ENHANCING SOIL HEALTH IN MICHIGAN POTATO CROPPING SYSTEMS

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ABSTRACT

Limited rotational crop diversity and frequent mechanical disturbance may promote soilborne disease and limit soil productivity in Michigan potato cropping systems. The objective of this four-year study was to evaluate alternative production practices that may impact soil health and potato production. To examine practices in both a two- and three- year potato rotation, two field studies were established near Clarksville, MI. Treatments within each cropping rotation represented practices hypothesized to impact soil health and include: fumigation (i.e., prior to first-year potato or none), annual applications of 2.2 or 4.4 Mg poultry litter ha^{-1} depending on crop rotation, and cover crop (i.e., cereal rye following corn/prior to potato). Soil health indicators included standard chemical indicators, 24hr microbial burst CO_2 respiration, permanganate oxidizable carbon, autoclaved citrate extractable protein, water stable aggregates, Potato Early Die (PED) pathogen population densities and soil microbial community composition (16S and ITS sequencing and phospholipid fatty acid analysis). Crop productivity was evaluated by crop yield and tuber quality.

Management practices hypothesized to improve soil health did interact in some cases to influence soil health and crop productivity, but in different and at times unpredictable ways. There were no observed changes in soil organic matter under any of the management strategies. Over the four years, annual poultry litter application produced increasingly greater midseason microbial respiration burst and soil test P values as well as greater yields. Adding a rye cover crop inconsistently weakened the positive effect of manure on yields, likely due to the suppressive effect of spring incorporated rye biomass on midseason respiration burst. Despite greater diversity and limited effect on PED pathogen levels, eliminating soil fumigation resulted in greater disease severity and reduced yields in the first year of potatoes. The fumigation effect dissipated by the second year of potatoes in both rotations.

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1. INTRODUCTION

Soil health broadly defined by the U.S. Department of Agriculture, National Resources Conservation Service (USDA NRCS) is the "continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans" (USDA NRCS, 2018). The definition expands on previous efforts to define the value of soil similar to both soil fertility and soil quality, which characterize soil at the field-scale in terms of crop productivity using chemical and physical properties. Soil health framework connects soil to human and ecosystem health further emphasizing the importance of the services soil provides to society, the environment and, recognizes these services are a product of processes mediated by soil biota (Blum, 2005; Janzen et al., 2021; Lehmann et al., 2020). Although soil health is not a new concept, improvements in abilities to characterize soil organisms and measure the fluxes and byproducts they control have allowed the concept of soil health to gain additional traction (Baveye, 2021; Fierer et al., 2021).

From an ecological perspective, soil health highlights the complex resource fluxes that connect soil to the broader ecosystem and define the relative functionality. Soil biological activity plays a previously underestimated role in controlling resource fluxes and helps determine whether soil is a source of pollutants and resources or a sink. The connection between soil biota and the processes that determine soil function can be particularly evident in agroecosystems where management can have significant and measurable effects on microbial activity and the fluxes of water, carbon (C), and nitrogen (N). For example, soil is the largest terrestrial sink for C and an important component of the global N cycle, however, changes in soil microbial activity driven by land use change that accelerates respiration of soil C have made agricultural soil a source of greenhouse gases CO_2 and N_2O, contributing 14% of anthropogenic CO_2 emissions and

60% of anthropogenic N_2O emissions (Eswaran et al., 1993; Janzen, 2004; Lal, 2003; Reay et al., 2012; Schulze et al., 2010). The status of soil as a source or sink or C and N is influenced by microbially mitigated transformations including SOM formation and decomposition, nitrification, mineralization, immobilization, and denitrification (Robertson & Vitousek, 2009). The capacity of soil to prevent air and water pollution at a global scale is important for the mitigation of climate change and the preservation of water resources.

The soil health framework can also be helpful at the field-scale to understand the complex interactions that drive soil function in agricultural soils. Soil C is not only important for climate change mitigation but may also serve as the base of the soil food web, which enhances the capacity of soil to support soil functions that are crucial to sustainable agroecosystems, including plant productivity and pathogen suppression. Carbon is generally added to soil as plant residue, some of which is respired as CO_2 and some of which is incorporated into microbial residue, a major component of the stable pool of SOM (Grandy & Neff, 2008; Liang et al., 2017; Miltner et al., 2012). The biological process of C turnover from plant residue to necromass releases important nutrients from plant residue, which can contribute to plant productivity (Jilling et al., 2018; Robertson & Vitousek, 2009; M. Yan et al., 2020). Further, greater SOM can indicate the presence of higher trophic community members that promote more rapid nutrient turnover and can be important predators to plant pathogens (Ferris, 2010; Ferris et al., 2012; Gao et al., 2019; Kopecky et al., 2021; Zak et al., 2003). Agroecosystems with frequent mechanical disturbance, low diversity rotations, and low C inputs can suppress diverse and active microbial communities, which limits soil function and reduces agroecosystem resiliency (Abdullah et al., 2017; Puissant et al., 2021; Vitousek et al., 1997).

To guide management decisions that will build soil health in U.S. agroecosystems, the USDA NRCS has developed four pillars as guiding principles when making management decisions (USDA NRCS, n.d.). The first principle, “maximize presence of living roots” suggests extending the season by planting cover crops and incorporating perennials into a rotation. The second principle is to “maximize soil cover”, often by leaving residue on the surface or planting cover crops during fallow periods. “Maximizing diversity” is a guiding ecological principle for ecosystem resiliency and, in the context of an agroecosystem, NRCS suggests promoting diverse microbial activity by planting diverse rotations, especially with cover crops. The final principle is to “minimize disturbances”, which is generally achieved with reduced or eliminated tillage and soil fumigation. By promoting an active and diverse microbiome, these practices may improve agricultural soils capacity to support C accrual and storage, plant productivity, and soilborne pathogen suppression, however, in practice, their effect on soil health can vary.

Increased focus on managing for improved soil health has led to the need for a soil health testing framework with accurate and relevant indicators to determine how management practices impact soil health across a broad variety of agroecosystems (Lehmann et al., 2020; Moebius-Clune et al., 2016; Wade et al., 2022). Chemical and biological indicators measure concentrations of the substrates and products of chemical and biological processes and can be used to indirectly measure rates of flux. Additionally, some biological indicators characterize the microbes that influence the fluxes. Physical indicators characterize the physical environment in which chemical and biological processes occur and has significant influence over the rates of flux.

1.1 Soil Health Metrics

Soil health assessments commonly include chemical indicators extractable nutrients, pH, and SOM because they are foundational to soil function. Soil organic matter can improve yield stability by improving water holding capacity and further, may serve as a reservoir of nutrients that undergo transformation from biotic (i.e., mineralization and immobilization) and abiotic (i.e., adsorption and desorption) processes (Cookson et al., 2005; Eeswaran et al., 2021; Pan et al., 2009; Rawls et al., 2003). Extractable nutrient concentrations are real time estimates of plant available nutrients that offer insight into the fate of nutrients mineralized from SOM, fertilizers, and organic amendments (Crants et al., 2021; D. Yan et al., 2017). Soil pH is a strong determinant of plant available nutrients as it impacts the chemical sorption and desorption of nutrients and influences some biological nutrient transformations including mineralization, nitrification, and N fixation (Amarasiri & Olsen, 1973; Cookson et al., 2007). Additionally, increasing pH correlates closely with greater soil microbial diversity pH (Barak et al., 1997; Fierer & Jackson, 2006; Rousk et al., 2010). While pH and nutrient concentrations are sensitive to management practices, SOM accumulation is relatively slow. Chemical indicators continue to be tools for managing soil fertility and can offer important insight for soil health evaluation.

Biological indicators of soil health may help assess the actual or potential functioning of the soil microbiome and include permanganate oxidizable carbon (POX-C; active C), autoclaved citrate extractable protein (ACE protein; soil protein), and mineralizable C (microbial CO^2 respiration). Though POX-C and ACE protein are chemical fractions of soil C and N, respectively, both are substrates and products relevant to microbial metabolism. Permanganate oxidizable C is a pool of C in SOM that is particularly available to microbes and has been used to predict C accrual, microbial biomass, and N mineralization (Culman et al., 2012; Hurisso et al., 2016). Autoclaved citrate extractable protein correlates closely with soil protein, which is an important source of organic N for microbes and an indicator of potentially mineralizable N contained in SOM (Hurisso et al., 2018). Both POX-C and ACE protein respond more rapidly to management practices than SOM and have been found to correlate with crop yields, though to a lesser degree than SOM (Culman et al., 2012, 2013; Lucas & Weil, 2012; Sprunger et al., 2021; Wade et al., 2020; Wright et al., 1999). To estimate general biological activity, burst CO_2 respiration can be measured by rewetting air-dried soil and determining the increase in CO_2 concentration in a closed vessel after 24 hours (Franzluebbers et al., 2000). A low CO_2 burst indicates a smaller or C-limited soil microbiome with overall lower metabolic activity suggesting reduced nutrient turnover. Between-site and in-season variability has previously limited the utility of the respiration burst test as a soil health indicator, however once soil texture, a strong determinant of a soil's capacity for microbial activity, is accounted for respiration burst indicates management-driven changes in soil health and predicts crop productivity(Sprunger et al., 2021; Wade et al., 2020). Measuring the substrates available for microbial activity offers a snapshot in time of soil function.

The composition of the microbial community via gene sequencing and phospholipid fatty acid (PLFA) analysis can help characterize the functional potential of the soil microbiome. Management driven changes in microbiome composition can correspond with changes in soil function (Jiao et al., 2019; Rieke et al., 2022; Wilhelm et al., 2023). For example, Fang et al. (2018) reported soil fumigation suppressed microbial diversity as well as N-cycling gene expression that corresponded to increased N_2O emissions. Gene sequencing allows researchers to estimate differences in species richness and evenness (α-diversity) and community structure (β-diversity), which are important because it is generally thought that greater biodiversity produces the ecosystem multifunctionality that drives soil health (Chen, Ding, Zhu, et al., 2020; Delgado-Baquerizo et al., 2016). In a grassland biodiversity gradient experiment, Wagg et al. (2014) observed a consistent trend of greater rates of decomposition, C storage, and net primary productivity with more diverse soil microbiomes. However, because of redundancies in soil microbe niche and function, taxonomic diversity and functional diversity do not always correlate (Banerjee et al., 2016; Chen, Ding, Li, et al., 2020).

Phospholipid fatty acid analysis, which uses unique PLFA biomarkers to identify and quantify broad functional groups of microbes and estimate total microbial biomass, is also a promising method to characterize the soil microbiome (Zelles, 1999). The relative abundance of groups is both functionally informative and sensitive to management. For example, PLFA can be used to estimate the fungal:bacterial (F:B) ratio of the soil microbial community, which can indicate improved C accrual potential (Malik et al., 2016). Studies have connected crop rotational diversity, manure application and eliminating fumigation to increases in F:B (Dangi et al., 2017; Esperschütz et al., 2007; Romaniuk et al., 2011). Further, shifts in microbial communities measured by PLFA analysis can be linked to changes in other soil health metrics indicating improvement in soil function. Shifts in PLFA groups driven by greater rotational crop diversity corresponded to increases in soil aggregation, POX-C and mineralizable C (Tiemann et al., 2015). While 16S and ITS amplicon sequencing offers detailed taxonomic information about the microbiome, the results are biased by primer selection as well as the persistence of extracellular, ‘relic’ DNA (Baker et al., 2003; Carini et al., 2016). Phospholipid fatty acids degrade rapidly outside the cell and thus represent more closely, albeit less specifically, the viable microbial community and thus quicker to reveal changes in microbial community after a change in management (Willers et al., 2015). Microbial community characterization, whether from PLFA or 16S/ITS sequencing, may be a crucial component of illustrating the connection between management and soil health as sequencing and analysis methods become more accessible and affordable.

An additional soil microbiome characterization method is quantifying soilborne pathogens. Multiple studies report decreases in pathogen populations with soil health practice implementation, a pattern often attributed to increases in soil microbial diversity (Bailey & Lazarovits, 2003; Mazzola, 2002). Further some management practices have been shown to increase pathogen suppression through increases in microbial interaction complexity, despite decreases or no change in microbial diversity (Bonilla et al., 2012; Kopecky et al., 2021; Peralta et al., 2018; A. R. Van Bruggen & Semenov, 1999). Results indicate multiple mechanisms connect increase in soil health to soilborne pathogen suppression and further investigation is required to understand the relationship between microbial diversity and specific soilborne disease complexes. An economically important and ecologically interesting system to investigate microbial diversity, soil health, and soilborne pathogens is potato cropping systems where proliferation of soilborne diseases like Potato Early Die complex and common scab pose significant agronomic and economic challenges. Potato Early Die Complex (PED) is a soilborne disease caused by *Verticillium dahliae* fungal infection facilitated by root lesion nematodes (RLN; *Pratylenchus penetrans*) can cause 30-50% yield loss (Powelson & Rowe, 1993). Though common scab, caused by *Streptomycetes scabies* infection, limits yield only in severe cases, surface lesions do impact marketable yield, especially in chipping potatoes commonly grown in Michigan. Both diseases are ubiquitous in all major potato growing regions in the US and previous research has suggested PED and Scab pathogens are susceptible to various soil health practices (Bailey & Lazarovits, 2003; Molina et al., 2014; Ochiai et al., 2008; Wiggins & Kinkel, 2005). Pathogen suppression is important to the productivity of potato cropping systems and can be estimated by tracking *V. dahliae* and *P. penetrans* populations.

The physical matrix in which biological and chemical processes occur in soils is also a major determinant of soil health. Soil texture measured by sand, clay, and silt content, helps predict expected values of soil health metrics. Coarse textured soils provide limited mineral associated nutrients and minimal protection from predation for bacteria resulting in lower overall microbial biomass and lower bacterial diversity (Sessitsch et al., 2001). On the other hand, the porosity of sand means more aerobic spaces and thus greater fungal diversity in coarse texture soils (Xia et al., 2020). Not only does soil texture shape the potential microbial community, but also is an important factor in what they are doing. Mineral surfaces and pore connectivity, determined by soil texture classes, significantly influence movement and transformations of chemical and biological nutrient transformations and as well as nutrient movement. A soil's intrinsic capacity to build SOM and store C is determined by soil texture, parent material, as well as environmental conditions. Within this capacity, the actual amount of SOM that is stored and in which form is determined by biological processes driven by microbes (Kallenbach & Grandy, 2011; Schmidt et al., 2011). This is reflected in the strength of correlation between soil texture and extractable nutrients, POX-C and mineralizable C revealed in a survey of 195 agricultural soils in the eastern Corn Belt, (Sprunger et al., 2021). Together, this means soil texture exerts a large influence on soil health.

