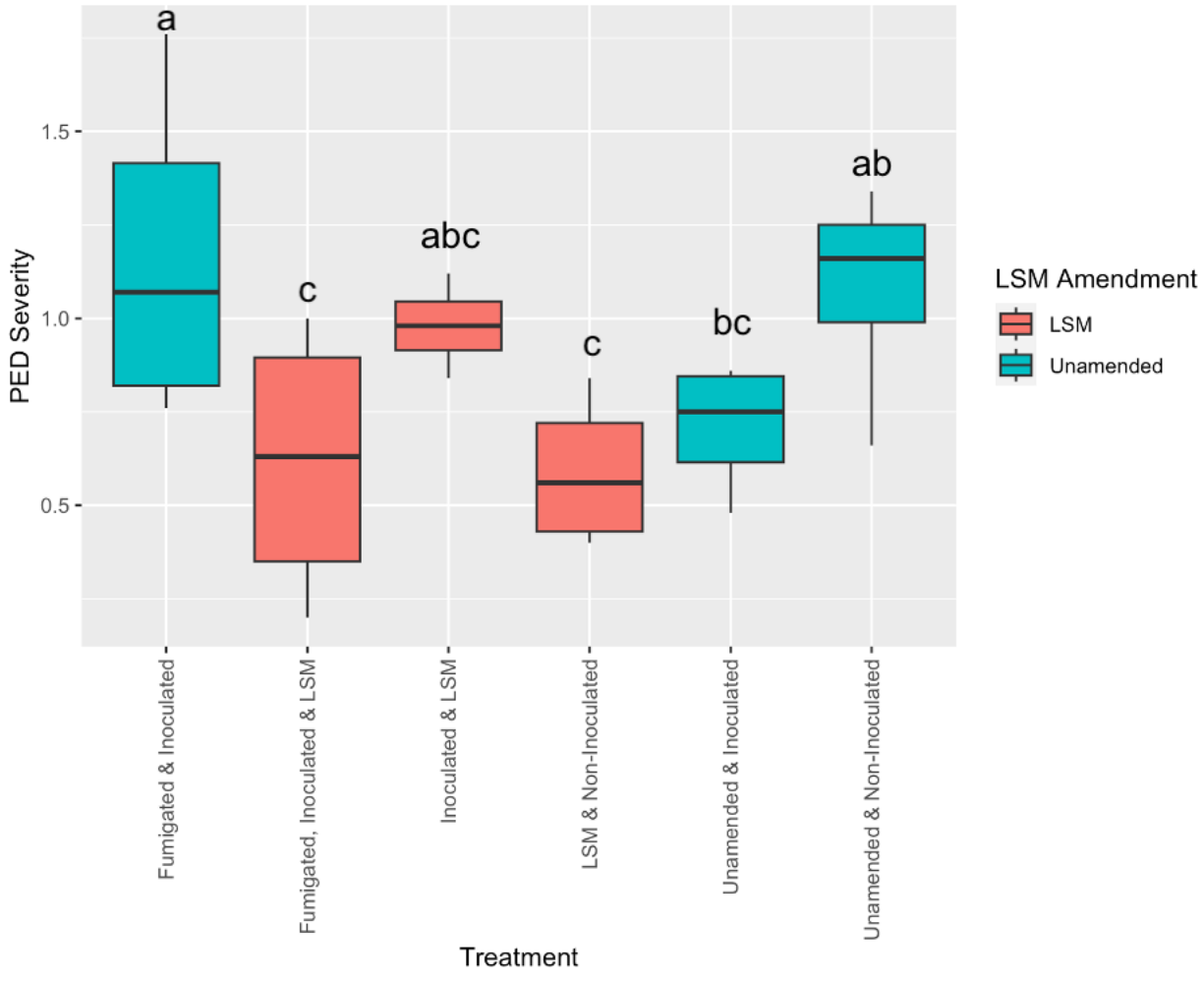


Figure 4.6 PED severity in tubers harvested from a field amended with lobster shell meal (LSM) in the fall, salmon-colored bars indicate LSM amendment. Other treatments were combinations of unamended with LSM, fumigated one month prior to LSM amendment, and inoculated with *Verticillium dahliae*. PED severities were significantly different by the treatments ($p = 0.048$). Bars topped by the same letter are not significantly different from each other based on Fisher's protected LSD test at $p < 0.05$



4.3.3 Lobster shell meal effects to soil microbial communities in the field

Over both fall and spring amended trial samples, 9,180,019 bacterial sequences were delivered which resulted in 6,049 bacterial ASVs across 155 samples. Within the fungal sequences, 3,028,243 reads were delivered, resulting in 14,246 ASVs across 155 samples. In spring-LSM-amended soils, microbial community diversity metrics, including observed ASVs, Shannon diversity index, evenness, and richness indicators ACE and Chao1, were not significantly different for soil bacterial communities among treatments. Fungal Shannon diversity was significantly different among soil treatments and was the lowest in soils amended with LSM and highest in the unamended, uninoculated treatment (Table 4.2). Within bacterial communities in soils amended with LSM in the spring, 127 ASVs were core with LSM, and 135 ASVs were core without LSM amendments (Table 4.3). By excluding ASVs which were common core members, and likely represented simply common soil microorganisms, 13 were conditionally core to LSM amended soils only and 20 were conditionally core to soils without LSM amendment. Of the top three bacterial phyla of ASVs which were conditionally core with LSM amendment, 30.7% were in the phylum Bacteriodota, followed by 23.1% Chloroflexi, and 23.1% Planctomycetota (Figure 4.7). The top two bacterial phyla which were conditionally core without LSM amendment, were Acidobacteriota and Chloroflexi (20% and 15%, respectively; Figure 4.7). For fungal core communities, 79 were found to be core with LSM amendment, and 78 were core without LSM amendment. After common core fungal ASVs were removed, 4 remained as conditionally core to LSM amended treatments, and 6 remained conditionally core to that without LSM amendment (Table 4.3). Of those fungal ASVs which were conditionally core with LSM amendments, three were unidentified, but one was identified by FungalTraits as a litter saprotroph. Of the fungal ASVs which were conditionally core without LSM, two were

unidentified, two were saprotrophs, one was a plant pathogen, and one was an animal parasite. In spring amended treatments, only one bacterial ASV was more abundant with LSM amendment and was identified in the order Solirubrobacterales and family *67-14* and one fungal ASV was more abundant with LSM and was identified to the family *Nectriaceae*.

In fall-amended microbial communities, treatment alone was responsible for significant differences in bacterial evenness and fungal Shannon diversity and evenness (Table 4.2). When different factors within the treatments were analyzed, fumigation was found to significantly reduce bacterial evenness, particularly in the treatment which was fumigated and amended with LSM (Table 4.2). When core communities were identified, 123 bacterial ASVs were core to LSM-amended soils, and 109 bacterial ASVs were core to treatments without LSM amendment. When common core members were removed, 20 ASVs were found to be conditionally core with LSM amendment, and 7 were found to be conditionally core without LSM amendment (Table 4.3). The top three bacterial phyla of conditionally core ASVs with LSM amendment, were Proteobacteria, Verrucomicrobiota, and Acidobacteriota (30%, 20%, and 20%, respectively; Figure 4.7). The top two bacterial phyla of conditionally core ASVs without LSM amendment were Acidobacteriota and Planctomycetota (42.9% and 28.6%, respectively; Figure 4.7). For fall amended fungal core communities, 61 ASVs were identified as core with LSM amendment and 69 were identified as core without LSM amendment (Table 4.3). Once common core members were removed, 5 fungal ASVs were conditionally core with LSM amendment and 8 were conditionally core without LSM amendment. FungalTraits identified those conditionally core ASVs with LSM amendment as having the primary lifestyle as two saprotrophs, one mycoparasite, one plant pathogen, and one was unidentified. Of those conditionally core ASVs without LSM amendments, FungalTraits identified five to be saprotrophs, one to be a

mycoparasite, and two were unidentified. There were not any differentially abundant bacterial ASVs in the fall amended microbial communities, however one fungal ASV was significantly less abundant with LSM amendment and was identified as *Linnemannia hyalina*.