While soil texture does not change readily, soil aggregation, an additional soil physical indicator, is very sensitive to management practices, with macroaggregates (250-2000 μm) responding more immediately after management change followed by microaggregates (53-250 μm) (Tiemann & Grandy, 2015; Tisdall & Oades, 1982). Physical disturbance from tillage breaks up existing aggregates and disrupts hyphae and roots, slowing future aggregation(Six et al., 1999). On the other hand, organic amendment and increased rotational crop diversity can promote aggregate formation, even in frequently tilled systems (Grandy et al., 2002; Osborne et al., 2014; Shepherd et al., 2002). Aggregation is an important soil health indicator because soil aggregates offer niche heterogeneity which promotes microbial diversity (Smith et al., 2014; Vos et al., 2013). Further, macroaggregates are sites of concentrated microbial activity, POX-C, and soil protein while microaggregates, the more stable class, are important for long term C accrual and storage (Grandy & Robertson, 2007; Six et al., 2009; Tisdall & Oades, 1982). Both the sensitivity of aggregates to management and the strong connection between aggregates and soil function make soil aggregation a useful soil health metric.

Previous research has attempted to combine soil health metrics into unified soil health indices, however, the interpretation of soil health metrics is context dependent, and values change throughout the season, making a universal soil health index difficult to define (Fierer et al., 2021; Rinot et al., 2019). "Healthy" values in one cropping system taken at a certain point in the season may be "unhealthy" in a cropping system with different soil texture and environmental conditions or measured at a different timing. Further, interpretation of soil health metrics varies depending on the goals of an individual grower and improvement of one function can result in the loss of another function, thus it is important to validate the utility of soil health indicators across a wide range of cropping systems. The soil health indicators used in the present study were selected for the following criteria: 1) expected to change with the adoption of soil health building practices relevant to potato cultivation within the time limits of the experiment and, 2) indicate changes in the capacity of soil to support C accrual and storage, plant productivity, and pathogen suppression functions. Soil health management strategies were evaluated on the extent to which they affected the individual soil health metrics as well as crop yield.

1.2 Soil Health Improvement in Michigan Potato Cropping Systems

Potato growers in Michigan are interested in building soil health to improve the economic and environmental sustainability of their operations. Soil health in potato production areas is often fragile and easily degraded as much of Michigan potato acreage is on coarse textured soil with a low capacity for supporting SOM protection, soil aggregation, and microbial activity. Frequent mechanical disturbance at preplant, hilling, and harvest destroys soil structure and accelerates the decomposition of SOM (Angers et al., 1999; Po et al., 2009). These factors make improvement of soil health in potato systems difficult and options for soil health building practices are limited by climate, soil texture, disease pressure, and economic context.

One of the first management practices that is targeted when discussing soil health improvement in potato systems is the widespread use of soil fumigants. Control of PED by extended rotation is often unsuccessful because *V. dahliae* microsclerotia can persist for 10 years in the soil and *P. penetrans* will accept a wide range of hosts, including corn, rye, and radish (Florini & Loria, 1990; Johnson et al., 2010; Miller, 1978; Wheeler & Johnson, 2016). Therefore, soil fumigation and nematicides have been important tools in potato systems for controlling PED, as well as other soilborne potato diseases like common scab. Chloropicrin (CP) and metam sodium (MS) are popular fumigants applied in the fall or spring before planting potatoes. While CP and MS have been found to be able to control *V. dahliae*, there is increased interest in understanding the effect of fumigation on nonpathogenic soil biota and soil health in the short and long term (Castellano-Hinojosa et al., 2022; Harris, 1990; Ślusarski & Spotti, 2016). Though the active ingredients in CP and MS themselves degrade within days of application, field and lab incubations suggest soil fumigation impacts non-pathogenic soil microbes and the functions they provide in the short term (i.e., days and weeks after fumigation) and long term (i.e., months after fumigation) (Crants et al., 2021; Sennett et al., 2021; Shen et al., 1984; Spokas et al., 2005; Zhang et al., 2017). In particular, microbes involved in N cycling seem to recover slowly after soil fumigation, impacting the N dynamics throughout the subsequent growing season (Shen et al., 1984; Spokas et al., 2005). Potato growers are interested in ways to both mitigate the effect of fumigants on the soil microbiome and extend the disease suppressive effect of soil fumigants.

Building soil health is a promising way to reduce yield loss from PED as soil health practices can improve both the capacity of soil to suppress pathogens (i.e. reduce the relative and absolute abundance of pathogen populations) or suppress disease (i.e. reduce disease severity without reducing pathogen populations). A survey of 100 Idaho farms found SOM, not soil *V. dahliae* population density, was correlated with wilt severity. Further, unlike most fungal diseases, PED severity can be reduced when N availability and tuber N uptake are in synch (Davis et al., 1994, 2001). These results indicate healthier soil, with improved C accrual and nutrient provisioning, may suppress the severity of PED.

A promising soil health practice for potato producers in Michigan is spring manure application. While high rates of manure applied over multiple years has been shown to build SOM, even low rates can have a significant effect on microbial community composition, soil health, and potato yield (Griffin & Porter, 2004; Ninh et al., 2014). When manure and mineral fertilizers are applied together, the manure can mitigate the effect of mineral fertilizer on the bacterial community composition (Rutan et al., 2022). This mitigating effect may stem from the positive effect of organic amendment on nutrient solubilizing microbial activity as well as the addition of limiting micronutrients contained in manure (Kopecky et al., 2021; Ninh et al., 2014). The impact of manure on plant productivity depends greatly on synchrony between nutrient mineralization and crop uptake, which is affected by manure C:N and C:P and, like all microbial processes, soil temperature and moisture (Griffin & Porter, 2004; Ninh et al., 2014; Nyiraneza et al., 2021; Rosen et al., 2014; Waddell et al., 2000). If nutrients are not all mineralized during the subsequent growing season or if certain nutrients are overapplied and soil nutrient concentrations accumulate, manure application poses increased risk of N loading in ground water and P loading in surface runoff (Stark & Porter, 2005; Waddell et al., 2000)

Planting a cover crop after corn harvest in a potato rotation increases crop rotational diversity and can reduce soil erosion and nutrient leaching in the fall through early spring (Nyiraneza et al., 2015; Zhou & Butterbach-Bahl, 2014). Additionally, cover crop biomass contributes C through root and aboveground residue that support microbial activity throughout the growing season as they decompose (Jahanzad et al., 2017; Larkin et al., 2010; Nyiraneza et al., 2021; R. Schmidt et al., 2018). While increasing crop diversity in general supports diverse microbial communities and improves disease suppression and SOM accumulation in soil, the use of cover crops to increase rotational diversity is especially important for SOM accumulation (McDaniel et al., 2014). This is reflected in the greater POX-C observed after multiple year with cover crops (Jokela et al., 2009; White et al., 2020). Like in manure, the C:N ratio of cover crop residue is a significant factor in determining the timing of cover crop biomass decomposition and organic nutrient mineralization after termination. Cereal rye has a high C:N ratio that can result in N immobilization during periods of crop uptake and reduce crop yields (Nyiraneza & Snapp, 2007; Poffenbarger et al., 2015). For pathogen suppression, there are two contrasting hypotheses that predict the influence of cover crops on pathogen populations. One being that increased rotational diversity builds general suppression of soilborne pathogens by sustaining greater nutrient solubilizing microbial activity and higher trophic level microbes that act as predators to plant pathogens (Kopecky et al., 2021; Latz et al., 2012; Mendes et al., 2011). The other being that a cover crop may act as a “green bridge”, allowing for pathogen proliferation during the fallow season if it is a suitable host (Grabau et al., 2017).

Further research is needed to understand the extent to which each of these practices can impact soil health in potato cropping systems individually, as well as the interactive effect of soil health practices implemented in combination. Practices were selected for the present study as realistic examples of management strategies for building soil health in Michigan potato cropping systems. Poultry litter applied annually at a low rate can supplement mineral nutrients while promoting microbial activity and diversity throughout the rotation. Cereal rye is a winter hard grass planted following corn harvest and terminated prior to potato planting, adding diversity to the crop rotation, physical protection of the soil during winter, and contribute biomass all of which support microbial processes in the early spring and throughout the growing season.

1.3 Research Objectives and Hypotheses

The objective of the present study was to evaluate the impacts of soil fumigation, manure application, and cover crops on soil health, soilborne pathogens, crop yield, and tuber quality throughout four years of both a 2-year and a 3-year potato rotation. I predicted that annual poultry litter amendment, cover crop planted prior to potatoes, and eliminating fumigation would all singly or in combination improve soil health as assessed using standard soil health chemical, physical and biological indicators. I predicted eliminating fumigation, applying poultry litter, and planting a cereal rye cover crop would all singly or in combination improve soil health as assessed by standard soil chemical, biological, and physical indicators. Specifically, I expected increase in microbiome diversity, decreases in soilborne pathogens, and increases in C and nutrient cycling. Further, I expected soil health improvement would correspond with improvements in the soil's capacity to accrue and store C (i.e., SOM), support plant productivity (i.e., crop yields), and suppress soilborne disease (i.e., disease severity and tuber quality). Though I expect these practices to have some measurable impact on soil health indicators in four years, previous research shows that short-term research may underestimate the overall impacts of soil health building practices on soil (Carter et al., 2003; Wood & Bowman, 2021).

2. METHODS

2.1 Experiment Design and Study Site Management

This study was part of a national collaboration across 10 major potato-producing states in the United States that included Michigan as well as Colorado, Idaho, Maine, Minnesota, Montana, North Dakota, Oregon, Washington, and Wisconsin. In Michigan, two field studies were conducted 2019-2022 at the Clarksville Research Center in Clarksville, MI (42° 52' 25.975" N 85° 15' 16.837" W) on a Lapeer sandy loam (coarse-loamy, mixed, semiactive, mesic Typic Hapludalf). Preceding the establishment of the study, the fields had been managed in continuous potato 2016-2018 with fallow winters and no history of manure applications. Plots measuring 3.5 m by 15.25 m were arranged in a randomized complete-block design with five replicate blocks.

Soil health management and control treatments were designed to represent a range of realistic management strategies for potato production in the U.S. and Michigan specifically (Table 1). Potatoes were planted every two (2YR) or every three (3YR) years in rotation with corn and winter wheat (Figure 1, Table 2, Table 3). Center pivot irrigation applied around 20 cm of water throughout the potato growing seasons in 2019, 2020, and 2022. In rotation years, corn and wheat were supplemented with irrigation as needed. The Michigan specific soil health management strategies used in this study included manure application (poultry litter; MANURE), a cover crop (cereal rye; CCR), a combination of manure and a cereal rye cover crop (MAN/CCR), and a nonfumigated control with no other soil health improvement practices (NOFUM) along with a treatment that followed grower standards for the region with no soil health management (GRSTAND). A national control treatment (NATCTRL) was included for comparison with studies across all the other collaborating states. Fumigation, planting, harvesting and sample dates for the 2YR and 3YR study are outlined in Table 2 and Table 3, respectively.

For the initial round of potatoes in both studies, beds were prepared for fumigation with early spring tillage with a disc harrow. Plots designated for chloropicrin (CP) fumigation received 131 kg chloropicrin ha^{-1} Strike85 and plots designated for metam sodium (MS) fumigation received 420 L ha^{-1} Vapam HL (178 kg metam sodium ha^{-1}). In the second round of potatoes, all plots were planted without fumigation. Seed tubers were planted in five rows per plot with 0.9 m spacing between rows and 0.3 m within rows. Treatments NOFUM, MANURE, MAN/CCR and GRSTAND, were planted with 'Superior' potatoes, a mid-season maturity (90-130 days), round white variety that is susceptible to Verticillium wilt and moderately tolerant to common scab (Zebarth et al., 2004). 'Snowden', a late maturity (> 130 days) chipping variety that is tolerant to Verticillium wilt and susceptible to common scab was planted in the COVER treatment (Zebarth et al., 2004). For the national control treatment (NATCTRL), 'Russet Burbank' tubers were cultivated with conventional management, with mineral fertilizer and spring soil fumigation. While 'Superior' and 'Snowden' are regionally relevant, production of 'Russet Burbank', a long season baking variety, is limited in Michigan. Fertility management was adjusted to account for nutrients contained in organic amendments and for differences in nutrient uptake between varieties (Table 4, Table 5). Pelletized litter from a commercial layer operation served as the organic amendment. Michigan State University recommended best practices guided pesticide, fungicide, and herbicide applications to control insect pests, foliar fungal pathogens, and weeds for all treatments.

For corn years, spring tillage prepared the fields for planting 76 cm rows at a population of 79,000 seeds ha^{-1} (hybrid line DKC51-38 or DK52-18). Treatments with mineral fertilizer only (NATCTRL, GRSTAND, NOFUM, COVER) received 56 kg N ha^{-1} from urea at planting and MANURE and MAN/CCR received 2.2. Mg ha^{-1} poultry litter. At V4 stage all treatments received 180 kg N ha^{-1} from 28% urea ammonium nitrate (UAN) for an estimated total 246 kg N ha^{-1} applied to all treatments (based on first year availability of nutrients form manure) and 72 kg P_2O_5 ha^{-1} from poultry litter on the manure treatments. In the treatments with cover crops (COVER and MAN/CCR), rye was broadcast seeded at 62 kg ha^{-1} following corn harvest (2YR: 25 October 2019, 3YR: 13 October 2021).

For wheat years, mineral fertilizer only treatments (NATCTRL, NOFUM, GRSTAND, COVER) received 112 kg N ha^{-1} from a broadcast application of urea at green up in the spring while MANURE and MAN/CCR received 2.2 Mg ha poultry litter and 50 kg N ha^{-1} from urea. After wheat harvest (2YR:14 July 2021, 3YR: 15 July 2020), all plots were broadcast seeded with tillage radishes and oats at 11 and 28 kg seed ha^{-1}, respectively, as a winter-killed cover crop.

2.2 Soil Sampling

Sample dates for the 2YR and 3YR studies are outlined in Table 2 and Table 3, respectively. At each sample date, around 30 cores of bulk soil were collected from each plot to a depth of 15 cm with a 2.54 cm diameter probe, homogenized cores by plot and separated into several sub-samples for various analyses. For soil health chemical indicators, samples were air dried at 20°C and sent to Agvise Laboratories (Northwood, ND) for determination of soil pH_{water}, soil organic matter (SOM; via loss on ignition), total organic C (TOC), total carbon (TC), and extractable nutrients (Combs & Nathan, 2012; Eliason et al., 2012; Mulvaney, 1996). Three soil health biological indicators were also measured at Agvise Laboratories including burst respiration (total CO_2 efflux measured from 40 g of soil for 24 hours following rewetting of the air dried soil) as well as POX-C and ACE protein using standard methods (Franzluebbers et al., 2000; Hurisso et al., 2018; Weil et al., 2002).

Sub-samples of soil used for pathogen analyses were kept at 4°C and sent within 48 hours of sampling to Pest Pros (Allied Cooperative, Plainfield, Wisconsin) for enumeration of *Verticillium dahliae* propagules and *Pratylenchus penetrans*, along with other pathogenic nematodes. Nematodes were extracted from 100cc soil with a Baermann funnel then identified and quantified. To quantify the potential *V. dahliae* population in the soil, plates with *V. dahliae* selective growth media were inoculated with a solution containing 0.5 g soil from each plot. After 14 days of incubation, colonies of *V. dahliae* were identified and counts were reported as Verticillium propagules per gram of soil (VPPG).

For soil microbiome analyses, field moist soil was passed through a 2 mm sieve and kept at -80 °C before I extracted DNA from 0.25 g of soil using the DNeasy PowerSoil Kit (Qiagen GmbH, Germany) according to the manufacturer's protocol. The structure of the soil microbiome was assessed using standard amplicon sequencing methods that target bacteria and fungi. Briefly, for bacteria, the V3/V4 region of the 16S rRNA gene was amplified using the 341F/806R primers and for fungi, the ITS2 region of the rRNA gene was amplified using the ITS3F/ITS4R primers. The University of Minnesota Genomics Center indexed and prepared libraries for sequencing with Illumina MiSeq 600 cycle v3 kits as described in (Gohl et al., 2016). To minimize run-to-run variation, each library was split across 3 runs and then each run was pooled accordingly to achieve a mean sequencing depth of 25,000 sequences. Using the DADA2 pipeline, raw sequence reads were processed into individual amplicon sequence variants (ASVs). Amplicons from the same year were pooled for sequencing (e.g. June 2019 and August 2019 amplicons were pooled together) allowing for comparison between sampling dates within a year. No mock community was included for standardization in any of the sequencing runs so comparison of community structure between years is limited.

Additional soil microbiome analyses were conducted using soils sampled 60 days after planting (DAP) in 2022. I sent fresh soil samples from four of the five replicate blocks and from both the 2YR and 3YR study to Ward Laboratories Inc. (Kearney, NE) for phospholipid fatty acid (PLFA) extraction and analysis using standard methods (Hamel et al., 2006).

To indicate changes in physical soil health, water stable aggregates were evaluated. At preplant sampling dates, I collected a slice of soil (15cm wide x 2.54 cm thick x 15 cm deep) from each plot and sent them to Agvise Laboratories (Northwood, ND) for water stable aggregate sizes and proportions (Kemper & Rosenau, 1986).

Preplant soil samples, taken at potato planting, were analyzed for soil chemical properties, soil biological properties, soil pathogen enumeration, soil bacterial and fungal community characterizations, and water stable aggregates. Midseason samples were taken 60 DAP potatoes and were analyzed for soil chemical properties (all except OM, TC, TOC), soil biological properties, soil pathogen populations, and fungal and bacterial community characterization. One exception, the 60 DAP 2019 samples were not analyzed for soil pathogens. In rotation crop years (corn or wheat planted), all after 2019, samples were taken in the fall after harvest. Fall samples were analyzed for soil chemical and biological metrics and soil bacterial and fungal community characterization. In Fall 2020, samples were also sent for determination of water stable aggregates.

2.3 Crop Yield and Disease Evaluation

To evaluate potato early senescence, a characteristic of Potato Early Die, green cover was estimated by visual evaluation at vine kill in 2019 and 2020 and 7 weeks before harvest in 2022 as fractional green canopy cover using pictures analyzed in the Canopeo smartphone application (Patrignani & Ochsner, 2015).

After potato harvest, U.S. No. 1 and U.S. No. 2 tubers were separated and graded. U.S. No. 1 tubers were characterized as well-formed with less than 5% of the surface with defect. U.S. No. 2 tubers were characterized as mishappen or with major defect. Tubers were graded for size by local standards for chipping potatoes. Specific gravity was determined with a subsample of 20 U.S. No. 1 tubers in 6-10 oz size class. The subsample was weighed in air and in water then specific gravity was calculated as the weight in air divided by the difference between the weight in air and weight in water. A subset of 50 tubers, with tubers from each size class was examined to estimate percentage of tubers with *V. dahliae* infection, hollow heart and Type 3 to 5 common scab lesions. Corn and wheat yields in rotation years were measured on the combine.

2.4 Statistical Analysis

All data was analyzed with R version 4.2.2 For each sample time in each rotation, I modelled the effect of management practices on soil properties and production metrics using analysis of variance (ANOVA). Linear mixed models were specified with treatment as the fixed effect and replication as the random effect in the lme4 package in R (Bates, Mächler, et al., 2015). Using the emmeans package in R, I determined the significance of the treatment effect by a Type III test with Kenward-Roger adjusted degrees of freedom (Lenth, 2022). When I found treatment effect to be significant ($\alpha<0.05$), I conducted pairwise comparisons among treatments using the cld function in multcomp package in R (McMurdie P J & Holmes S, 2013).

For variables with non-normally distributed data, linear models were constructed based on Poisson (*Verticillium* counts) or negative binomial (*Verticillium* and Scab infection rates) distributions using the glmer function in lme4 package in R (Bates, Mächler, et al., 2015). Fixed effect was defined as treatment and replication as the random effect. Wald's Type III Chi-squared (χ^2) test is reported to estimate treatment effect ($Pr>\chi^2$) and I back calculated estimated marginal means in the response scale along with 95% confidence intervals using the emmeans package in R to compare differences between treatments.

To look closer at effect of fumigation across fertility programs, I constructed multiple degree of freedom contrasts comparing nonfumigated 'Superior', NOFUM and all CP fumigation 'Superior' treatments (GRSTAND, MANURE, and MAN/CCR). The NATCTRL and CCR treatments were left out of these contrasts because of the confounding effect of innate varietal differences in yield potential, nutrient uptake, and growth stage during sampling.

To model changes in microbial community across treatments, metrics of both α- and β-diversity were calculated and analyzed. α- diversity was estimated with the Shannon index using *estimate_richness* in the phyloseq package in R and modeled with treatment as the fixed effect and replication as the random effect using lme4 package in R (Bates, Machler, et al., 2015). β-diversity, estimated by dissimilarities in relative abundance of ASVs across treatments and sample dates taken within each year were determined with the Bray-Curtis distance method using *ordinate*, a phyloseq function (McMurdie P J & Holmes S, 2013). Dissimilarity was visualized with Principle Coordinates Analysis (PCoA) plots using *plot_ordination* function with *stat_ellipse* option also from the phyloseq package. Differences in community structure between treatments were characterized further with permutational multivariate analysis of variance (PERMANOVA) conducted based on a Bray-Curtis distance matrix as the input data matrix and treatment and sample time as grouping with 99,999 permutations using the *adonis2* function in vegan package version 2.6-2 (Anderson, 2017; Oksanen J et al., 2022). When the main effect of treatment was significant and sample time by treatment interaction was not significant, I conducted a pairwise PERMANOVA with 99,999 permutations to look at differences in community structure between treatments across sample times using the *pairwise.adonis* wrapper function in pairwiseAdonis package (Martinez Arbizu, 2017).

3. RESULTS

In the present study, I primarily discuss results from the treatments planted with 'Superior' tubers (GRSTAND, NOFUM, MANURE, and MAN/CCR) in order to isolate the effect of soil health building practices. The CCR and NATCTRL treatments offer valuable information about the effect of potato cultivar on soil health indicators and data from these treatments will be important in the context of the full national study.

3.1 Environmental Conditions

Mean daily temperatures during the growing season were within 10% of 30-year averages in every year of the study, however, the number of July 2019 and 2020 days with maximum temperature above 26°C was +25% and +40% greater than the 30-year average indicating possible heat stress during early tuber bulking (Table 6). Soil temperatures remained above 10°C for the growing seasons after 26 April 2020 and 4 May 2022 (Table 7). June 2019 and 2020 were +50% and +34% above the 30-year average total monthly precipitation. August 2019 was only 27% drier than the 3-yr average, while August 2020 was 50% drier. Compared to 2019 and 2020, precipitation trended in the opposite direction in 2022. June 2022 precipitation was 70% below the 30-year mean likely impacting tuber set and August 2022 precipitation was 80% greater than the 30-year mean, indicating potentially conducive conditions for PED pathogen development (Table 8).

3.2 Yield, tuber quality, and disease severity

3.2.1 Two Year Rotation (Corn-Potato-Wheat-Potato)

<u>1st Round of Potatoes 2020:</u> In the 2YR rotation experiment, tuber yield in the first year of potatoes, 2020, was significantly affected by the soil health management treatments (Table 9). 'Superior' planted 30 days after CP fumigation (GRSTAND, MANURE, MAN/CCR treatments)

yielded 13.3 Mg ha^{-1} greater than those not fumigated (NOFUM; Table 9, p = 0.0001). Further, the proportion of the yield > 6 oz. harvested after CP fumigation was on average 1.8 times greater compared to the nonfumigated control indicating fumigation prolonged tuber bulking (Table 9, p = 0.0011). Of the treatments that were fumigated with CP, there was no difference in yield between treatments that received spring poultry litter (MANURE and MAN/CCR) and those that were fertilized with mineral fertilizer only (GRSTAND). 'Russet Burbank' in NATCTRL and 'Snowden' in the CCR treatment yielded, expectedly greater than the treatments with 'Superior'. Proportion of yield that was graded as U.S. No.1 varied only by variety.

Common scab and PED infection rates were not measured after the 2020 harvest. Potato Early Die 2020 severity was estimated by the observed percent green cover at vine kill, which was 11% greater in CP fumigated plots compared to the nonfumigated control (Table 10; p = 0.0196). 'Russet Burbank' from NATCTRL had the highest rate of survival with 32% remaining green cover at vine kill while 'Snowden' survival from the CCR treatment was the same as NOFUM at 5%. Specific gravity varied only by potato variety.

<u>Rotation Crops 2019 and 2021:</u> Corn yields in 2019 ranged 7.47-8.48 Mg ha^{-1} with no differences between treatment. Wheat yields following the 2020 potatoes varied by treatment (data not shown). The wheat following 'Superior' in treatments NOFUM, GRSTAND, MANURE, and MAN/CCR yielded 5.99-6.46 Mg ha^{-1}, significantly greater than wheat following the long season potato varieties planted in CCR and NATCTRL that yielded 5.32 and 5.52 Mg ha^{-1}, respectively. Of the 'Superior' treatments, MANURE yields were the greatest, followed by MAN/CCR, though both were only marginally greater than GRSTAND and NOFUM.

<u>2nd Round of Potatoes 2022:</u> In the 2YR rotation, 2022 potatoes were planted 30 days earlier than 2020. Earlier planting can likely explain the greater yields seen in NOFUM, MANURE, and

MAN/CCR in 2022 compared to 2020. Despite the advantages to earlier planting, however, GRSTAND yields were not greater than 2020 and 6.6 and 10.2 Mg ha^{-1} lower than the 2022 yields of other 'Superior' treatments that received annual poultry litter (Table 9; p = 0.0423 and 0.0031 for MANURE and MAN/CCR, respectively). Fumigation in 2020 had no residual effect on 2022 yields. The proportion of yield graded as U.S. No. 1 was similar across all 'Superior' treatments. 'Snowden' in the CCR treatment were 100% graded as U.S. No. 1. proportion of yield graded > 6 oz. ranged 78-84% across 'Superior' and 'Snowden' treatments. Only 65 and 38% of the NATCTRL yield graded as U.S. No. 1 and > 6 oz., respectively, which is expected because of the shortened Michigan potato growing season.

All 2022 treatments had fully senesced by harvest so green cover evaluation occurred four weeks prior to harvest. In early August, the NATCTRL and CCR treatments were at 33 and 30% green cover, respectively, while the GRSTAND, MANURE, and MAN/CCR were significantly more senesced with 17, 18, and 13% green cover, respectively (Table 10). The NOFUM treatment resulted in the least green cover at only 5%. *Verticillium* tuber infection rates were <10% across treatments. 'Snowden' in the CCR treatment were the most severely infected with common scab. Common scab infection rates in 'Superior' treatments that had been fumigated in 2020 were 7-14%, lower than infection in the NOFUM treatment (Table 10; p = 0.0410). Specific gravity varied only by potato variety.

3.2.2 Three-Year Rotation (Potato-Wheat-Corn-Potato)

<u>1st Round of Potatoes 2019:</u> In 2019 within the 3YR rotation, yields from CP fumigated plots, including GRSTAND, MANURE, and MAN/CCR, were 8.5 Mg ha^{-1} greater than the nonfumigated control (NOFUM) (Table 9; p < 0.0001). Yields from NATCTRL and CCR were only marginally greater than the fumigated 'Superior' treatments. The largest proportions of U.S.

No. 1 tubers were produced by ‘Russet Burbank’ in NATCTRL. Little variation in U.S. No. 1 grading occurred among ‘Superior’ treatments except for NOFUM, which had significantly greater proportion of U.S. No. 1 tubers, likely due to especially low total yields. Tubers were not graded by size at this harvest date.

Green cover at 2019 vine kill varied only by variety (Table 10). ‘Superior’ treatments (NOFUM, GRSTAND, MANURE, and MAN/CCR) had greater senescence than NATCTRL or CCR with no significant differences among ‘Superior’ treatments. *Verticillium* tuber infection did not vary by treatment and specific gravity varied only by potato variety.

<u>Rotation Crops 2020 and 2021:</u> Wheat yields following potato in 2020 varied by treatment (data not shown). The treatments following long season variety potatoes, NATCTRL and CCR, appeared to yield lower than the treatments following ‘Superior’ with 5.18 and 5.05 Mg ha, respectively, though not statistically significant in each comparison. Wheat yields following ‘Superior’ ranged 5.45-6.39 Mg ha^{-1}, with MANURE yielding the highest. In 2021, corn yields ranged 13.06-14.76 Mg ha^{-1} with no significant differences between treatments.

<u>2nd Round of Potatoes 2022:</u> Due to earlier planting date, 2022 potatoes yielded greater than 2019 across all treatments. Across ‘Superior’ treatments, only yields in MANURE treatment were significantly greater than the other ‘Superior’ yields (NOFUM, GRSTAND, MAN/CCR) (Table 9). Similar to the 2YR rotation, the proportion of NATCTRL graded as U.S. No.1 and > 6 oz was lower than all other treatments due to of the high rate of misshapen ‘Russet Burbank’ tubers. There were no significant differences in size or quality grading among ‘Superior’ and ‘Snowden’ potato treatments.