PCoA ordinations of bacterial and fungal communities in both fall and spring amended trials did not indicate differentiation by LSM amendment, except by sampling timepoint (Figure 4.8).

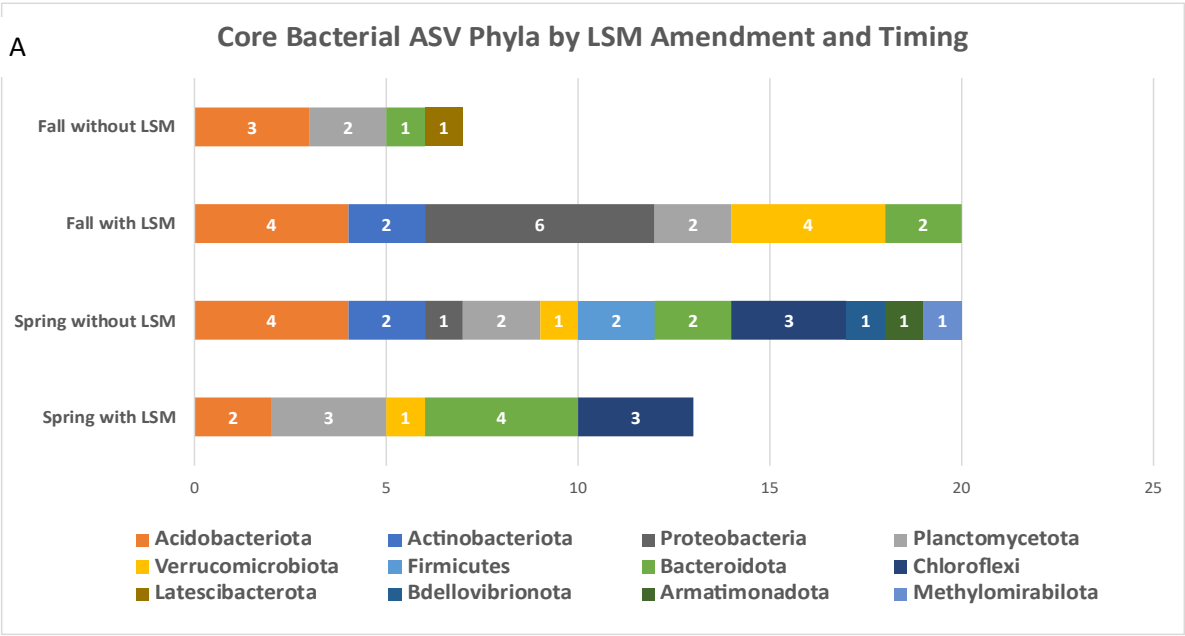
Table 4.2 Alpha and beta diversity indicators of soil bacterial and fungal microbial communities, including Shannon diversity index, observed ASVs, evenness, and richness indicators ACE and Chao, by treatment for the spring and fall LSM amended field trials. Values followed by the same letter within each parameter are not significantly different based on Fisher's protected LSD test at $p < 0.05$. Fall and spring amendment time points were analyzed separately.

Bacterial Diversity Statistics							
Amendment Timing	Tmt.	Description	Average Shannon Diversity	Average Observed ASV Diversity	Average Evenness	Average ACE	Average Chao1
Spring	1	Unamended; Non-inoculated	5.80	493.50	0.94	494.84	494.94
	2	Unamended; Inoculated	5.75	453.25	0.94	455.01	456.28
	3	LSM; Non-inoculated	5.76	445.38	0.94	446.23	446.92
	4	LSM; Inoculated	5.73	451.63	0.94	452.70	453.66
p =			0.59	0.57	0.11	0.58	0.60
Fall	5	Unamended; Non-inoculated	5.68	422.00	0.94 a	423.01	423.96
	6	Unamended; Inoculated	5.64	409.75	0.94 a	410.50	410.60
	7	LSM; Non-inoculated	5.67	412.38	0.94 a	413.08	413.57
	8	LSM; Inoculated	5.61	388.63	0.94 a	389.21	389.45
	9	Fumigated; Inoculated	5.55	378.13	0.94 a	378.59	378.44
	10	LSM & Fumigation; Inoculated	5.62	455.63	0.92 b	457.70	457.62
p =			0.79	0.50	0.01	0.49	0.49
Fungal Diversity Statistics							
Amendment Timing	Tmt.	Description	Average Shannon Diversity	Average Observed Diversity	Average Evenness	Average ACE	Average Chao1
Spring	1	Unamended; Non-inoculated	4.94 ab	435.38	0.82	466.76	462.45
	2	Unamended; Inoculated	4.95 a	419.13	0.82	449.75	448.42
	3	LSM; Non-inoculated	4.82 bc	383.00	0.81	403.78	399.76
	4	LSM; Inoculated	4.79 c	401.00	0.80	428.56	422.92
p =			0.02	0.49	0.07	0.60	0.58
Fall	5	Unamended; Non-inoculated	4.84 ab	431.88	0.80 a	467.89	461.44
	6	Unamended; Inoculated	4.73 abc	439.63	0.78 abc	493.21	489.67
	7	LSM; Non-inoculated	4.62 bc	430.75	0.76 bc	482.93	476.16
	8	LSM; Inoculated	4.51 c	419.13	0.75 c	466.42	463.18
	9	Fumigated; Inoculated	4.88 a	478.25	0.79 ab	532.77	526.96
	10	LSM & Fumigation; Inoculated	4.64 bc	444.25	0.76 bc	502.81	498.21
p =			0.02	0.30	0.02	0.36	0.37

Table 4.3 Core community members of bacteria and fungi in soils treated with lobster shell meal (LSM) amendment by spring and fall. Initial total included those which were common core members overall. Conditionally core members were core to that management factor level. Uniquely core members were not core to any other management factor or level, but only core to that specific management factor level.

Factor		**16S**			**ITS**		
		Initial Total	Number w/o shared	Uniquely Core	Initial Total	Number w/o shared	Uniquely Core
Fall Amended	With LSM	123	28	20	61	6	5
	Without LSM	109	14	7	69	14	8
Spring Amended	With LSM	127	24	13	79	13	4
	Without LSM	135	32	20	78	12	6

Figure 4.7 Phyla of amplicon sequence variants (ASVs), of conditionally core bacteria (A) and primary lifestyle assigned by FungalTraits of conditionally core fungal ASVs (B) in soils either amended with lobster shell meal (LSM) or not and amended in the fall or spring. Conditionally core members were core to that management factor level.