Green cover in the MANURE and MAN/CCR treatment plots was greater than in GRSTAND in 2022, though only significant in the case of MANURE (Table 10; $p = 0.0282$). In

2022, green cover measured in August was 33% in non- ‘Superior’ treatments CCR and NATCTRL. Fumigation had no residual effect on 2022 green cover. *Verticillium* tuber infection was under 10% in NOFUM and MAN/CCR, significantly lower than infection rates in both GRSTAND and MANURE (Table 10). Manure had no significant effect on *Verticillium* tuber infection. Common scab infection rate was greatest in the GRSTAND and MANURE treatments as compared to merely 6 and 12% infection rates in NOFUM and MAN/CCR (Table 10). Specific gravity varied only by potato variety (Table 10).

3.3 Soil Health Metrics

3.3.1 Two-Year Rotation Chemical Soil Health Metrics

After 4 years of soil health management, SOM and TC did not change significantly from the initial concentrations of 1.7% SOM and 1.0% TC at the 2YR site with no differences between treatments at any sample date (data not shown). Initial soil pH for the 2YR study measured 6.6 during 2020 potato planting (Table 12). Soil texture composition was 59% sand, 28% silt, and 13% clay with 5.50 meq $100g^{-1}$ CEC. Through the duration of the 2YR rotation, pH varied by sample date though remained ±1 the initially measured pH.

Mean preplant soil nitrate (NO_3^-) concentrations in 2020 in the 2YR study were 3-5 times greater than preplant nitrate concentrations from other sampling years (Table 11). Treatments NOFUM and NATCTRL had significantly lower preplant soil NO_3^- concentrations than the treatments that had been fumigated by CP 30 days prior (GRSTAND, MANURE, CCR, and MAN/CCR). Though poultry litter had been applied 25 days prior to sampling to MANURE and MAN/CCR, soil NO_3^- concentrations in the manure treatments did not differ from GRSTAND. At 60DAP 2020, soil NO_3^- concentrations were the greatest recorded at any sample date (Table 12). Long season variety treatments NATCTRL and CCR had the lower soil NO_3^- compared to the ‘Superior’ treatments. NOFUM had greater inorganic N concentrations than all other treatments, though not significantly greater than GRSTAND or MANURE treatments.

Following 2021 wheat harvest and pre-potato 2022 planting soil NO_3^- concentrations were under 10 mg kg^{-1} across all treatments (Table 11; data not shown for 2021 sample). As in 2020, midseason 2022 soil NO_3^- concentrations were greater than preplant 2022 levels, however the difference between treatments in 2022 were different than the trends seen at 60DAP 2020 (Table 12). Soil NO_3^- concentrations in NOFUM, GRSTAND, MANURE and CCR were significantly lower than MAN/CCR and greater than NATCTRL with no differences within the treatments.

Prior to 2020 potatoes, mean soil test P concentrations ranged from 83-102 mg kg^{-1} and did not vary by treatment, despite the fact that preplant fertilizer and manure had been applied 25 days prior to sampling (Table 11). At 60DAP, soil test P increased 15% from preplant concentrations with no differences across treatments (Table 12). Following wheat harvest 2021, soil test P decreased back to levels similar to preplant 2020 ranging from 79-98 mg kg^{-1} with no significant differences between treatments. Preplant 2022 soil test P concentrations were greater than preplant 2020 and there were no differences between treatment (Table 11). At 60DAP 2022, soil test P concentrations were 24 and 43% greater in manure treatments compared to GRSTAND (Table 12; $p = 0.0171$ and 0.0013 for MANURE and MAN/CCR, respectively).

3.3.2 Two-Year Rotation Biological Soil Health Metrics

Biochemical: June 2020 24 hr CO_2 burst rates varied significantly by treatment (Table 13). Fastest rates were from NOFUM plots which averaged 127 ppm CO_2 day^{-1} though not significantly faster than rates found in MANURE and MAN/CCR plots. NATCTRL, GRSTAND, and CCR had the lowest rates ranging 90-99 ppm CO_2 day^{-1}. Similar patterns were observed in 60DAP 2020 samples, with the exception that NATCTRL burst respiration rates recovered to be similar to NOFUM, MANURE, and MAN/CCR. GRSTAND and CCR rates remained low at 65 and 66 ppm CO_2 day^{-1} (Table 14). No treatment effects occurred following 2021 wheat harvest and rates ranged 155-181 ppm CO_2 day^{-1}. Preplant 2022 burst rates were similar across treatments and greater than preplant 2020 (Table 13). By 60DAP 2022, burst rates were slightly lower across treatments than preplant rates and dependent on treatment (Table 14). Notably, the MANURE and MAN/CCR had greater burst rates than GRSTAND (Table 14; $p = 0.0008$ and 0.0.0103, for MANURE and MAN/CCR, respectively).

Preplant 2020 POX-C concentrations were the greatest across all sampling times, ranging from 421-464 mg POX-C kg^{-1} soil (Table 13). Midseason POX-C concentrations were lower than preplant across treatments, though NOFUM POX-C was significantly greater than MANURE, MAN/CCR, NATCTRL, and CCR, and similar to GRSTAND (Table 14). By October 2021, 3 months after wheat harvest, between treatment variation disappeared and concentrations ranged from 312-370 mg POX-C kg^{-1} soil. During spring 2022, POX-C concentrations were the lowest values measured in any sample time from the 2YR study with means ranging from mg POX-C kg^{-1} soil (Table 13). Growing season accumulation of POX-C in 2022 produced a 236% increase across treatments but only slightly marginal differences between treatments (Table 14; $p = 0.1105$). Soil protein concentration did not vary by treatment but did vary by sample date (Table 13, Table 14, fall sample data not shown). Growing season accumulation of ACE protein was consistent but slight increasing only by 0.5 mg protein g soil^{-1} and 0.3 mg protein g soil^{-1} between preplant and 60DAP samples in 2020 and 2022, respectively. Samples taken post wheat harvest in 2021 are greater than those taken 60DAP 2020 and comparable to those found at 60DAP 2022, indicating the accumulation in soil protein concentration occurred during the rotation year and was maintained through the 2022 potato growing season.

Microbiome: Total microbial biomass, estimated by PLFA sampled 60DAP 2022, was not affected by treatment in the 2YR study, nor was the ratio of fungi to bacteria (F:B) (Table 14).

An average of 24,724 and 21,208 non-chimeric reads per sample were obtained in 2020 samples for 16S and ITS sequencing, respectively. Both preplant 2020 bacterial and fungal diversity, estimated by Shannon diversity indices, varied by treatment (Table 15). At preplant, one month after soil fumigation, bacterial and fungal communities were more diverse in the NATCTRL and NOFUM treatments compared to treatments fumigated with CP (GRSTAND, MANURE, CCR, and MAN/CCR) (Table 15; $p = 0.0026$ and $p = 0.0004$ for bacterial and fungal diversity, respectively). Both bacterial and fungal diversity in CP fumigated treatments remained lower than NATCTRL and NOFUM through the subsequent sampling at 60DAP (Table 15; $p = 0.0074$ and $p = 0.0119$ for bacterial and fungal diversity, respectively).

Trends observed in α-diversity are reflected in the structure of the sampled microbial communities. Permutational analysis of variance did not result in significant interaction between sample time and treatment effects so treatment effects on β-diversity are reported across the preplant and 60DAP sample times (Table 16)**.** A principal coordinates ordination illustrates divergence in the bacterial (16S) and fungal (ITS) communities sampled from NATCTRL and NOFUM and those sampled from GRSTAND, MANURE, CCR, and MAN/CCR in the 2YR rotation (Figure 1). Results from the pairwise PERMANOVA confirm this divergence showing significant differences between both NATCTRL and NOFUM and all other treatments, GRSTAND, MANURE, CCR, and MAN/CCR (Table 17).

Potato Early Die Pathogen Populations: Initial potato soil *V. dahliae* populations sampled in June 2020 and August 2020, were near 0 Verticillium propagules g^{-1} (VPPG) across treatments (Table 18). Following wheat harvest in 2021, soil *V. dahliae* populations were greater than preplant and midseason 2020 samples, ranging from 23-42 VPPG with no significant difference among treatments.

By spring 2022, *V. dahliae* populations decreased across all treatments (Table 18). Compared to GRSTAND, MANURE and MAN/CCR treatments, NOFUM, NATCTRL, and CCR plots had lower *V. dahliae* counts, though the difference was only marginal in NATCTRL. When measured 60 days later, the treatment difference had dissipated.

Initial RLN populations at preplant 2020 and 60DAP 2020 in the 2YR study ranged just below the threshold for expected PED damage (>100 nematodes with 0 VPPG) across treatments (Table 18). However, by October 2021 following wheat, RLN counts increased to 164-417 RLN 100cc $soil^{-1}$ with no difference between treatments. Populations peaked in April 2022 with the largest quantity observed in GRSTAND. By 60DAP 2022, RLN counts decreased from preplant counts but GRSTAND still had a significantly greater population than all other treatments besides MANURE.

3.3.3 Two-Year Rotation Physical Soil Health Metrics

Water stable aggregates, measured in the spring of every potato year, were not significantly affected by treatments across the 4 years, but the proportions of aggregates sizes do appear to be changing over time across treatments. Between the initial potato planting in 2020 and the 2022 planting, macroaggregates increased from 60% and microaggregates decreased slightly, though significantly (Figure 2).

3.3.4 Three-Year Rotation Chemical Soil Health Metrics

After 4 years of soil health management, SOM and TC did not change significantly from the initial concentrations of 1.0% SOM and 0.7% TC at the 3YR rotation site with no differences between treatments at any sample time (data not shown). Soil pH at the 3YR rotation was more acidic than the 2YR location. Though from the same soil series, the 3YR study contained coarser ground (70% sand, 25% silt, 5% clay) with 5.9 pH and 3.55 meq $100g^{-1}$ CEC. Treatments had no effect on soil pH across any of the sampling dates (Table 11, 12).

Despite differences in fertility management, inorganic soil concentrations only varied by treatment at the 60DAP 2022 sample (Table 11, Table 12). At 60DAP 2022 sampling, NATCTRL and CCR had the lowest soil nitrate concentrations, 32 and 38 mg kg^{-1}, respectively, while GRSTAND, NOFUM, MANURE, and MAN/CCR all had similar soil NO_3^- concentrations (60-73 mg kg^{-1}).

Preplant 2019 soil test P concentrations ranged from 117-154 mg kg^{-1} (Table 11). By 60DAP, soil test P increased on average 53% from preplant concentrations to range from 182-235 mg kg^{-1}. Though a broad range, soil P did not vary by treatment at 60DAP in 2019 (Table 12). Following 2020 wheat harvest, no treatment differences on soil test P occurred with concentrations ranging from 144-180 mg kg^{-1}. Following corn harvest 2021 soil test P in all treatments ranged 140-158 mg kg^{-1} except for MAN/CCR, which reached 204 mg kg^{-1} soil test P. Preplant 2022 soil test P concentrations were the greatest measured across any study year ranging 194-223 mg kg^{-1} (Table 11). By 60DAP 2022, soil test P concentrations decreased 11-22% in all treatments except the MANURE treatment, which increased 16% from preplant soil P concentrations resulting in greater soil P concentrations in the MANURE and MAN/CCR treatments compared to GRSTAND (Table 12; $p = 0.0030$ and $p = 0.0159$ for MANURE and MAN/CCR, respectively).

3.3.5 Three-Year Rotation Biological Soil Health Metrics

Biochemical: 24-hour CO_2 burst rates did not significantly differ between treatments at any sample date except for the final 60DAP sample in July 2022 at which point the MANURE treatment produced a microbial burst respiration rate 30% greater on average than all other treatments (Table 13; Table 14). Permanganate oxidizable C concentrations did not differ between treatments at any sample date. In both the 2019 and 2022, POX-C increased 72% and 112%, respectively, between potato planting to 60DAP (Table 13, Table 14). Concentrations peaked in October 2020 following wheat harvest with mean values ranging mg POX-C kg^{-1} soil. Soil protein concentrations did not vary by treatment but did vary by sample date (Table 13, Table 14). Similar to the 2YR rotation, soil protein increased within the potato growing season in both 2019 and 2022. However, there were no measured soil protein increases during the 3 years between potato growing seasons. Soil protein concentrations measured in 2022 at preplant and 60DAP were similar to those measured in 2019.

Potato Early Die Pathogen Populations: Soil *V. dahliae* populations were undetectable or low throughout the 3YR rotation with mean VPPG counts ranging from 0-4 VPPG. Samples taken prior to 2022 potato planting were statistically but likely not biologically different (Table 18). No treatment effects occurred on RLN populations through the duration of the 3YR study. Like the initial 2YR study preplant RLN count, preplant 2019 RLN counts for the 3YR study were below the threshold for expected PED damage, ranging from 51-83 RLN 100cc $soil^{-1}$ (Table 18). Marginal count increases occurred during preplant 2022 but did not exceed the commercial lab recommended threshold for fumigation. Root lesion nematode 60DAP populations increased 1-9 times preplant counts but no differences occurred between treatments.

Microbiome: The PLFA profile varied by treatment in the 3YR study when sampled at 60DAP 2022 (Table 14). The MANURE treatment resulted in the greatest total microbial biomass with 2119 ng microbial biomass g^{-1} soil. MANURE and MAN/CCR treatments provided the widest F:B ratio despite MAN/CCR significantly differing from than NATCTRL and NOFUM.

An average of 20,870 and 24,724 non-chimeric reads per sample were obtained for 16S in 2019 and 2020, respectively. An average of 24,331 and 21,208 non-chimeric reads per sample were obtained for ITS in 2019 and 2020, respectively. Estimates of June 2019 bacterial and fungal diversity were not impacted by treatment prior to initial potato planting (Table 15). By 60DAP, fungal diversity was still same across all treatments, but bacterial diversity varied by treatment (Table 15). The NOFUM treatment produced the bacterial community with greatest diversity at 60DAP (Table 15).

Analyses examining β-diversity further indicated the lack of treatment effects on bacterial communities and subtle treatments effect on fungal community. Only the main effect of sample date was found to significantly impact the bacteria β-diversity in the 3YR study, with no interaction between management and sample time (Table 16). Fungal β-diversity, however, significantly varied by both main effects of management and sample date with no significant interaction so treatment effects on β-diversity are reported across the preplant and 60DAP sample times (Table 16). Principle coordinates analysis indicated a less dramatic separation between NOFUM and NATCTRL and the other treatments compared to that seen in the 2YR rotation (Figure 1). Pairwise comparison suggests the fungal community in GRSTAND and MAN/CCR were different than NATCTRL and NOFUM (Table 17). No other differences in fungal community structure nor any differences in bacterial community structure were observed between treatments in the 3YR rotation study (Table 17).

3.3.6 Three-Year Rotation Physical Soil Health Metrics

Water stable aggregates, measured in the spring of every potato year, were not significantly affected by treatments across the 4 years, but the proportions of aggregates sizes do appear to be changing over time across treatments. Between the initial potato planting in 2019 and the 2022 planting, the macroaggregates and microaggregates decreased in the 3YR rotation (Figure 2).

4. DISCUSSION

I sought to understand how different management strategies, aimed at improving soil health in potato cropping systems, would impact soil chemical, biological, and physical properties as well as potato production. I tested the effects of various management practices that have been shown to improve soil health, such as organic matter amendments, increased cropping system diversity through increased rotation length and inclusion of cover crops, and eliminating fumigation. I hypothesized these practices to varying degrees would increase soil microbial activity and diversity that could translate into improvements in soil function, as measured by soil health indicators. Though I found eliminating fumigation to have the strongest effects on soil health, made evident by shifts in microbial communities and changes in C and N dynamics, only annual manure application significantly improved potato yields after four years of annual application.

4.1 Management Effects on Soil Health Indicators

Neither chemical indicators related to long-term changes in soil health nor physical indicators were impacted by soil health building practices implemented during the four-year study. During the first year of study, the manure effect on soil health indicators were marginal in the 2YR rotation study and negligible in the 3YR study. After 4 years of implementation, manure increased midseason microbial activity across both the 2YR and 3YR rotations as well as the F:B ratio of the microbial community in the 3YR rotation further indicating increasing benefit over time. However, the effects of eliminating fumigation and the cover crop on soil health indicators were limited to only to growing season following fumigation and cover crop termination, respectively.

4.1.1 Chemical Indicators of Soil Health

Effects of soil health building practices on soil chemical indicators were generally short-lived and contained within the growing season. Following four years of study, none of the tested soil health management practices impacted total SOM or TC accumulation. Results of the present study agree with previous work reporting no change in SOM with low rates of annual C input in potato cropping systems (Rees et al., 2014).

Eliminating fumigation was the only tested practice to impact soil NO_3^-. At preplant, soil NO_3^- concentrations were greater in fumigated plots as rapid microbial biomass turnover after fumigation can produce net N mineralization. De Neve et al. (2004) reported increased net N mineralization for up to 4 weeks across following 1,3 dichloropropane fumigation resulting in soil NO_3^- concentrations 20 mg kg^{-1} greater than the nonfumigated control. By midseason (i.e., 90 days after fumigation), NO_3^- concentrations were lower with fumigation. Previous studies have revealed two simultaneous effects of soil fumigation that may explain reduced NO_3^- concentrations. Soil fumigation may suppress soilborne pathogens leading to vigorous root growth and thus crop N uptake early in the season. Another possibility may be fumigation suppresses nitrifying microbes (Crants et al., 2021; Fang et al., 2018; Sennett et al., 2021; Yan et al., 2015, 2017). The effect of fumigation on midseason NO_3^- concentrations was not observed in subsequent sampling, indicating only a short-term effect of chloropicrin fumigation on nitrification.

The addition of a cover crop had no measured impact on extractable nutrient concentrations at preplant or 60DAP across any study year. Previous studies have found that combinations of rye and manure reduced nitrate leaching and increased crop N uptake (Nyiraneza & Snapp, 2007). The decomposition of high C:N cover crop biomass may result in net immobilization of nutrients during crop uptake (Rutan & Steinke, 2019). Preplant and 60DAP samples for extractable nutrient concentrations only offer a limited understanding of the full effect of the cover crop on nutrient dynamics.

Despite differences in N source and total N between treatments, the addition of manure did not impact preplant or 60DAP soil NO_3^- concentrations across either study year. Manure treatments did, however, correspond with significantly greater concentrations of soil test P. The poultry litter provided an additional 100 kg P_2O_5 ha^{-1} compared to the mineral fertilizer only treatments. Studies have found that manure applications can result in available P greater than the sum of what is applied (Laboski & Lamb, 2003). As microbes metabolize manure, organic acids produced can decrease sorption of P to soil binding sites, increasing the availability of soil P, especially on low CEC soils without a history of manure (Kafkafi et al., 1988; Toor & Bahl, 1997). The accumulation of soil test P poses an environmental concern especially in coarse textured soils.

In-season soil pH dynamics, though slight, were significantly different with the annual application of poultry litter and reflect a stabilizing effect of manure on soil pH. Acidification following the application of ammonium-forming fertilizers including UAN28%, urea, and poultry litter, is well documented (Barak et al., 1997; Geisseler & Scow, 2014; Schroder et al., 2011). In the present study, soil pH decreases from preplant to 60DAP in every study year though to a greater degree in the 2YR rotation. Though manure and other organic amendments, produce NH_4^+ that is later nitrified, four consecutive years of manure application in the 2YR rotation mitigated soil acidification. By 2022 in the 2YR rotation, pH at both preplant and 60DAP were 0.3 greater in MANURE as compared to GRSTAND (Table 11; Table 12; $p = 0.0014$ and 0.0102 at preplant and 60DAP, respectively). Increased soil pH with manure application is well documented as properties intrinsic to various sources of manure, including Ca content and ash alkalinity can produce a net increase in pH (Materechera & Mkhabela, 2002; Pettit, 2017). For example, the poultry litter product used in this study contained 71 kg Ca Mg^{-1}, which has a $CaCO_3$ equivalent of 11%, as estimated by a regression equation from Pettit (2017) based on 139 samples of poultry litter from various sources. Although $CaCO_3$ equivalent is not a commonly tested property in manure analysis, it may be helpful to include as it can vary greatly by manure source and has important implications for soil pH, soil health and crop productivity.

4.1.2 Biological Indicators of Soil Health

Microbial Communities: Though manure and the rye crop did not impact microbial community composition in the first two years of the study, both impacted soil C and nutrient cycling, as indicated by biochemical properties. Increased F:B with four years of poultry litter suggests the impacts of organic amendments on the microbial community may be cumulative as evidenced by increasing magnitude of the effect of manure on the microbial burst respiration. Eliminating fumigation did not increase pathogen populations, but rather may have preserved microbial diversity and altered soil biochemical properties, though all measured effects were contained within the year following fumigation.

Neither the addition of manure, nor rye cover crop impacted microbial community diversity or community structure in the first two years of study. Supplementing manure with mineral fertilizer may weaken the effect of manure on the microbial community. In a long-term field experiment, the manure only treatment produced a distinct microbial community with especially low Acidobacteria, whereas the microbial community that resulted from manure and mineral fertilizer combination overlapped closely with that of the mineral fertilizer only treatment (Wang et al., 2016). However, without supplementing poultry litter with mineral fertilizers, it is difficult for growers to achieve an ideal balance of nutrients. For example, if manure application rates are based on N requirements, then there is overapplication of P that presents a critical risk for P pollution. Additionally, the effects of soil health building practices on microbial communities may depend on environmental conditions. Rutan et al. (2022) observed the initial year of cover crop adoption had limited effect on bacterial community structure in the growing season following termination, particularly in the study year in which there was sufficient precipitation.

The magnitude of the shift in microbial community structures may be more dependent on duration of implementation as well as upon the quantity and quality of C added to soils. Larkin et al. (2022) observed stronger shifts in microbial activity and community structure in response to dairy manure (high C) vs. swine manure (low C) when application rates were equalized by N content. Long term (> 20 years) application of manure and mineral fertilizer led to greater Shannon diversity and distinct PLFA community structure compared to a mineral fertilizer treatment (Zhong et al., 2010). Though only marginal shifts in PLFA profile occurred after one year of a cover crop in vegetable production, significant shifts and increases in microbial biomass transpired after six years of annual cover crop usage (2017). These results and the lack of large changes in the current, relatively short study show the importance of long-term monitoring with regards to microbial communities' responses to soil health building practices.

Although manure and rye cover crop impacts on microbial communities were limited, eliminating CP fumigation increased overall microbial diversity and produced distinct fungal and bacterial communities in the 2YR rotation for at least 90 days after fumigation. Soil fumigants are nonselective biocides and the communities that recover post-fumigation are often distinct from where fumigation did not occur (Drenovsky et al., 2005; Huang et al., 2020; Sederholm et al., 2018; Zhang et al., 2017). Interestingly, the microbial communities after metam sodium fumigation were not different than the non-fumigated treatment. The effects of MS and CP fumigation on microbial communities have been reported to vary in magnitude and duration and the long-term effects are still not well understood (Sennett et al., 2021).

Phospholipid fatty acid analysis from 2022 samples indicated four years of annual manure application had some measurable effects on the microbial community while the effects from fumigation on microbial communities may have only been temporary. The MANURE treatment in the 3YR rotation produced greater total microbial biomass and both MANURE and MAN/CCR appeared to be more favorable for fungi, resulting in wider F:B ratios. Reported responses of F:B ratios from manure application varies with some studies demonstrating long-term manure application increased fungal relative to bacterial biomass, while others observed increased bacterial relative to fungal biomass (Larkin, 2022; Lupwayi et al., 2018; Ozlu et al., 2019). Using F:B ratios to characterize the function of a microbial community is increasingly contested as our understanding of the physiological and functional redundancies between fungi and bacteria deepens (Strickland & Rousk, 2010). Forthcoming 16S and ITS sequence data from 2022 samples may help characterize any slower microbial community shifts driven by the soil health practices.

<u>Potato Early Die Pathogens:</u> The impact of soil health practices on PED pathogen populations varied by pathogen and rotation. While both rotations saw increases in root lesion nematodes (*P. penetrans*) across treatments, the 2YR rotation allowed for a more rapid proliferation of *V. dahliae*. Based on Michigan State University recommendations, 96% of the plots in the 2YR rotation fostered preplant PED pathogen populations greater than the threshold for fumigation while none of the preplant PED pathogen populations in the 3YR rotation warranted fumigation.

Although many growers are opting for more diverse and extended (3-5 year) potato rotations due to shorter and less diverse potato rotations being conducive to PED pathogens and other soilborne pathogens, 2-year rotations continue to be necessary in some areas of Michigan (Johnson et al., 2010).

Within the 2YR rotation, all soil health building practices reduced *P. penetrans* populations in 2022 as compared to the grower standard. However, in the 3YR rotation, soil health building practices did not affect the proliferation of *P. penetrans.* It is unlikely the suppressive effect of manure and cover crops observed in the 2YR rotation were due to fumigation properties of the organic amendments individually as the low rates of poultry litter and rye biomass were not sufficient to produce the concentrated accumulation of NH_3 or HNO_2 necessary for biofumigation (Bonanomi et al., 2007; Mahran et al., 2008; Tenuta & Lazarovits, 2002; Tubeileh & Stephenson, 2020). It is more likely that the root lesion nematodes suppression was related to overall increased microbial activity and diversity leading to niche exclusion of biocontrol (Bonilla et al., 2012; Peralta et al., 2018).

<u>Soil C Cycling via Microbial Activity:</u> Changes in microbial communities the year following fumigation corresponded with changes in POX-C and microbial burst respiration measurements offering insight into the short-term effects of eliminating fumigation on C cycling. Preplant burst rates decreased further in fumigated plots than nonfumigated plots indicating microbial activity suppression one month after fumigation in the 2YR rotation. Further, the POX-C measured 90 days after fumigation was 15% greater compared to the nonfumigated plots. Fumigation has been shown to reduce decomposition and suppress C mineralization in subsequent months, indicating reduced turnover of plant residue into the partially processed POX-C pool (Crants et al., 2021; Li et al., 2017; Sennett et al., 2021). Just as the impact of fumigation on microbial community

composition appeared to dissipate after one year as indicated by PLFA metrics, the impact of fumigation on biological soil properties also appeared to dissipate after 2019 and 2020 in the 3YR and 2YR rotations, respectively.

Microbial burst respiration measured preplant and midseason revealed shifts in microbial activity weeks and months following the incorporation of manure and cover crops biomass. Preplant samples taken following cover crop termination and manure incorporation reveal limited impact on early season microbial activity. When manure was applied one week prior to planting, there were no measured effects on microbial burst respiration across study years. However when manure was applied almost one month before sampling in 2020, the respiration burst was as high as the nonfumigated plots indicating that the positive impact of manure on microbial activity overcame the suppressive effect of fumigation. Cover crop prior to potato made no additional contribution to preplant microbial burst respiration, despite the reported importance of root exudate contributions to labile C and microbial activity (Finney et al., 2017).

Within the 3YR rotation, midseason 2022 microbial burst respiration revealed contrasting effects of manure and cover crops. While four years of annual manure applications produced a greater burst, the addition of the rye cover crop prior to potato planting effectively dissipated the manure effect. Greater concentrations of mineralizable C are a common product of manure application across multiple cropping systems (Hurisso et al., 2016). The pelletized poultry litter used in the present study has an estimated 10:1 C:N ratio indicating high availability to microbes (estimate from Nyiraneza & Snapp, 2007). Even at 60DAP, manure continued to cultivate a more active and abundant microbiome indicated by both microbial burst respiration and total microbial biomass in the 3YR rotation. Total microbial biomass estimated by PLFA correlates closely with microbial respiration (Orwin et al., 2018). When planting a cover crop, adding low

C:N manure will increase the rate of residue decomposition and nutrient release relative to no manure addition, but the rate of nutrient release is relatively slow if the cover crop biomass contains high C:N (Nyiraneza & Snapp, 2007; Poffenbarger et al., 2015). However, by 60DAP, decomposition of relatively high C:N (> 25:1) rye biomass may have produced a net N immobilization at midseason, restricting microbial activity and decreasing midseason microbial burst respiration with marginally lower NO_3^- concentrations. The C:N ratio of all organic amendments should be taken into consideration when predicting C and N mineralization dynamics.

Though microbial community diversity and structure are important indicators of soil health, results in this study reveal limitations in the connection between changes in microbial communities and long-term accumulation of the soil health-related biochemical fractions. Contrary to the hypothesis that the additional C inputs from manure and cover crops would increase labile C, neither manure nor cover crop influenced POX-C concentrations measured preplant or midseason in any study year. Pokhrel et al. (2021) also found limited influence of manure application on POX-C on sand and suggested a lack of mineral protection in coarse textured soil may leave POX-C more susceptible to being rapidly turned over into stable forms of C. The lack of effects may also have been due to the relatively short timeframe as previous studies report limited effect of manure or cover crops on POX-C in initial years of adoption (Decker et al., 2022; Hurisso et al., 2016). Long term studies (> six years) investigating increased rotation diversity and annual cover crops show a positive effect on POX-C even on coarse textured soil (Culman et al., 2013; White et al., 2020).