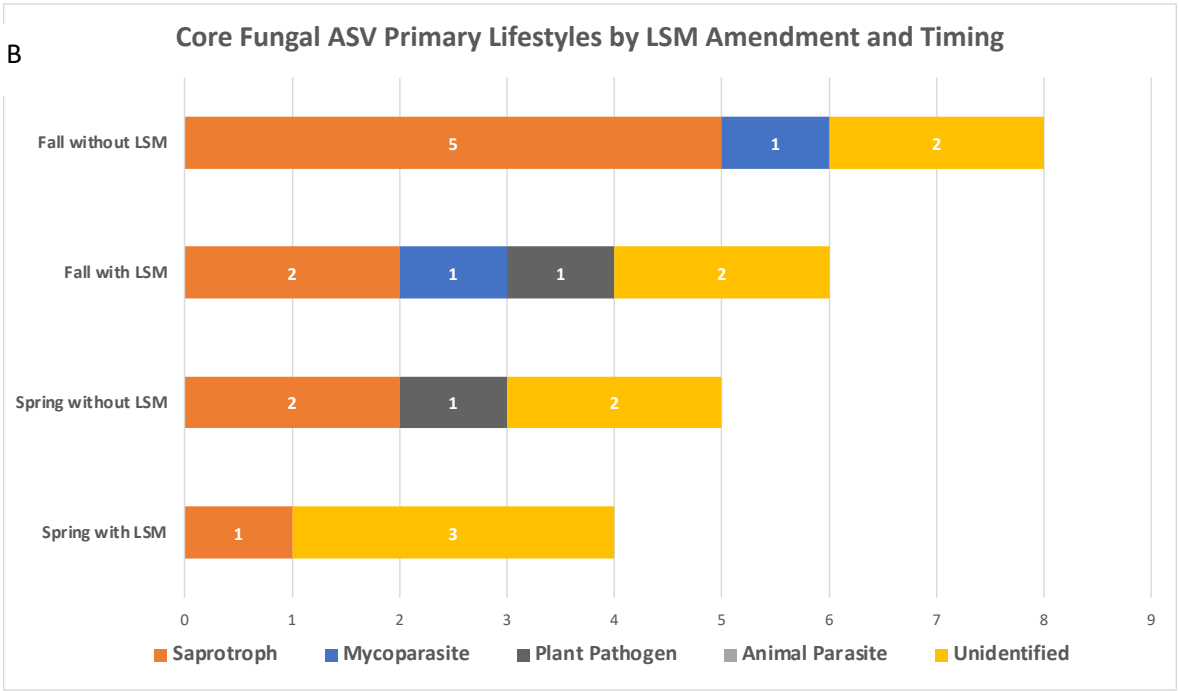
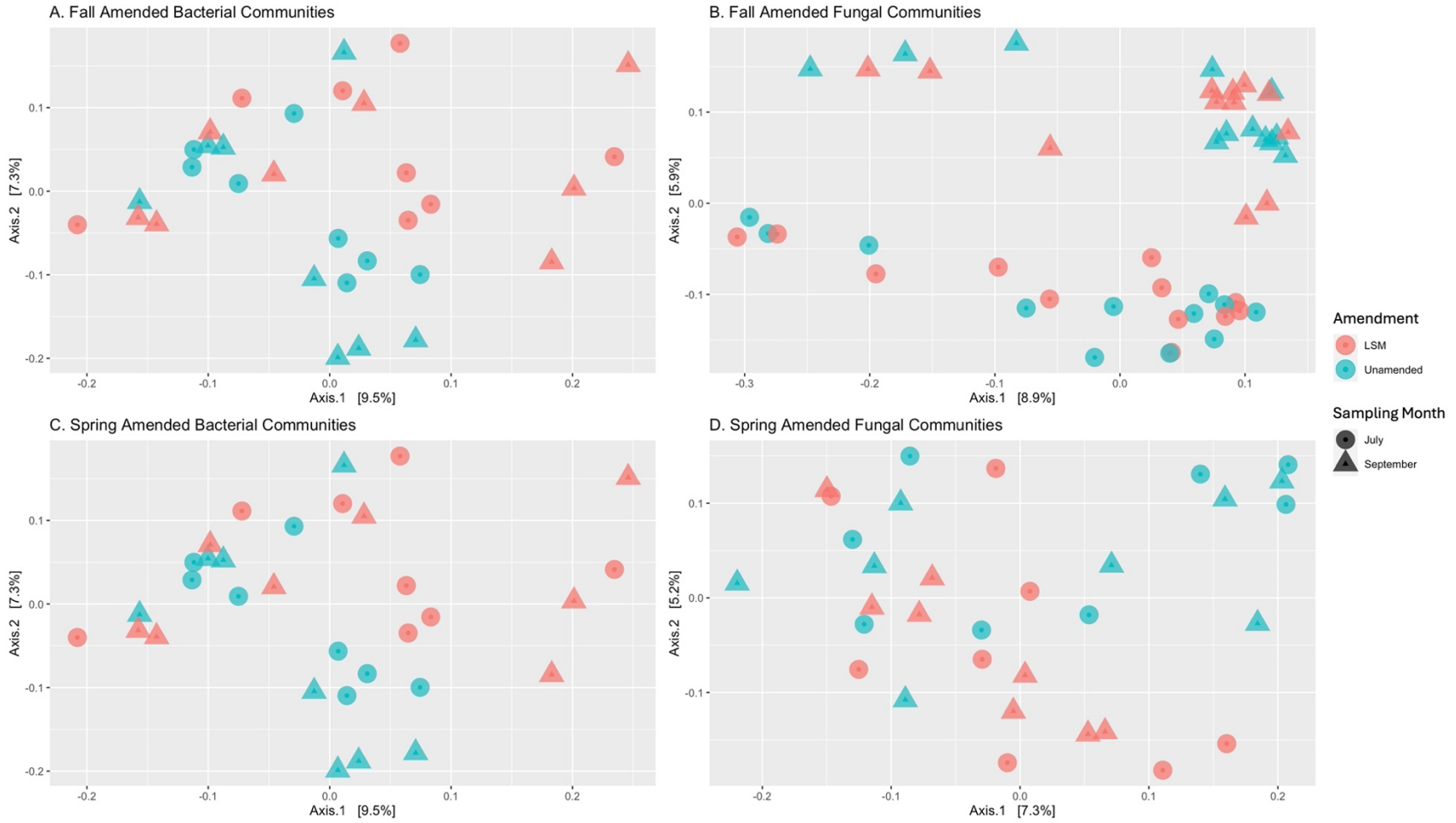


Figure 4.8 Principal Coordinate Analysis (PCoA) of Bray-Curtis dissimilarity of bacteria and fungi in trials amended with lobster shell meal (LSM) in the fall (A, $p = 0.501$; B, $p = 0.371$) and spring (C, $p = 0.646$; D, $p = 0.948$). Clusters are not significantly different by LSM amendment.



4.4 Discussion

In this study, the use of LSM as a soil amendment to reduce soilborne diseases and increase yield was evaluated in potato production. Unfortunately, there were no consistent trends across both the greenhouse and field trials regarding treatment effects on soilborne disease and potato growth. However, there were some significant treatment effects in individual trials, which suggest more research is needed to understand the role of LSM as a soil amendment. The impacts of LSM amendment clearly promoted the growth of aboveground tissues of potato plants in greenhouse settings. This is similar to other studies, which found crawfish shell powder to significantly increase radish growth in greenhouse studies (Chen et al. 2023). However, this did not translate to effects on tuber yield in either the greenhouse or field trials, as there were no significant differences in potato yield. In field trials, PED incidence and severity was not consistently lower in uninoculated soils, which indicates that the field inoculation did not increase the disease pressure. However, in the fall amended field trial, fumigated soil with LSM amendment significantly reduced the disease severity compared to unamended and fumigated treatments. However, this was not consistent across non-fumigated treatments. The long-term effects are yet to be investigated, especially with respect to impacts to native microbial populations and disease pressure.

The rate of LSM used in the field trial was determined based on recommendations by Coast of Maine, a Maine-based soil amendment company (Coast of Maine, Portland, Maine). However, in comparison with other studies of shellfish-based soil amendments showing significant effects on disease suppression and plant growth promotion, the rates used in this study were about half or less than half of those found in other studies using chitin derived from crustacean shells (Debode et al. 2016; Inderbitzin et al. 2018). For example, crab shell meal as a

soil amendment to soils which were originally conducive of Verticillium wilt in eggplant resulted in the soils becoming disease suppressive after amendment with crab shell meal at a rate approximately two times the amount used in this study (Inderbitzin et al. 2018). In the greenhouse study, higher rates of LSM increased aboveground biomass, however, disease incidences and severities were not consistently impacted by the treatments in both the greenhouse and field trials. Additionally, the 2023 field season, when the trials were planted in potatoes, presented many challenges, including excess rainfall, very cool temperatures, and employment turnover at the farm resulting in reduced field maintenance. These field season issues may have impacted these results.

Fungal communities had lower Shannon diversity when amended with LSM, regardless of amendment timeline, and LSM reduced fungal evenness when amended in the fall. Similarly, fumigation decreased bacterial evenness, specifically when LSM amendments were also added. While there were no clear consistent trends, it was found that LSM was more impactful to fungal communities while fumigation was impacting bacterial evenness. Additionally, the LSM treatments did not significantly alter bacterial diversity, richness, or evenness. Evenness is a measurement of the distribution of different species within a sample, and a reduction indicates that the treatments impacted different microbial community members unequally (Zhang and Xu 2008). Additionally, fall and spring amended soil microbial communities did not follow similar patterns. This could be the result of the delayed amendment timeline, or it could be an artifact of the microbially distinct fields. Either of these could be supported by literature, as Rose et al. found that immediately amending inoculated soils with chitin could quickly increase available nitrogen, promoting seedling growth, but not provide time for antagonistic populations to build and suppress pathogenic populations, and result in more disease incidence (Rose et al. 2003).