Protein Turnover and Soil N Cycling: None of the tested soil health practices had a measurable impact on the accumulation of ACE protein which is an important source of organic N. Soil protein accumulation and aggregate stability have been found to improve with manure application and crop diversity, but the disruptive effect of tillage on aggregate formation and soil protein accumulation may counteract any positive impacts from soil health practices on soil protein (Sprunger et al., 2021; Tiemann et al., 2015; Wright et al., 1999, 2007). A study comparing organic, conventional, and no-till corn-soy systems, found only no-till management resulted in soil aggregate formation and soil protein accumulation suggesting that soil protein accumulation is limited by disturbance not organic inputs (Wright et al., 2007). When applied in a no-till system, Pokhrel et al. (2021) found 13% greater concentrations of ACE protein after 3 years of poultry manure applied at 4.5 Mg ha^{-1} compared to the mineral fertilizer conventional control. The frequent mechanical disturbance used in the current study may have restricted the expected increases in soil aggregation and ACE protein with poultry litter and a rye cover crop.

Overall, the temporality of the soil health practices' effects on microbial community diversity and structure seems to be reflected in shifting microbial activity as indicated by some, though not all, biological indicators of soil health. Eliminating fumigation produced a distinct microbial community in the weeks and months following fumigation as characterized by gene sequencing, and the community shifts corresponded with changes in biochemical properties linked to microbial activity and C cycling, including POX-C and microbial burst respiration. Just as the effect of manure on microbial communities was observed only in the final year of the study, the impact of low rates of poultry litter on microbial activity seemed to increase in magnitude after four years of implementation.

4.2 Management Effects on Soil Functioning

4.2.1 Plant Productivity

Despite having no effect on PED pathogen suppression or SOM accumulation during the four years of this study, soil health practices did impact the capacity of soil to sustain plant productivity as measured by crop yield. While the effect of manure and cover crops on C and nutrient cycling within the growing season is likely what drives crop yield, the connection between eliminating fumigation and crop yields is unclear and warrants further study.

Manure effect on yield was cumulative across the four years. Despite reduced rates of mineral fertilizer, manure treatments produced similar yields to the mineral only treatment in the first year of implementation, indicating nutrients from manure sufficiently supplemented those from mineral sources. By the final study year, annual poultry litter improved yields 5.6 and 7.3 Mg ha^{-1} compared to mineral only in the 2YR and 3YR studies, respectively (Table 9). The present work agrees with previous work that tuber yield benefit can be seen even at relatively low rates of manure application in combination with mineral fertilizers (Ninh et al., 2014; Nyiraneza & Snapp, 2007; Rees et al., 2014).

When accounting for the chemical composition of the manure source, manure treatments received additional 100-143 kg P_2O_5 ha^{-1} in both potato years compared to the mineral fertilizer treatments. The greater concentrations of available midseason P in manure treatments may have contributed to greater yields. Even when preplant soil P levels exceed critical levels, yield response to applied P has been observed because of increased P availability during peak potato uptake, when daughter tubers produce a round of fine root hairs during tuber bulking around 60-80DAP (Rosen et al., 2014).

Beyond greater rates of P application with manure, there may be an advantage of location of manure P in the soil profile and timing of manure P mineralization during tuber bulking. Unlike other crops that develop deep rooting systems to take advantage of nutrients that move deeper in the profile as the season matures, potato roots are shallow (< 20 cm from surface) and require shallow-placed nutrients even late into the season. It is possible that mineral-sourced P had been taken up, adsorbed, or moved below the rooting zone by tuber bulking, while manure-sourced organic P continued to be mineralized in the rooting zone (Schwartz et al., 2011).

The magnitude of the effect of manure on yield appeared to correspond with the magnitude of the effect on microbial burst respiration. Studies suggest preplant mineralizable C, estimated by microbial burst respiration, may be used to predict yield or help determine in-season N recommendations, though recent findings suggest the high variability of the metric limits its predictive power (Wade et al., 2020; Yost et al., 2018). The results in the present study suggest that midseason mineralizable C may be a useful tool to quantify the nutrient provisioning function of soil and possibly better predict yields, though further research is required to establish this relationship.

Total 2022 potato yields for treatments receiving manure in the 3YR rotation were -7.7 Mg ha^{-1} lower following spring incorporation of a cereal rye cover crop as compared to the same treatments that were left fallow the previous winter (i.e., no rye cover crop) (Table 9). Lower yields may be explained by decreased N availability during tuber initiation as cover crop significantly suppressed microbial burst respiration resulting in marginally lower soil NO_3^- (Lazicki et al., 2020). Sample timing may have led to an underestimation of N immobilization as the 60DAP sample was likely taken after tuber initiation, which is the growth period where N immobilization would be most yield limiting.

Nitrogen management strategies used in the current study may have eliminated expected improvements in N synchrony from the rye cover crop. Potato yield advantages due to the timing of N released from cover crop biomass may only be observed when fertilizer N is applied at a single timing, which is not a best management practice for coarse soil textures in irrigated agroecosystems (Jahanzad et al., 2017).

Yield losses due to eliminating fumigation were hypothesized to stem from loss of pathogen control, but despite a neutral or positive impact on PED pathogen populations, CP fumigation produced greater yields and decreased wilt severity compared to the nonfumigated treatment. Eliminating fumigation did impact other soil biological (POX-C, microbial burst respiration) and chemical indicators (soil NO_3^-) suggesting decreases in C turnover and nitrification in the 3 months following fumigation, however, it is unclear how these changes produced reduced crop yields. Early season N availability can delay tuber formation, however further research is necessary to determine if the magnitude of the effect of fumigation on delaying soil NO_3^- accumulation via nitrification was enough to significantly promote tuber formation and yield (Koch et al., 2020).

4.2.2 Pathogen and Disease Suppression

We observed only limited connections between PED pathogen suppression and disease severity. The observed suppression of root lesion nematode (*P. penetrans*) by manure, cover crops, and eliminating fumigation in the 2YR rotation did not correspond with reduced *Verticillium* tuber infection or wilt severity. Additionally, despite the few effects of soil health building practices on PED pathogen populations in the 3YR rotation, four years of annual poultry litter application reduced wilt severity as indicated by 8% greater vine cover when measured late season 2022. Results agree with a previous study finding little correlation between

PED pathogen populations and PED disease severity (Davis et al., 2001). My results along with others finding low correlation between pathogen populations and disease severity suggest growers may find greater PED disease control by managing for general disease suppression achieved with improved soil health, more so than pathogen control.

Effects of soil health building practices on common scab disease severity were relatively predictable. Eliminating CP fumigation produced greater scab incidence in 2022, indicating a longer-term effect of fumigation on *Streptomycetes scabies* pathogenicity. Though CP is labelled for use in scab control, few data are available pertaining to efficacy in Michigan (Merlington, 2014; Wharton et al., 2007). My data suggests CS did not control scab in the growing season following fumigation, nor in the subsequent years. Although growers may be hesitant to apply manure to potato cropping systems due to disease concerns in particular common scab, my data show that manure alone did not impact tuber scab infection in either rotation (Braun et al., 2017). However, reduced scab infection with the addition of both manure and a rye cover crop in the 3YR rotation suggest a suppressive effect of the rye cover crop. Wiggins and Kinkel (2005) report a winter buckwheat cover crop increased the proportion of nonpathogenic *Streptomycetes* and though this did not correspond with decreases in scab disease severity, nonpathogenic *Streptomycetes* have been shown to effectively reduce pathogenic scab infection through both competitive exclusion and antibiosis (Neeno-Eckwall et al., 2001).

Increases in SOM, microbial burst respiration, total microbial biomass, or microbial diversity are generally linked to soilborne disease suppression through direct or indirect mechanisms. Increases in microbial biomass and/or diversity can lead to direct disease suppression or pathogen suppression as abundance or activity of pathogens is reduced through niche exclusion or biocontrol. Alternatively, disease suppression is achieved indirectly as plant defenses are upregulated or enhanced. Both mechanisms require further research to establish a framework for predicting disease suppression (A. H. C. Van Bruggen & Semenov, 2000). My results show soil health building practices had only limited effects on the disease suppression capacity of the soil, which may stem from the relatively small adjustments in management as well as the shortened timeframe. In a long-term potato cropping system experiment in Maine, researchers have observed significant increases in soilborne disease suppression as well as SOM, microbial biomass C, and greater tuber yields after almost 20 years of integrated soil health management strategies (Larkin, 2022; Larkin, Griffin, et al., 2021; Larkin, Honeycutt, et al., 2021).

4.2.3 Soil C Accrual and Storage

Soil health building practices tested in the present study did not have a measured impact on the soil's capacity to accrue and store C as indicated by a lack of SOM, aggregate and POX-C accumulation. Previous studies suggest inputs of 2,000-2,400 kg C ha^{-1} yr^{-1} are required to maintain SOM in coarse textured soil in high disturbance systems (Angers et al., 1999; Carter et al., 2004). Though C contributions by crop and cover crop residue were not directly measured in the current study, previously reported biomass data from crops grown in similar conditions and an assumption of 40% C concentration for plant biomass allow comparison of estimated annual C inputs between treatments. All tested management strategies were expected to maintain or accumulate SOM with crop residue contributing an average of 2,930 kg C ha^{-1} yr^{-1} though potato years C contributions from crop residues were below the maintenance threshold at an estimated 1,500 kg C ha^{-1} (Alford et al., 1996; Al-Sheikh et al., 2005; Bolinder et al., 1999; Grabau et al., 2017). In addition to the crop residues, poultry litter added 1,000-2,000 kg C ha^{-1} annually and rye cover crop added a one-time addition of 600 kg C ha^{-1}, resulting in total average annual C inputs of 4,415 kg C ha^{-1} for MANURE and 4,565 kg C ha^{-1} for MAN/CCR. Estimates are based on Nyiraneza & Snapp (2007), who reported C inputs from rye biomass cultivated in similar conditions to the current study and poultry litter from the same source. It is also significant to note rye cover crop C contributions may be underestimated as root biomass is not included in this estimate. Even though C inputs in the MANURE and MAN/CCR treatments well exceeded the suggested 2,000-2,400 kg C ha^{-1} yr^{-1} for SOM maintenance, in this study I found that SOM were just maintained, and not increase. This suggests more time may be necessary to see SOM accumulation. Previous studies observed SOM accumulation after > 10 years of practice implementation (Angers et al., 1999; Mitchell et al., 2015).

Although I found no increase in soil C stocks with manure application, there has been strong positive relationship between the quantity of C input and C accrual and storage potential in potato cropping systems previously observed (Griffin & Porter, 2004). Mallory and Porter (2007) also observed SOM accumulation in a potato cropping system when manure was applied at significantly greater rates than my study (20,000 kg C ha^{-1} from beef manure). Although greater rates of manure application may deliver greater C accrual benefits, there are economic and environmental considerations that may prevent Michigan commercial potato growers from adopting the practice. In Michigan, guidelines established by Michigan Department of Agriculture and Rural Development, "Generally Accepted Agricultural and Management Practices", restrict manure P rates to those that match expected crop P removal when soil test P concentrations are above 100 mg P kg^{-1} soil (2021). It would also be difficult to increase C inputs through cover crop because to allow for greater biomass to accumulate, the rye cover crop would need to be planted earlier, potentially before corn harvest, and intercropping cover crops has not been widely adopted in Michigan.

5. CONCLUSION

Annual low-rate applications of poultry manure consistently produced 26-30% yield increases, presumably driven by significant impacts on soil health indicated by greater midseason microbial burst respiration and soil test P. The effects of manure on both potato yields and soil health indicators were diminished by the addition of the winter rye cover crop the year prior to potato planting. Given additional time, the increased rotational diversity of the cover crop treatment may result in greater rates of SOM accumulation as timeframes > four years may be required for measurable SOM increases. Eliminating fumigation shifted microbial community structure and had measurable effects on C and N cycling, but also greater disease severity in the year following no fumigation, which limited yields.

Even without SOM accumulation, management practices resulted in measurable impacts on soil health as well as crop productivity within the four years of the experiment. While SOM is a key indicator of soil function, changes in total SOM alone does not capture all changes in soil characteristics important for soil health, further emphasizing the importance of using multiple indicators to gain a comprehensive picture of soil health with multiple, integrated changes in management in potato cropping systems.

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APPENDIX

Table 1. Description of treatments replicated in both the 2-Year and 3-Year studies.

Treatment	Abbreviation	Variety	Fumigation	Manure	Cover Crop
No fumigation control	NOFUM	Superior † PED susceptible	None	N	N
Grower Standard	GRSTAND	Superior PED susceptible	Chloropicrin	N	N
Fumigated/manure	MANURE	Superior PED susceptible	Chloropicrin	Poultry litter	N
“Kitchen Sink”	MAN/CCR	Superior PED susceptible	Chloropicrin	Poultry litter	Winter rye
Fumigated/cover crop	CCR	Snowden PED tolerant	Chloropicrin	N	Winter rye
National control	NATCTRL	Russet burbank	Metam sodium	N	N

† PED: Potato Early Die

Table 2. Fertilizer applications and crop production schedule at the 2-Year rotation study site. Nutrient rates applied in the potato years are listed in Table 4 and Table 5.

	Corn 2019	Potato 2020	Wheat 2021	Potato 2022
Fumigation	NA †	13 May 2020	NA	NA
Main crop planting	7 June 2019	15 June 2020	7 October 2020	12 May 2022
Main crop fertility management †	**PPI**: ‡ **V4**: ‡	**PPI**: 20 May 2020 **Infurrow**: 15 June 2020 **Emergence:** 7 July 2020 **Hill**: 21 July 2020 **7 DAH**: 28 July 2020	**Green Up**: 4 May 2021	**PPI**: 10 May 2022 **Infurrow**: 12 May 2022 **Emergence**: 2 June 2022 **Hill**: 13 June 2022 **7 DAH**: 20 June 2022 **14 DAH**: 27 June 2022
Green Cover Evaluation	NA	22 September 2020	NA	20 July 2022
Main crop harvest	25 October 2019	5 October 2020	14 July 2021	10 September 2022
Cover crop planting	25 October 2019 Broadcast (CCR & MAN/CCR)	NA	11 August 2021 Broadcast	NA
Cover crop termination	‡ Tillage (CCR & MAN/CCR)	NA	Winter kill	NA
Preplant soil sample	NA	15 June 2020	NA	28 April 2022
60 DAP soil sample	NA	‡	NA	11 July 2022
Fall soil sample	NA	NA	6 October 2021	NA

† **PPI**: broadcast and incorporated with field cultivator prior to planting main crop. **Infurrow**: band applied in furrow at planting. **Emergence**: surface band applied at emergence of potato plants. **Hill**: surface band applied before hilling. **7 DAH**: surface band applied 7-10 days after hilling. **14 DAH**: surface band applied 14 days after hilling. **V4**: surface band applied at v4 stage of corn. **Green Up**: broadcast applied in spring as wheat plants begin to show new growth.

‡ Date unknown.

Table 3. Fertilizer applications and crop production schedule at the 3-Year rotation study site. Nutrient rates applied in potato years are listed in Table 4 and Table 5.

	Potato 2019	Wheat 2020	Corn 2021	Potato 2022
Fumigation	7 May 2019	NA	NA	NA
Main crop planting	7 June 2019	29 October 2019	11 May 2021	12 May 2022
Main crop fertility management †	**PPI**: ‡ **Infurrow**: 7 June 2019 **Emergence**: 26 June 2019 **Hill**: 8 July 2019 **7 DAH**: 15 July 2019	**Green Up**: 20 May 2020	**PPI**: 4 June 2021 **V4**: ‡	**PPI**: 10 May 2022 **Infurrow**: 12 May 2022 **Emergence**: 2 June 2022 **Hill**: 13 June 2022 **7 DAH**: 20 June 2022 **14 DAH**: 27 June 2022
Green Cover Evaluation	23 September 2019	NA	NA	20 July 2022
Main crop harvest	7 October 2019	15 July 2020	13 October 2021	10 September 2022
Cover crop planting	NA	11 August 2020 Broadcast	13 October 2021 Broadcast (CCR and MAN/CCR)	NA
Cover crop termination	NA	Winter kill	9 May 2022 Tillage (CCR & MAN/CCR)	NA
Preplant soil sample	‡	NA	NA	28 April 2022
60 DAP soil sample	‡	NA	NA	11 July 2022
Fall soil sample	NA	‡	6 October 2021	NA

† **PPI**: broadcast and incorporated with field cultivator prior to planting main crop. **Infurrow**: band applied in furrow at planting. **Emergence**: surface band applied at emergence of potato plants. **Hill**: surface band applied before hilling. **7 DAH**: surface band applied 7-10 days after hilling. **14 DAH**: surface band applied 14 days after hilling. **V4**: surface band applied at v4 stage of corn. **Green Up**: broadcast applied in spring as wheat plants begin to show new growth.

‡ Date unknown.

Table 4. Rates of applied nutrients in the first round of potatoes varied by potato variety and fertilizer source. The initial rounds of potatoes were planted 2020 for the 2 Year rotation study and 2019 for the 3 Year Rotation study. Application dates can be found in Table 2 and Table 3.

Application Timing †	**PED Tolerant Variety with Mineral Fertilizer** NATCTRL and CCR§	**PED Susceptible Variety with Mineral Fertilizer** NOFUM and GRSTAND§	**PED Susceptible Variety with Manure and Mineral Fertilizer** MANURE and MAN/CCR§
	-----**kg ha^{-1}**-----		
PPI	0-0-62: 252K	0-0-62: 252K	0-0-62: 144K Poultry litter: 123N, 143P, 108K
In-furrow	10-34-0: 34N, 112 P UAN 28%: 34N Liquid K_2O: 84K	10-34-0: 34N, 112 P UAN 28%: 60N Liquid K_2O: 84K	10-34-0: 20N, 67P UAN 28%: 74N Liquid K_2O: 84K
Emergence	UAN 28%: 100N	UAN 28%: 100N	UAN 28%: 100N
Hill	UAN 28%: 73N	UAN 28%: 95N	UAN 28%: 34N
7 DAH	UAN 28%: 67N	UAN 28%: 95N	UAN 28%: 34N
14 DAH	-	-	-
Season Total (N, P, K)	**(275, 100, 300)**	**(344, 100, 300)**	**(344, 188, 300)**

† **PPI**: broadcast and incorporated with field cultivator prior to planting main crop. **In-furrow**: band applied in furrow at planting. **Emergence**: surface band applied at emergence of potato plants. **Hill**: surface band applied before hilling. **7-10 DAH**: surface band applied 7 days after hilling. **14 DAH**: surface band applied 14 days after hilling.

§ NATCTRL, Russet Burbank potatoes planted after spring metam sodium fumigation and with mineral fertilizer only. NOFUM, Superior potatoes planted without fumigation and with mineral fertilizer only. GRSTAND, Superior potatoes planted after spring chloropicrin (CP) fumigation and with mineral fertilizer only. MANURE, Superior potatoes planted after spring CP fumigation and with combination mineral fertilizers and annual application of poultry litter. CCR, Snowden potatoes planted after spring CP fumigation and with mineral fertilizer only, winter rye cover crop planted after corn harvest and incorporated in the spring. MAN/CCR, Superior potatoes planted after spring CP fumigation and with combination mineral fertilizer and annual poultry litter, winter rye cover crop planted after corn harvest and incorporated in the spring.

Table 5. Rates of applied nutrients in the second round of potatoes varied by potato variety and fertilizer source. The second round of potatoes for both the 2-Year and 3-Year Rotation studies were planted in 2022. Application dates can be found in Table 2 and Table 3.

Application Timing †	**PED Tolerant Variety with Mineral Fertilizer** NATCTRL and CCR§	**PED Susceptible Variety with Mineral Fertilizer** NOFUM and GRSTAND§	**PED Susceptible Variety with Manure and Mineral Fertilizer** MANURE and MAN/CCR§
	kg ha^{-1}		
PPI	0-0-62: 336K	0-0-62: 336 K	0-0-62: 336K Poultry litter: 123N, 143P, 108K
In-furrow	10-34-0: 34N, 112P	10-34-0: 34N, 112P	10-34-0: 34N, 112P
Emergence	UAN 28%: 56N	UAN 28%: 56N	UAN 28%: 56N
Hill	UAN 28%: 90N	UAN 28%: 90N	UAN 28%: 90N
7 DAH	UAN 28%: 123N	UAN 28%: 123N	UAN 28%: 123N
14 DAH	-	Urea: 52N	-
Season Total (N, P, K)	**(302, 112, 336)**	**(354, 112, 336)**	**(426, 255, 444)**

† **PPI**: broadcast and incorporated with field cultivator prior to planting main crop. **In-furrow**: band applied in furrow at planting. **Emergence**: surface band applied at emergence of potato plants. **Hill**: surface band applied before hilling. **7-10 DAH**: surface band applied 7 days after hilling. **14 DAH**: surface band applied 14 days after hilling.

§ NATCTRL, Russet Burbank potatoes planted after spring metam sodium fumigation and with mineral fertilizer only. NOFUM, Superior potatoes planted without fumigation and with mineral fertilizer only. GRSTAND, Superior potatoes planted after spring chloropicrin (CP) fumigation and with mineral fertilizer only. MANURE, Superior potatoes planted after spring CP fumigation and with combination mineral fertilizers and annual application of poultry litter. CCR, Snowden potatoes planted after spring CP fumigation and with mineral fertilizer only, winter rye cover crop planted after corn harvest and incorporated in the spring. MAN/CCR, Superior potatoes planted after spring CP fumigation and with combination mineral fertilizer and annual poultry litter, winter rye cover crop planted after corn harvest and incorporated in the spring.

Table 6. Mean Daily Temp [Days Max Temp >26°C]. Monthly temperature data from environwether.msu.edu observed at Clarksville, MI station and 30 yr avg compiled from NOAA data observed 1991-2020 at Hastings Station. Potato growing season months are in bold.

Year	**2019**	**2020**	**2021**	**2022**	**30 yr avg**
	-------------------------°C [# of days] ---------------------------				
April	7.6 [0]	5.9 [0]	8.4 [0]	5.8 [1]	7.4 [1]
May	12.8 [3]	13.2 [4]	12.9 [8]	**15.2 [9]**	13.9 [6]
June	**18.4 [10]**	**19.7 [17]**	20.8 [17]	**19.7 [13]**	19.3 [14]
July	**23.2 [28]**	**23.3 [31]**	21.4 [18]	**21.8 [19]**	21.4 [22]
August	**20.0 [21]**	**21.1 [19]**	22.5 [22]	**21.0 [18]**	20.4 [19]
September	**18.2 [11]**	**15.6 [2]**	17.7 [4]	17.2 [5]	16.5 [9]

Table 7. Days with Max Soil Temp >10°C based on Michigan Automated Weather Station data in Clarksville, MI.

Month	**2019**	**2020**	**2021**	**2022**
	-------------------# of days-------------------			
April	-	19	24	15
May	-	30	31	29
June	NA	30	30	30
July	31	31	30	31
August	30	31	30	30
September	30	NA	30	30
Date after which soil temperature remains >10°C	**NA**	**26-Apr**	**21-Apr**	**4-May**

Table 8. Total monthly precipitation (cm). Monthly precipitation data and 30 yr average from NOAA data observed 1991-2020 at Hastings, MI station. Potato growing season months are in bold.

Year	2019	2020	2021	2022	**30 yr avg**
	----------------------- cm ------------------------				
April	10.7	8.8	3.3	12.4	9.7
May	15.2	18.5	4.0	**14.6**	11.2
June	**16.4**	**14.2**	18.5	**3.1**	10.6
July	**8.3**	**7.2**	17.9	**7.6**	8.7
August	**7.1**	**5.8**	8.8	**17.8**	9.8
September	**18.6**	**7.6**	8.3	2.9	8.4
Total	90.3	71.5	75.4	66.1	68.5

Table 9. Potato yield along with the proportion of total yield graded as U.S. No 1 and the proportion of total yield graded as >6oz from the 2-Year (2YR) and 3-Year (3YR) Rotation sites.

Site		Year	GRSTAND§ SUP ¶	NOFUM SUP	MANURE SUP	MAN/CCR SUP	CCR SNO	NATCTRL RSB	Pr>F	Fum[a]
2YR	Total Yield Mg ha^{-1}	2020	19.0 b	6.9 a †	21.4 b	20.5 b	31.7 c	31.1 c	<0.0001	+13.3 ***
		2022	17.6 a	22.5 ab	24.2 bc	27.8 bc	29.9 c	24.8 bc	0.0116	ns
	US No 1 %	2020	87 b	89 b	90 b	85 b	97 c	73 a	<0.0001	ns
		2022	92 b	90 b	94 b	94 b	100 b	65 a	<0.0001	ns
	>6oz %	2020	18 ab	8 a	25 b	27 b	26 b	26 b	0.0058	+15 **
		2022	80 bc	78 b	84 c	81 bc	80 bc	38 a	<0.0001	ns
3YR	Total Yield Mg ha^{-1}	2019	17.8 ab	9.8 a	15.0 ab	22.0 b	23.4 b	22.5 b	0.024	+8.5 ***
		2022	26.7 a	28.4 a	37.7 b	30.2 a	32.2 ab	31.6 a	0.0178	ns
	US No 1 %	2019	32 a	50 b	32 a	31 a	39 a	73 c	<0.0001	-18 ***
		2022	93 b	95 b	94 b	93 b	98 b	74 a	<0.0001	ns
	>6oz %	2019	-	-	-	-	-	-	-	-
		2022	85 bc	85 bc	91 d	89 cd	84 b	60 a	<0.0001	ns

§ GRSTAND, 'Superior' potatoes planted after spring chloropicrin (CP) fumigation and with mineral fertilizer only. NOFUM, 'Superior' potatoes planted without fumigation and with mineral fertilizer only. MANURE, 'Superior' potatoes planted after spring CP fumigation and with combination mineral fertilizers and annual application of poultry litter. MAN/CCR, 'Superior' potatoes planted after spring CP fumigation and with combination mineral fertilizer and annual poultry litter, winter rye cover crop planted after corn harvest and incorporated in the spring. CCR, 'Snowden' potatoes planted after spring CP fumigation and with mineral fertilizer only, winter rye cover crop planted after corn harvest and incorporated in the spring. NATCTRL, 'Russet Burbank' potatoes planted after spring metam sodium fumigation and with mineral fertilizer only.

¶ SUP, 'Superior' variety. SNO 'Snowden' variety. RSB, 'Russet Burbank' variety.

† Values within a row followed by the same lowercase letter are not significantly different at α=0.05.

‡ ns, not significant at α=0.05

[a] Multiple degree of freedom contrast GRSTAND, MANURE & MAN/CCR vs NOFUM. Compares treatments that were fumigated prior to 2019/2020 planting to that which was planted without fumigation. Asterisks indicate thresholds of significance (ns, P > 0.05; *, P < 0.05; **, P < 0.01; ***, P < 0.001).

Table 10. Potato tuber quality and crop health. Tuber infection rates and specific gravity were determined post-harvest. Green cover was determined at vine kill in 2019 and 2020 and 4 weeks before harvest in 2022.

		Year	GRSTAND § SUP¶	NOFUM SUP	MANURE SUP	MAN/CCR SUP	CCR SNO	NATCTRL RSB	Pr>F	Fum [a]
2YR	***Verticillium* Infection** % tubers	2022	3 abc †	2 ab	7 bc	2 ab	14 c	1 a	0.0492	ns
	Vine Green Cover %	2020	14 ab	5 a	15 ab	18 b	6 a	32 c	0.0009	+11*
		2022	18 b	5 a	18 b	13 ab	30 c	33 c	0.0001	+10*
	Specific Gravity	2020	1.068 a	1.068 a	1.069 a	1.067 a	1.082 c	1.072 b	<0.0001	ns
		2022	1.061 ab	1.064 b	1.062 b	1.062 ab	1.074 c	1.059 a	0.0022	ns
	Scab Infection % tubers	2022	8 a	26 ab	14 a	7 a	52 b	30 ab	0.0082	-16*
3YR	***Verticillium* Infection** % tubers	2019	5	16	7	7	16	22	ns ‡	ns
		2022	44 b	7 a	48 b	8 a	38 b	3 a	<0.0001	+26*
	Vine Green Cover %	2019	15 a	8 a	13 a	13 a	32 b	42 c	<0.0001	ns
		2022	18 a	21 ab	29 bc	25 abc	33 c	33 c	<0.0001	ns
	Specific Gravity	2019	1.072 ab	1.071 a	1.075 b	1.074 ab	1.085 d	1.080 c	<0.0001	ns
		2022	1.061 a	1.063 ab	1.063 ab	1.062 a	1.076 c	1.066 b	<0.0001	ns
	Scab Infection % tubers	2022	54 b	6 a	57 b	12 a	58 b	12 a	0.0001	+35**

§ NOFUM, 'Superior' potatoes planted without fumigation and with mineral fertilizer only. GRSTAND, 'Superior' potatoes planted after spring chloropicrin (CP) fumigation and with mineral fertilizer only. MANURE, 'Superior' potatoes planted after spring CP fumigation and with combination mineral fertilizers and annual application of poultry litter. MAN/CCR, 'Superior' potatoes planted after spring CP fumigation and with combination mineral fertilizer and annual poultry litter, winter rye cover crop planted after corn harvest and incorporated in the spring. CCR, 'Snowden' potatoes planted after spring CP fumigation and with mineral fertilizer only, winter rye cover crop planted after corn harvest and incorporated in the spring. NATCTRL, 'Russet Burbank' potatoes planted after spring metam sodium fumigation and with mineral fertilizer only.