This was not observed in this study directly, but core community member distribution, as well as alpha diversity stats varied between the two amendment timepoints.

While disease incidences were not significantly different by treatment, fall amended treatments applied to fumigated soils showed significantly less severe. It has been observed that beneficial bacterial phyla can rapidly rebound following fumigation, but it has also been observed that fumigation can negatively impact soil function and health by altering microbial communities (Sederholm et al. 2018; Sennett et al. 2022). Alterations to the soil microbial communities is often considered to be balanced by functional redundancy, but microbial diversity differences can impact the functions performed by soil microbiota (Juarez et al. 2013; Philippot et al. 2013). For this reason, the restructuring of the soil microbiome following fumigation to promote soil health and suppressiveness is an area of ongoing research, and the use of biological control agents or organic amendments following fumigation has been shown to reduce both *Fusarium* spp. and *Phytophthora* spp. populations in tomato production compared to fumigation alone (Cheng et al. 2021). While these results did not indicate that LSM was significantly and consistently restructuring bacterial microbial communities, there were differences related to fumigation and LSM amendment. When soils were amended with LSM in the fall, fungal Shannon diversity was lower than non-amended treatment pairs with or without *V. dahliae* inoculum, respectively. However, in treatments which were fumigated, bacterial diversity was higher with LSM amendment compared to unamended. This could imply that in the absence of a high stress event, LSM could be altering communities by reducing diversity, as has been seen in other studies of chitin-amended soils (Randall et al. 2020). However, after a high stress event which impacts soil bacteria, such as fumigation which significantly reduced the evenness of bacterial communities, LSM could be serving to restructure and promote bacterial

communities. Further research is required to determine if LSM can be a useful tool in restructuring and potentially restoring bacterial communities after stress.

There were more bacterial core community members with fall amendment compared to spring amendment with LSM, which is unsurprising because bacterial diversity was numerically, but not significantly, reduced with LSM amendment. With LSM amendments, the top phyla of bacteria with LSM amendment were Bacteroidota and Proteobacteria, with spring and fall amendment, respectively. Furthermore, of all the bacterial core members overall regardless of amendment timing, Bacteroidota had consistently more core members with LSM amendment than without. Bacteria in the Bacteroidota phylum are a highly varied group which are commonly found in soils and perform a wide range of ecosystem services ranging from metabolizing carbohydrates, antagonizing plant pathogens, and some are plant growth promoting rhizobacteria (Kruczyńska et al. 2023).

Overall, there were many fewer fungal core members compared to bacterial core members, which is unsurprising, as there were much fewer fungal ASVs detected compared to bacterial ASVs. However, of those ASVs representing core members in LSM-amended soils, there were no strong trends of specific fungal primary lifestyles being enhanced by LSM, as there were equal numbers of plant pathogens and mycoparasites both with and without LSM amendment. There were consistently more uniquely core fungi regardless of amendment timeline in those treatments without LSM amendment, with the majority representing various saprotrophs. The larger number of these saprotrophs without LSM treatment could indicate that the amendments could be inadvertently impacting these fungi, as fungal diversity and evenness were also both significantly reduced with the fall LSM amended treatments. Fungal populations have previously been found to be significantly reduced through the addition of chitin

amendments, and this finding is consistent with other studies (Bell et al. 1998). Only one ASV was significantly less abundant with fall amendment, and this fungal ASV was identified as *Linnemannia hyalina.* This fungus is known to be both adapted to the cold, capable of ecosystem services such as nitrate reduction in soil, and degradation of cellulose (Aldossari and Ishii 2021, 2022).

In spring amended treatments, one bacterial ASV and one fungal ASV were found to be significantly more abundant with LSM and were identified in the families *67-14* (of the order Solirubrobacterales) and *Nectriaceae*, respectively. The family *67-14* is in the order Solirubrobacterales, which is poorly described, but is known to contain gram positive bacteria which can tolerate a wide range of temperatures (Whitman and Suzuki 2015). However, this family also is within the better studied phylum Actinobacteriota, which are common to aquatic and terrestrial environments, produce mycelium, and are known as antagonists as they are capable of producing antibiotics and enzymes to decompose various organic substances (Hazarika and Thakur 2020). Nearly all bacteria in the phylum Actinobacteriota are capable of decomposing chitin, and have been found to more effectively use shrimp shells than colloidal chitin for the production of chitinases, which could explain why this ASV is significantly more abundant with LSM amendment than without (Swiontek Brzezinska et al. 2014). Fungal members in the family *Nectriaceae* are not well defined, but include plant and animal pathogens, biodegraders, and biological control agents (Lombard et al. 2015). However, because this particular ASV was not identified further, it is difficult to determine the role it is playing in the soil environment.

4.5 Conclusions

In greenhouse studies, LSM treatments increased aboveground plant growth proportionately with increasing rates, although this trend did not translate to enhanced potato yields in either the greenhouse or field trials. Additionally, in the initial greenhouse trial PED incidence was found to be significantly reduced when planting was time delayed when amended with the higher rate of LSM, however this was not seen in the second greenhouse trial and could point to the stress of these plants not being fertilized. Disease incidences within tubers were not significantly different in either greenhouse or field trials by the treatments, although PED severity was significantly reduced by LSM amendment within fumigated soils. In general, fungi were more sensitive to the addition of LSM. LSM was found to alter fungal communities by reducing evenness in both the fall and spring amended trials, and by reducing Shannon diversity in the fall amended trial only. Bacterial diversity metrics were not significantly affected by LSM amendment, except one bacterial ASV in the phylum Actinobacteriota was found to be significantly more abundant with LSM amendment. Although trial limitations and environmental anomalies may not have resulted in a clear or consistent demonstration of the potential benefits of LSM treatments in this study, LSM as a soil amendment may be a useful tool in restructuring soil microbial communities following fumigation, reducing PED severity, and enhancing plant growth, but requires much additional research. In the future, better field maintenance, increased rates of LSM, and enhanced investigations of the role of LSM in PED disease severity suppression following fumigation could improve its efficacy and use as a soil amendment.

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APPENDICES

Appendix A. Aggregate data

Table A.1 Aggregate data collected in the first two years of the study of management impacts to soil health in potato producing fields in Maine. Summer of year one and spring and fall of year two, depending on the crop which was planted. See full treatment descriptions in Table 2.1.

Treatment	Year 1 Summer 2019 (2-year in rotations; 3-year in potatoes)			Year 2 (2-year potatoes in Spring; 3-year rotations in Fall)		
	Large Macroaggregate Percent	Macroaggregate Percent	Microaggregate Percent	Large Macroaggregate Percent	Macroaggregate Percent	Microaggregate Percent
1[bc]	18.654	44.906	21.9	16.33	37.318	16.674
2[bd]	17.578	41.102	24.976	16.262	33.948	17.218
3[bce]	19.66	40.504	24.906	18.604	31.454	14.666
4[bdg]	18.454	42	24.9	16.77	30.894	14.798
5[bcf]	19.616	44.348	22.45	18.052	41.258	16.064
6[bdf]	18.568	41.574	26.008	18.516	32.82	15.1
7[ac]	17.622	28.01	35.872	15.496	36.63	19.22
8[ad]	18.002	28.802	36.642	13.54	34.344	18.106
9[ace]	16.596	29.852	39.518	18.094	35.314	19.784
10[adg]	14.704	32.312	40.582	16.262	38.148	17.344
11[adef]	13.33	32.602	37.174	14.508	35.608	20.082
12[ach]	14.922	39.096	31.704	13.814	33.772	19.096

[a] Two-year rotation
[b] Three-year rotation
[c] Russet Burbank
[d] Caribou Russet
[e] Compost
[f] Non-grain Rotation Crops
[g] Green manure
[h] Fumigation

Table A.2. Aggregate data collected in the last two years of the study of management impacts to soil health in potato producing fields in Maine. In year three soil was sampled in the fall and all treatments were in rotation crops. In year four, soil was sampled in the summer and all treatments were in potatoes. See full treatment descriptions in Table 2.1.

Treatment	Year 3 (Fall 2021; all rotations)			Year 4 (Summer 2022; all potatoes)		
	Large Macroaggregate Percent	Macroaggregate Percent	Microaggregate Percent	Large Macroaggregate Percent	Macroaggregate Percent	Microaggregate Percent
1[bc]	17.664	29.352	18.704	14.124	32.792	11.792
2[bd]	17.034	27.81	18.414	14.928	32.208	11.644
3[bce]	17.85	25.386	15.528	14.032	30.728	11.212
4[bdg]	15.564	27.304	17.884	16.068	33.408	10.772
5[bcf]	16.04	29.416	18.934	14.628	37.48	11.056
6[bdf]	16.994	29.316	19.218	14.48	33.444	11.044
7[ac]	17.246	31.906	16.514	16.368	35.104	11.432
8[ad]	17.916	30.006	20.184	16.064	34.452	10.56
9[ace]	17.824	31.242	17.68	15.716	34.74	11.436
10[adg]	15.108	33.088	22.182	15.98	35.24	11.252
11[adef]	16.972	32.47	17.824	14.604	42.352	11.204
12[ach]	15.028	34.886	18.816	15.216	38.64	10.928

[a] Two-year rotation
[b] Three-year rotation
[c] Russet Burbank
[d] Caribou Russet
[e] Compost
[f] Non-grain Rotation Crops
[g] Green manure
[h] Fumigation

Appendix B. All potato early dying assay data

Table B.1. Nematode and Verticillium propagule counts collected as part of the potato early dying screening analysis results from soils collected in a study of management impacts to soil health in potato producing fields in Maine. See full treatment descriptions in Table 2.1.

Treatment	Study Year One (2-year Fall 2019 in Rotations; 3-year Spring 2019 in potatoes)		All Second Season of Rotations (Fall 2021)		All Second year of Potatoes (Summer 2022)	
	Root Lesion Nematodes (per 100cc of soil)	Verticillium Propagules per gram of soil	Root Lesion Nematodes (per 100cc of soil)	Verticillium Propagules per gram of soil	Root Lesion Nematodes (per 100cc of soil)	Verticillium Propagules per gram of soil
1[bc]	340	0	94.6	0	74.2	0
2[bd]	416.2	0	6.8	0	15.8	0
3[bce]	330.8	0	6.8	0	6	0
4[bdg]	389.6	0	42.8	0	33	0
5[bcf]	322.2	0	132.8	0	83.2	0.4
6[bdf]	420.8	0	74.4	0	32.4	0
7[ac]	162.2	0	128.6	2	96.8	0
8[ad]	216.8	0	142	0	34	0
9[ace]	232.8	0	60.8	0	21.2	0.4
10[adg]	170.2	0	92.6	0	83.6	0
11[adef]	144.2	0	187	0.4	22.6	0.4
12[ach]	223.4	0.4	38.4	0	15.2	0

[a] Two-year rotation
[b] Three-year rotation
[c] Russet Burbank
[d] Caribou Russet
[e] Compost
[f] Non-grain Rotation Crops
[g] Green manure
[h] Fumigation

Appendix C. All physiochemical data collected while plots were planted in rotations

Table C.1 Physiochemical data of results of soil sampled collected from plots during year one of rotations (part 1) in a study of management impacts to soil health in potato producing fields in Maine. This occurred in 2019 for two-year rotations and 2020 for three-year rotations. See full treatment descriptions in Table 1.

Rotation Year One Collected in the Fall at Harvest (2019 for 2-year rotations; 2020 for 3-year rotations): Part 1															
Tmt	ACE Protein (mg/gm)	NH4 (ppm)	Boron (ppm)	Bray Phosphorus (ppm)	Base Saturations (percent)					Calcium (ppm)	Cation Exchange Capacity (meg/100g)	Cation Exchange Capacity (Percent)	Copper (ppm)	Iron (ppm)	Magnesium (ppm)
					Calcium	Hydrogen	Magnesium	Potassium	Sodium						
1[bc]	7.76	4.54	0.26	172.8	42.24	41.14	12.68	3.82	0.44	883.2	10.8	0.74	5.952	70.04	156.8
2[bd]	7.34	5.62	0.26	164.6	40.74	43.88	11.34	3.52	0.42	828	10.84	0.76	6.418	67.48	139.8
3[bce]	8.28	4.8	0.24	183.2	51.36	28.86	14.16	4.54	0.94	1048	10.64	0.76	5.44	59.94	173
4[bdg]	7.48	4.34	0.2	177	51.36	29.54	15.12	3.64	0.48	1058.4	10.42	0.72	5.544	47.7	185.8
5[bcf]	7.9	4.44	0.28	184.6	42.24	39.9	13.18	4.18	0.46	842.4	10.66	0.74	6.108	81.2	158.2
6[bdf]	7.42	7.74	0.2	168.6	43.28	40.16	12.22	3.94	0.44	909.4	11.06	0.76	6.218	53.94	155.6
7[ac]	8.24	3.56	0.34	146	46.28	39.2	10.68	3	0.7	818.2	8.94	0.18	6.964	86.16	114.6
8[ad]	7.68	4.52	0.2	146.2	49.22	35.82	11.18	3.08	0.78	833.2	8.56	0.16	6.608	54.84	115.2
9[ace]	8.14	3.92	0.34	142	43.56	42.02	10.62	2.94	0.74	796.2	9.3	0.24	6.866	91.32	116.6
10[adg]	8.54	3.76	0.24	145.2	46.34	39.44	10.3	3.12	0.72	819.4	9.12	0.18	7.194	62.08	107.6
11[adef]	8.12	4	0.22	145.8	49.12	34.76	12.28	3.22	0.84	839.6	8.7	0.14	6.79	58.26	126.8
12[ach]	8.5	3.52	0.26	141.2	25.34	63.64	8.04	2.38	0.62	536.2	10.98	0.02	7.5	70.14	103.2

[a] Two-year rotation
[b] Three-year rotation
[c] Russet Burbank
[d] Caribou Russet
[e] Compost
[f] Non-grain Rotation Crops
[g] Green manure
[h] Fumigation

Table C.2 Physiochemical results of soil collected from plots during year one of rotations (part 2) in a study of management impacts to soil health in potato producing fields in Maine. This occurred in 2019 for two-year rotations and 2020 for three-year rotations. See full treatment descriptions in Table 1.

Rotation Year One Collected in the Fall at Harvest (2019 for 2-year rotations; 2020 for 3-year rotations): Part 2															
Tmt	Manganese (ppm)	Nitrate-N (ppm)	Olsen Phosphorus (ppm)	Organic Matter (Percent)	pH	pH (Buffer)	Potassium (ppm)	POXC (ppm)	Salts (mmhos/cm)	Sodium (ppm)	Solvita (ppm)	Sulfur (ppm)	Total Carbon (Percent)	Total Organic Carbon (Percent)	Zinc (ppm)
1[bc]	3.882	8.4	59.4	4.24	5.74	6.48	153.4	503.6	0.096	10.6	157.36	3	1.74	1.66	0.32
2[bd]	3.878	8	58.2	4.1	5.7	6.44	143	444	0.096	10	144.54	2.6	1.68	1.6	0.294
3[bce]	3.478	9	60.6	4.48	5.9	6.64	182.2	477.6	0.154	21.8	173.06	6.2	1.92	1.86	0.816
4[bdg]	2.804	45.4	61	3.86	5.86	6.64	143.6	418.2	0.27	11.4	122.5	4.2	1.72	1.62	0.252
5[bcf]	4.9	17.9	61.2	4.2	5.62	6.48	160.4	420.4	0.12	10	127.36	3	1.74	1.64	0.33
6[bdf]	4.218	29.9	61	4.16	5.62	6.48	167.4	414.8	0.222	10.2	170.6	3	1.66	1.58	0.274
7[ac]	3.424	7.3	48.2	4.1	6.22	6.58	103	437.4	0.092	14	270.8	3.8	1.76	1.76	0.348
8[ad]	3.33	7.1	50.4	3.96	6.22	6.62	102.4	437.2	0.102	15.2	259.1	4.2	1.74	1.7	0.26
9[ace]	3.588	8.3	49.4	4	6.12	6.54	105.4	434.6	0.094	16	238.5	8.2	1.76	1.7	0.366
10[adg]	3.04	8.9	47.6	4.12	6.18	6.56	108.4	452	0.094	14.8	238.96	3.6	1.74	1.72	0.286
11[adef]	2.932	8.9	48.4	4	6.3	6.62	108.6	474.6	0.1	16.4	234.54	3.8	1.76	1.74	0.264
12[ach]	4.04	7.9	52.4	4	5.74	6.26	99.8	394.2	0.07	15.4	166.68	3	1.68	1.68	0.32

[a] Two-year rotation
[b] Three-year rotation
[c] Russet Burbank
[d] Caribou Russet
[e] Compost
[f] Non-grain Rotation Crops
[g] Green manure
[h] Fumigation

Table C.3 Physiochemical results of soil collected from plots during year two of rotations (part 1) of a study of management impacts to soil health in potato producing fields in Maine. All sampling occurred in 2021. See full treatment descriptions in Table 2.1.

Year Two Rotations Collected in the Fall at Harvest (all in 2021) Part 1														
				Base Saturations										
Tmt	ACE Protein (mg/gm)	NH4 (ppm)	Boron (ppm)	Calcium (Percent)	Hydrogen (Percent)	Magnesium (Percent)	Potassium (Percent)	Sodium (Percent)	Calcium (ppm)	Cation Exchange Capacity (meg/100g)	Cation Exchange Capacity (Percent)	Copper (ppm)	Iron (ppm)	Magnesium (ppm)
1[bc]	7.32	1.78	0.24	59.96	16.3	18.98	4.04	0.78	1007.4	8.42	0.6	6.178	70.74	191.2
2[bd]	7.02	1.96	0.26	64.42	10.04	20.4	4.34	0.88	1064.4	8.22	0.64	6.122	82	201
3[bce]	7.38	2.24	0.34	64.66	14.2	16.72	3.66	0.76	1184.4	9.18	0.78	6.19	101.46	184
4[bdg]	6.86	1.84	0.18	64.76	11.96	19	3.64	0.8	1149.2	8.92	0.64	5.916	56.62	203
5[bcf]	7.2	1.98	0.28	60.24	16.94	17.96	4.2	0.9	1059	9.06	0.66	6.302	83.44	193.2
6[bdf]	7.14	1.92	0.24	62.8	14.84	17.2	4.64	0.6	1017.2	8.16	0.64	6.052	76.68	167.4
7[ac]	7.2	2.4	0.3	52.08	27.52	15.24	4.64	0.58	825.6	7.96	0.74	7.276	100.1	144.6
8[ad]	7.08	2.36	0.42	55.22	24.46	15.18	4.6	0.58	870.4	7.96	0.68	7.528	153.96	144.6
9[ace]	8.18	2.94	0.48	64.5	13.22	17.28	4.54	0.66	1010	7.92	0.68	7.836	166.82	161.6
10[adg]	7.44	3.26	0.34	55.56	25.3	14.14	4.54	0.58	836.2	7.7	0.7	7.902	117.94	127.2
11[adef]	8.56	2.24	0.36	63.18	14.32	17.3	4.6	0.72	1039.2	8.24	0.64	7.724	139.48	170.6
12[ach]	7.42	4.02	0.16	60.66	15.82	17.54	5	0.7	918.8	7.62	0.64	7.47	64.46	158.6

[a] Two-year rotation
[b] Three-year rotation
[c] Russet Burbank
[d] Caribou Russet
[e] Compost
[f] Non-grain Rotation Crops
[g] Green manure
[h] Fumigation

Table C.4. Physiochemical results of soil collected from plots during year two of rotations (part 2) of a study of management impacts to soil health in potato producing fields in Maine. All sampling occurred in 2021. See full treatment descriptions in Table 2.1.

Year Two Rotations Collected in the Fall at Harvest (all in 2021) Part 2															
Tmt	Manganese (ppm)	Nitrate-N (ppm)	Olsen Phosphorus (ppm)	Organic Matter (Percent)	pH	pH (Buffer)	Potassium (ppm)	POXC (ppm)	Salts (mmhos/cm)	Sodium (ppm)	Solvita (ppm)	Sulfur (ppm)	Total Carbon (Percent)	Total Organic Carbon (Percent)	Zinc (ppm)
1[bc]	3.26	11.7	50	3.96	6.42	6.82	132.6	382.2	0.122	15.4	172.4	4	1.72	1.66	0.258
2[bd]	3.36	11.8	52.4	3.82	6.46	6.88	138.2	370.2	0.136	17	166.64	7.2	1.64	1.56	0.292
3[bce]	3.86	10.2	53.6	4.16	6.38	6.84	129.6	411.4	0.118	15.8	155.52	4.8	1.82	1.76	0.504
4[bdg]	2.92	10.7	50.4	3.76	6.48	6.86	126	337.6	0.122	16	155.54	5	1.62	1.56	0.234
5[bcf]	4.3	15.2	54.4	4	6.32	6.76	146.8	411.2	0.152	18.4	173.16	8.2	1.72	1.62	0.304
6[bdf]	4.94	11.5	50.6	4	6.32	6.82	147.4	440.8	0.114	10.8	165.72	5.8	1.74	1.64	0.306
7[ac]	4.18	9	54.4	4.1	6.06	6.7	141.6	418	0.088	10.6	175.74	2.8	1.78	1.7	0.37
8[ad]	6.68	10.7	52	4	6.1	6.72	141.8	427.6	0.094	10	161.28	3.4	1.72	1.62	0.466
9[ace]	5.26	14.2	65.8	4.46	6.16	6.82	141	512.4	0.12	12	178.88	4.6	2.02	1.92	1.212
10[adg]	4.78	9.7	51	4.14	6	6.72	134	442	0.084	10.4	154.68	2.6	1.74	1.68	0.402
11[adef]	5.28	15	64.8	4.56	6.2	6.8	148.8	502.8	0.122	13.8	189.96	3.2	2.08	1.98	1.474
12[ach]	4.62	11.1	50.2	4.16	6.18	6.82	146.8	460.2	0.114	12	222.54	4.8	1.86	1.82	0.288

[a] Two-year rotation
[b] Three-year rotation
[c] Russet Burbank
[d] Caribou Russet
[e] Compost
[f] Non-grain Rotation Crops
[g] Green manure
[h] Fumigation

Appendix D. All physiochemical data collected while plots were planted in potatoes

Table D.1. Physiochemical results from soil collected during the first year of potatoes and sampled in the spring at planting in a study of management impacts to soil health in potato producing fields in Maine. Year one sampling occurred in 2020 for two-year rotations and in 2019 for three-year rotations. See full treatment descriptions in Table 2.1.

Tmt	Potato Year One (2019 in 3-year rotations; 2020 in 2-year rotations) Part 1													
	Spring Sampling Timepoint													
	ACE Protein (mg/gm)	NH4 (ppm)	Boron (ppm)	Base Saturations (percent)					Calcium (ppm)	Cation Exchange Capacity (meg/100g)	Cation Exchange Capacity (Percent)	Copper (ppm)	Iron (ppm)	Magnesium (ppm)
				Calcium	Hydrogen	Magnesium	Potassium	Sodium						
1[bc]	8.38	16.48	0.42	33.16	45.92	13.58	6.52	0.88	516.6	7.98	0.34	6.128	114.3	126.6
2[bd]	8.1	11.6	0.3	33.12	47.08	13.4	5.64	0.88	521.6	7.94	0.22	6.09	88.16	127.2
3[bce]	9	19.68	0.2	40.02	35.3	14.8	7.46	2.62	762.6	9.78	0.24	5.586	50.4	168.4
4[bdg]	8.26	6.32	0.2	33.34	48.46	12.74	4.5	0.88	601.6	9.08	0.2	5.452	65.58	136.8
5[bcf]	8.24	21.22	0.18	35.18	43.62	14.68	5.68	0.96	607.4	9.04	0.22	5.824	55.64	147.4
6[bdf]	8.08	19.36	0.28	38.8	38.42	15.44	6.14	0.9	567.4	8.2	0.22	5.782	85.9	137.4
7[ac]	6.64	21.7	0.2	35.88	45.74	12.6	5.3	0.5	766	10.96	0.14	6.956	50.06	164.8
8[ad]	6.44	56.76	0.22	40.74	40.12	12.92	5.62	0.6	792	10.04	0.1	6.828	58.02	151
9[ace]	7.12	66.6	0.2	45.08	27.22	16.42	9.36	2.24	913	10.26	0.12	7.024	45.04	198.8
10[adg]	6.54	55.08	0.22	36.2	46.34	11.6	5.32	0.52	790.6	10.98	0.12	7.45	65.68	151.8
11[adef]	6.54	25.24	0.36	42.72	33.18	15.64	6.86	1.62	783.4	9.46	0.04	7.196	112.1	170
12[ach]	6.46	67.82	0.22	43.2	33.5	15.96	6.56	0.78	873.4	10.28	0.02	6.966	61.36	193.8

[a] Two-year rotation
[b] Three-year rotation
[c] Russet Burbank
[d] Caribou Russet
[e] Compost
[f] Non-grain Rotation Crops
[g] Green manure
[h] Fumigation

Table D.2. Physiochemical results of soil collected during the first year of potatoes and sampled in the spring at planting in a study of management impacts to soil health in potato producing fields in Maine. Year one sample were collected in 2020 for two-year rotations and in 2019 for three-year rotations. See full treatment descriptions in Table 2.1.

Tmt	Potato Year One (2019 in 3-year rotations; 2020 in 2-year rotations) Part 2														
	Spring Sampling Timepoint														
	Manganese (ppm)	Nitrate-N (ppm)	Olsen Phosphorus (ppm)	Organic Matter (Percent)	pH	pH (Buffer)	Potassium (ppm)	POXC (ppm)	Salts (mmhos/cm)	Sodium (ppm)	Solvita (ppm)	Sulfur (ppm)	Total Carbon (Percent)	Total Organic Carbon (Percent)	Zinc (ppm)
1[bc]	8.794	60.7	62.2	4.12	5.02	6.56	195.4	428.6	0.376	15.6	139.06	3.8	1.78	1.7	0.43
2[bd]	8.324	48	54.8	4.02	5.16	6.56	168.6	407.8	0.274	15.4	151.46	3.8	1.74	1.7	0.374
3[bce]	9.544	83.9	72.6	4.72	5.14	6.58	270.6	515.4	0.65	57.2	151.86	12	2.12	2.08	1.906
4[bdg]	7.552	16.5	57.4	4.04	5.5	6.5	157	448.2	0.112	17.4	165.88	4.6	1.72	1.72	0.336
5[bcf]	8.648	75.3	64.2	4.12	5.12	6.52	185.6	419	0.462	19.2	154.08	4.6	1.74	1.72	0.318
6[bdf]	7.916	43.8	61	4.04	5.32	6.58	189.2	450	0.344	16	154.1	5	1.74	1.68	0.35
7[ac]	4.37	137.5	61.8	3.96	5.34	6.44	228.6	365.6	0.758	12.8	134.5	3.8	1.74	1.74	0.368
8[ad]	4.26	123.2	60.2	3.92	5.52	6.52	211.8	358.8	0.756	13.8	145.98	6.2	1.72	1.72	0.376
9[ace]	4.594	143.5	75.2	4.32	5.52	6.68	355	402.2	0.966	49	159.18	14.6	2	1.98	1.578
10[adg]	6.04	171.3	64.6	4.06	5.2	6.44	229.6	348.4	0.992	13.2	119.14	5.2	1.8	1.76	0.46
11[adef]	4.632	113.8	70.6	4.16	5.5	6.6	249.4	383	0.732	34.8	132.68	8	1.88	1.88	1.402
12[ach]	6.658	196	70.2	4.1	5.34	6.58	259.4	370.2	1.14	18.6	99.96	18	1.78	1.78	0.416

[a] Two-year rotation
[b] Three-year rotation
[c] Russet Burbank
[d] Caribou Russet
[e] Compost
[f] Non-grain Rotation Crops
[g] Green manure
[h] Fumigation

Table D.3. Physiochemical results of soil collected during year one of potatoes from soil sampled in the summer approximately 60 days after planting in a study of management impacts to soil health in potato producing fields in Maine. See full treatment descriptions in Table 2.1.

Tmt	Potato Year One (2019 in 3-year rotations; 2020 in 2-year rotations) Part 1											
	Summer Sampling Timepoint											
	ACE Protein (mg/gm)	NH4 (ppm)	Boron (ppm)	Base Saturations (percent)				Calcium (ppm)	Cation Exchange Capacity (meg/100g)	Copper (ppm)	Iron (ppm)	Magnesium (ppm)
				Calcium	Magnesium	Potassium	Sodium					
1[bc]	7.8	13.12	0.14	73.66	18.18	7.24	1	984.6	6.66	5.418	47.7	143.8
2[bd]	7.56	23.44	0.18	71.18	19.16	8.1	1.2	875.6	6.12	5.782	60.96	137.8
3[bce]	8.28	11.14	0.14	71.18	17.98	7.8	3.02	1004.4	7.02	5.36	52.74	152.2
4[bdg]	7.44	11.62	0.2	75.54	16.88	6.62	0.94	1156.4	7.6	4.866	52.82	151.8
5[bcf]	8.04	9.56	0.16	71.5	19.72	7.54	1.12	911	6.36	5.806	60.02	150.2
6[bdf]	7.5	15.04	0.84	71.72	19.12	7.66	0.96	975.2	6.7	5.76	136.16	148.8
7[ac]	6.54	13.64	0.36	66.74	24.52	7.04	1.5	833	6.2	7.252	114.4	179.2
8[ad]	6.26	11.26	0.24	69.32	22.46	6.42	1.36	816.2	5.88	7.194	69.24	158.2
9[ace]	7.34	3.42	0.24	65.94	23.94	6.9	3.24	946.6	7.16	7.378	63.78	205.2
10[adg]	6.7	3.66	0.18	70.34	21.54	6.46	1.54	810.2	5.76	7.642	65.12	149.2
11[adef]	7.32	3.38	0.38	66.94	23.08	6.84	2.88	937.8	7	7.58	120.06	193.8
12[ach]	6.62	3.84	0.24	69.58	23.48	5.68	1.52	859.6	6.18	7.204	73.68	174.4

[a] Two-year rotation
[b] Three-year rotation
[c] Russet Burbank
[d] Caribou Russet
[e] Compost
[f] Non-grain Rotation Crops
[g] Green manure
[h] Fumigation

Table D.4. Physiochemical results of soil collected during year one of potatoes from soil sampled in the summer approximately 60 days after planting in a study of management impacts to soil health in potato producing fields in Maine. See full treatment descriptions in Table 2.1.

Tmt	Potato Year One (2019 in 3-year rotations; 2020 in 2-year rotations) Part 2										
	Summer Sampling Timepoint										
	Manganese (ppm)	Nitrate-N (ppm)	Olsen Phosphorus (ppm)	pH	Potassium (ppm)	POXC (ppm)	Salts (mmhos/cm)	Sodium (ppm)	Solvita (ppm)	Sulfur (ppm)	Zinc (ppm)
1[bc]	7.828	102.5	52	5.48	186.6	482.2	0.558	14.8	236.04	2.6	0.24
2[bd]	8.044	100	51	5.4	195	440.2	0.6	16.6	223.14	2.8	0.296
3[bce]	9.422	98.1	59	5.68	206.4	518.2	0.594	43.2	271.62	9.8	1.866
4[bdg]	7.59	98.9	49.6	5.74	188.8	458	0.57	16	269.2	4.4	0.28
5[bcf]	7.86	95.2	52.6	5.54	183.4	439.8	0.474	16.2	253.62	2.4	0.266
6[bdf]	9.372	100.4	51.6	5.56	190.2	446.2	0.57	14.4	239.8	2.8	0.59
7[ac]	9.216	160	54.4	5.68	170.4	449.8	0.862	21.2	170.18	9.2	0.516
8[ad]	7.114	112.4	48.6	5.76	145.6	422.2	0.598	18.4	137.98	7.6	0.338
9[ace]	6.328	96.2	63.4	6.02	190.2	515.2	0.658	53.2	174.94	15	2.684
10[adg]	7.3	116.3	46.6	5.72	143	376.4	0.664	20.2	122	7.2	0.384
11[adef]	6.024	87.1	58.6	6	186	479.8	0.552	46.6	157.6	12	2.082
12[ach]	5.742	122.1	48.2	5.88	136.6	430.2	0.576	21.6	114.92	17	0.37

[a] Two-year rotation
[b] Three-year rotation
[c] Russet Burbank
[d] Caribou Russet
[e] Compost
[f] Non-grain Rotation Crops
[g] Green manure
[h] Fumigation

Table D.5. Physiochemical results of soil collected during year two of potatoes from soil sampled in the spring planting in a study of management impacts to soil health in potato producing fields in Maine. This occurred in 2022 for all treatments. See full treatment descriptions in Table 2.1.

Tmt	Year Two Potatoes (all in 2022) Part 1														
	Spring Sampling Timepoint														
	ACE Protein (mg/gm)	NH4 (ppm)	Boron (ppm)	Bray Phosphorus (ppm)	Base Saturations (percent)					Calcium (ppm)	Cation Exchange Capacity (meg/100g)	Cation Exchange Capacity (Percent)	Copper (ppm)	Iron (ppm)	Magnesium (ppm)
					Calcium	Hydrogen	Magnesium	Potassium	Sodium						
1[bc]	5.46	3.68	0.32	181.8	49.2	32.3	14.54	3.48	0.64	869	8.94	0.16	5.022	47.6	14.54
2[bd]	5.26	3.02	0.58	164.6	50.34	31.74	14.7	3.32	0.66	897.2	8.94	0.16	5.082	89.33	14.7
3[bce]	6.3	3.54	0.26	207.2	65.86	11.56	17.2	4.5	1.06	1246.6	9.44	0.16	4.864	40.51	17.2
4[bdg]	5.26	3.64	0.6	172.8	53.24	28.84	14.1	3.16	0.62	1018.6	9.48	0.22	4.928	95.93	14.1
5[bcf]	6.04	2.82	0.28	216.8	53.54	26.02	15.96	3.94	0.64	922	8.7	0.16	5.232	48.08	15.96
6[bdf]	5.44	2.6	0.32	183.4	51.28	28.42	16.04	3.7	0.76	912	8.74	0.14	5.15	51.53	16.04
7[ac]	6.98	3.76	0.18	190.4	45.04	38.1	12.68	3.88	0.48	779.6	8.92	0.08	6.012	51.58	12.68
8[ad]	5.94	3.76	0.22	150.6	43.58	39.96	12.06	3.7	0.64	722.8	8.32	0.06	5.742	61.92	12.06
9[ace]	7.18	3.56	0.3	217.4	55.34	24.6	14.5	4.78	0.82	983.4	8.92	0	6.16	76.24	14.5
10[adg]	6.98	4.1	0.26	155	44.06	40.46	11.22	3.44	0.72	774.2	9.02	0.06	6.25	69.16	11.22
11[adef]	6.84	3.98	0.2	182.4	51.14	30.94	13.38	4.16	0.56	837.4	8.32	0.04	5.946	61.78	13.38
12[ach]	7.5	4.22	0.28	192.4	58.74	19.1	16.38	5.14	0.56	947.4	8.08	0.02	5.636	70.58	16.38

[a] Two-year rotation
[b] Three-year rotation
[c] Russet Burbank
[d] Caribou Russet
[e] Compost
[f] Non-grain Rotation Crops
[g] Green manure
[h] Fumigation

Table D.6. Physiochemical results of soil collected during year two of potatoes from soil sampled in the spring planting in a study of management impacts to soil health in potato producing fields in Maine. This occurred in 2022 for all treatments. See full treatment descriptions in Table 1.

Tmt	Year Two Potatoes (2022) Part 2															
	Spring Sampling Timepoint															
	Manganese (ppm)	Nitrate-N (ppm)	Olsen Phosphorus (ppm)	Organic Matter (Percent)	pH	pH (Buffer)	Potassium (ppm)	POXC (ppm)	Salts (mmhos/cm)	Sodium (ppm)	Solvita (ppm)	Sulfur (ppm)	Total Carbon (Percent)	Total Organic Carbon (Percent)	Zinc (ppm)	Soil Moisture (Spring)
1[bc]	4.48	19.00	44.20	3.48	5.98	6.64	120.40	208.20	0.12	12.80	108.46	4.60	1.46	1.42	0.23	16.92
2[bd]	4.70	18.60	41.80	3.42	5.90	6.64	116.60	233.60	0.13	13.60	105.28	4.80	1.44	1.40	0.30	13.56
3[bce]	5.02	19.80	50.00	4.20	6.24	6.82	166.20	440.60	0.15	22.40	131.38	7.00	1.86	1.84	1.08	19.10
4[bdg]	6.21	15.40	40.40	3.50	5.90	6.66	113.20	218.00	0.12	13.40	111.58	4.80	1.36	1.36	0.32	16.28
5[bcf]	4.72	16.60	47.40	3.74	6.12	6.70	131.80	230.80	0.12	12.20	118.44	6.20	1.52	1.52	0.22	17.81
6[bdf]	4.51	17.20	42.40	3.52	6.10	6.68	123.20	232.20	0.12	14.40	124.70	5.60	1.44	1.40	0.21	16.82
7[ac]	4.78	18.50	56.40	4.24	5.72	6.58	134.20	338.60	0.13	9.60	111.60	3.80	1.54	1.52	0.24	17.41
8[ad]	5.10	13.90	47.40	3.90	5.70	6.60	119.80	342.00	0.11	12.80	120.00	4.00	1.48	1.48	0.26	17.02
9[ace]	5.94	19.10	62.20	4.50	5.88	6.70	166.40	369.00	0.15	16.80	125.62	5.80	1.84	1.84	1.22	18.77
10[adg]	5.29	18.10	47.80	4.18	5.68	6.56	120.60	327.60	0.12	14.20	119.34	4.60	1.56	1.56	0.31	17.51
11[adef]	5.36	17.90	54.60	4.26	5.86	6.66	134.80	369.00	0.13	10.80	111.68	4.60	1.66	1.66	0.49	17.35
12[ach]	5.74	20.30	51.00	4.38	6.20	6.76	162.00	381.80	0.17	10.80	118.16	7.00	1.72	1.72	0.27	16.95

[a] Two-year rotation
[b] Three-year rotation
[c] Russet Burbank
[d] Caribou Russet
[e] Compost
[f] Non-grain Rotation Crops
[g] Green manure
[h] Fumigation

BIOGRAPHY OF THE AUTHOR

Katherine (Katie) Ashley was born in Sebastian, Florida on January 9, 1992. She was raised in Vero Beach, Florida and graduated from Sebastian River High School in 2010. She attended the University of Central Florida and graduated in 2014 with a Bachelor's degree in Biology with a minor in Environmental Studies. She first moved to Maine in 2017 and earned a Master's of Science degree in Botany and Plant Pathology from The University of Maine in the spring of 2020. After receiving her Master's degree, Katie worked at North Carolina State University as a Horticulture Extension Agent in western North Carolina. Katie then returned to Maine in 2021 and joined the School of Food and Agriculture and the School of Biology and Ecology to pursue a doctoral degree at the University of Maine. Following her degree, Katie was awarded a Fulbright Postdoctoral Award to Logroño, La Rioja, Spain, where she will be working at the Institute of Grapevine and Wine Sciences. Katie is a candidate for the Doctor of Philosophy degree in Plant Science from the University of Maine in May 2024.