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† Values within a row followed by the same lowercase letter are not significantly different at α=0.05.

‡ ns, not significant at α=0.05

[a] Multiple degree of freedom contrast GRSTAND, MANURE & MAN/CCR vs NOFUM. Compares treatments that were fumigated prior to 2019/2020 planting to that which was planted without fumigation. Asterisks indicate thresholds of significance (ns, P > 0.05; *, P < 0.05; **, P < 0.01; ***, P < 0.001).

Table 11. Preplant nitrate (NO_3^-) and soil test phosphorus (Bray P1 Mehlich equivalent) concentrations and pH.

Site		Year	GRSTAND §	NOFUM	MANURE	MAN/CCR	CCR	NATCTRL	Pr>F	Fum [a]
2YR	N-NO_3^- mg kg^{-1}	2020	30.3 b	18.1 a	30.2 b	27.2 b	26.2 b	20.3 a †	<0.0001	+10.6***
		2022	7.2	6.6	6.6	5.4	5.5	6.6	ns	ns
	P-Mehlich mg kg^{-1}	2020	102	95	96	90	92	83	ns	ns
		2022	127	109	132	120	109	115	ns	ns
	pH	2020	6.4	6.7	6.5	6.6	6.5	6.7	ns	ns
		2022	6.5 a	6.6 a	6.8 bc	6.8 c	6.5 a	6.6 ab	0.0010	ns
3YR	N-NO_3^- mg kg^{-1}	2019	5.4	5.8	6.8	5.9	5.3	5.1	ns	ns
		2022	7.8	5.5	6.3	7.3	4.8	3.6	ns	ns
	P-Mehlich mg kg^{-1}	2019	135	151	154	142	117	149	ns	ns
		2022	217	221	209	251	194	223	ns	ns
	pH	2019	5.8	6	6.1	5.8	5.9	5.8	ns	ns
		2022	6	6.2	6.3	6.1	6.1	5.9	ns	ns

§ GRSTAND, 'Superior' potatoes planted after spring chloropicrin (CP) fumigation and with mineral fertilizer only. NOFUM, 'Superior' potatoes planted without fumigation and with mineral fertilizer only. MANURE, 'Superior' potatoes planted after spring CP fumigation and with combination mineral fertilizers and annual application of poultry litter. MAN/CCR, 'Superior' potatoes planted after spring CP fumigation and with combination mineral fertilizer and annual poultry litter, winter rye cover crop planted after corn harvest and incorporated in the spring. CCR, 'Snowden' potatoes planted after spring CP fumigation and with mineral fertilizer only, winter rye cover crop planted after corn harvest and incorporated in the spring. NATCTRL, 'Russet Burbank' potatoes planted after spring metam sodium fumigation and with mineral fertilizer only.

† Values within a row followed by the same lowercase letter are not significantly different at α=0.05.

‡ ns, not significant at α=0.05

[a] Multiple degree of freedom contrast GRSTAND, MANURE & MAN/CCR vs NOFUM. Compares treatments that were fumigated prior to 2020 planting to that which was planted without fumigation. Asterisks indicate thresholds of significance (ns, P > 0.05; *, P < 0.05; **, P < 0.01; ***, P < 0.001).

Table 12. Midseason soil nitrate (NO_3^-) and soil test phosphorus (Bray P1 Mehlich equivalent) concentrations and pH measured 60DAP potatoes.

Site		Year	GRSTAND §	NOFUM	MANURE	MAN/CCR	CCR	NATCTRL	Pr>F	Fum [a]
2YR	$N\text{-}NO_3^-$	2020	167 abc	223 c	184 bc	157 ab	114 a	113 a	0.0132	-53.6*
	mg kg^{-1}	2022	101 b	92 b	101 b	131 c	83 b	50 a	0.0003	ns
	P-Mehlich	2020	143	147	153	146	129	141	ns	ns
	mg kg^{-1}	2022	106 b	102 ab	132 c	152 d	95 ab	91 a	0.0002	+28**
	pH	2020	5.5	5.7	6	6	5.6	5.8	ns	ns
		2022	5.6 a	5.6 a	5.9 b	5.8 ab	5.9 ab	6.0 b	0.016	ns
3YR	$N\text{-}NO_3^-$	2019	40.8	45.1	38.9	28.4	32.5	34.8	ns	ns
	mg kg^{-1}	2022	72 b	60 b	73 b	62 b	38 a	32 a	0.0006	ns
	P-Mehlich	2019	207	217	225	235	186	221	ns	ns
	mg kg^{-1}	2022	169 a	187 ab	243 c	222 bc	155 a	181 ab	0.0054	ns
	pH	2019	6	5.7	5.9	6	5.6	5.5	ns	ns
		2022	5.6	5.6	5.9	5.6	5.7	5.6	ns	ns

§ NOFUM, 'Superior' potatoes planted without fumigation and with mineral fertilizer only. GRSTAND, 'Superior' potatoes planted after spring chloropicrin (CP) fumigation and with mineral fertilizer only. MANURE, 'Superior' potatoes planted after spring CP fumigation and with combination mineral fertilizers and annual application of poultry litter. MAN/CCR, 'Superior' potatoes planted after spring CP fumigation and with combination mineral fertilizer and annual poultry litter, winter rye cover crop planted after corn harvest and incorporated in the spring. CCR, 'Snowden' potatoes planted after spring CP fumigation and with mineral fertilizer only, winter rye cover crop planted after corn harvest and incorporated in the spring. NATCTRL, 'Russet Burbank' potatoes planted after spring metam sodium fumigation and with mineral fertilizer only.

† Values within a row followed by the same lowercase letter are not significantly different at α=0.05.

‡ ns, not significant at α=0.05

[a] Multiple degree of freedom contrast GRSTAND, MANURE & MAN/CCR vs NOFUM. Compares treatments that were fumigated prior to 2020 planting to that which was planted without fumigation. Asterisks indicate thresholds of significance (ns, P > 0.05; *, P < 0.05; **, P < 0.01; ***, P < 0.001).

Table 13. Preplant soil biological metrics. 24hr CO_2 respiration test measures the burst of CO_2 in a closed vessel 24 hours after rewetting air dried soils. POX-C measures mg permanganate oxidizable C per kg soil. ACE Protein measures the autoclaved-citrate extractable protein as a metric of available organic N in soil.

Site		Year	GRSTAND §	NOFUM	MANURE	MAN/CCR	CCR	NATCTRL	Pr>F	Fum [a]
2YR	**24hr CO_2 Respiration** ppm	2020	90 a	127 c †	113 bc	110 bc	96 ab	99 ab	0.0043	-22**
		2022	150	149	152	152	153	147	ns ‡	ns
	POX-C mg kg^{-1}	2020	464	455	441	441	428	421	ns	ns
		2022	69	117	106	98	65	88	ns	ns
	ACE protein mg g^{-1}	2020	3.4	3.3	3.3	3.2	3.2	3.0	ns	ns
		2022	4.5	4.2	4.3	4.1	4.2	4.0	ns	ns
3YR	**24hr CO_2 Respiration** ppm	2019	126	143	145	112	102	130	ns	ns
		2022	113	103	117	127	129	115	ns	ns
	POX-C mg kg^{-1}	2019	201	234	160	143	163	210	ns	ns
		2022	105	163	114	118	102	120	ns	ns
	ACE protein mg g^{-1}	2019	3.9	4.2	4	3.7	3.4	4.1	ns	ns
		2022	3.8	4	3.9	4	3.4	3.9	ns	ns

§ GRSTAND, 'Superior' potatoes planted after spring chloropicrin (CP) fumigation and with mineral fertilizer only. NOFUM, 'Superior' potatoes planted without fumigation and with mineral fertilizer only. MANURE, 'Superior' potatoes planted after spring CP fumigation and with combination mineral fertilizers and annual application of poultry litter. MAN/CCR, 'Superior' potatoes planted after spring CP fumigation and with combination mineral fertilizer and annual poultry litter, winter rye cover crop planted after corn harvest and incorporated in the spring. CCR, 'Snowden' potatoes planted after spring CP fumigation and with mineral fertilizer only, winter rye cover crop planted after corn harvest and incorporated in the spring. NATCTRL, 'Russet Burbank' potatoes planted after spring metam sodium fumigation and with mineral fertilizer only.

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‡ ns, not significant at α=0.05

[a] Multiple degree of freedom contrast GRSTAND, MANURE & MAN/CCR vs NOFUM. Compares treatments that were fumigated prior to 2020 planting to that which was planted without fumigation. Asterisks indicate thresholds of significance (ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

Table 14. 60 DAP soil biological metrics. 24hr CO_2 respiration test measures the burst of CO_2 in a closed vessel 24 hours after rewetting air dried soils. Autoclaved citrate extractable (ACE) protein measures the autoclaved-citrate extractable protein as a metric of available organic N in soil. Total microbial biomass and fungal:bacterial (F:B) ratio estimated by phospholipid fatty acid analysis.

Site		Year	GRSTAND §	NOFUM	MANURE	MAN/CCR	CCR	NATCTRL	Pr>F	Fum [a]
2YR	**24hr CO_2 Respiration**	2020	65 a †	70 ab	78 b	77 ab	66 a	81 b	0.0242	ns
	ppm	2022	86 a	101 ab	127 c	115 bc	97 ab	103 ab	0.0051	ns
	POX-C	2020	368 ab	406 b	354 a	351 a	336 a	365 a	0.0268	-54**
	mg kg^{-1}	2022	258	330	305	306	281	292	ns ‡	ns
	ACE protein	2020	4	3.6	3.9	3.7	3.8	3.6	ns	ns
	mg g^{-1}	2022	4.7	4.6	4.5	4.6	4.6	4.4	ns	ns
	Total Microbial Biomass ng g^{-1}	2022	2595	3104	2380	2905	2010	2717	ns	ns
	F:B	2022	0.17	0.19	0.18	0.21	0.18	0.15	ns	ns
3YR	**24hr CO_2 Respiration**	2019	155	147	152	167	133	134	ns	ns
	ppm	2022	99 a	112 a	140 b	110 a	112 a	105 a	0.0215	ns
	POX-C	2019	331	319	356	333	255	320	ns	ns
	mg kg^{-1}	2022	231	275	271	237	250	264	ns	ns
	ACE protein	2019	4	4.6	4.5	4.3	3.9	4.5	ns	ns
	mg g^{-1}	2022	4.5	4.9	4.5	4.6	4.3	4.3	ns	ns
	Total Microbial Biomass ng g^{-1}	2022	1628 a	1491 a	2119 b	1542 a	1511 a	1634 a†	0.0456	ns
	F:B	2022	0.04 a	0.08 ab	0.16 c	0.12 bc	0.07 ab	0.08 ab	0.0094	ns

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[a] Multiple degree of freedom contrast GRSTAND, MANURE & MAN/CCR vs NOFUM. Compares treatments that were fumigated prior to 2020 planting to that which was planted without fumigation. Asterisks indicate thresholds of significance (ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

Table 15. Shannon diversity (α-diversity) estimated from 16S (bacterial) and ITS (fungal) gene sequencing from 2020 and 2019 samples.

Site		Sample Time	GRSTAND§	NOFUM	MANURE	MAN/CCR	CCR	NATCTRL	Pr>F	Fum [a]
2YR	**Bacterial Shannon Diversity**	Preplant 2020	7.7 a †	8.0 b	7.6 a	7.5 a	7.7 ab	8.0 b	0.0061	-0.5**
		60DAP 2020	7.9 bc	8.1 c	7.8 ab	7.6 a	7.9 bc	8.1 c	0.0060	-0.3**
	Fungal Shannon Diversity	Preplant 2020	4.4 a	4.8 b	4.5 a	4.4 a	4.6 a	4.9 b†	0.0003	-0.4***
		60DAP 2020	4.4 abc	4.8 c	4.2 a	4.1 a	4.4 ab	4.6 bc	0.0117	-0.5**
3YR	**Bacterial Shannon Diversity**	Preplant 2019	7.8	8	7.9	8	7.9	7.8	ns	ns
		60DAP 2019	7.7 a	8.0 b	7.6 a	7.5 a	7.6 a	7.7 a	0.0131	-0.3***
	Fungal Shannon Diversity	Preplant 2019	4.9	5	4.8	4.8	4.9	4.7	ns	ns
		60DAP 2019	4.6	4.8	4.7	4.6	4.7	4.6	ns	ns

§ NOFUM, 'Superior' potatoes planted without fumigation and with mineral fertilizer only. GRSTAND, 'Superior' potatoes planted after spring chloropicrin (CP) fumigation and with mineral fertilizer only. MANURE, 'Superior' potatoes planted after spring CP fumigation and with combination mineral fertilizers and annual application of poultry litter. MAN/CCR, 'Superior' potatoes planted after spring CP fumigation and with combination mineral fertilizer and annual poultry litter, winter rye cover crop planted after corn harvest and incorporated in the spring. CCR, 'Snowden' potatoes planted after spring CP fumigation and with mineral fertilizer only, winter rye cover crop planted after corn harvest and incorporated in the spring. NATCTRL, 'Russet Burbank' potatoes planted after spring metam sodium fumigation and with mineral fertilizer only.

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‡ ns, not significant at α=0.05

[a] Multiple degree of freedom contrast GRSTAND, MANURE & MAN/CCR vs NOFUM. Compares treatments that were fumigated prior to 2020 planting to that which was planted without fumigation. Asterisks indicate thresholds of significance (ns, P > 0.05; *, P < 0.05; **, P < 0.01; ***, P < 0.001).

Table 16. Permutational analysis of variance results for Bray-Curtis difference based on 16S (bacteria) and ITS (fungi) amplicon sequencing data as affected by treatment and sample date based on 99,999 permutations. Differences in Bray-Curtis differences represent differences in community structure (β-diversity). Sequence data produced from soil DNA sampled in June and August 2020 for the 2-Year Rotation and June and August 2019 for the 3-Year rotation. Asterisks next to P-values indicate thresholds of significance (*, P < 0.05; **, P < 0.01; ***, P < 0.001).

Site		**Bacteria**		**Fungi**	
2YR	Sample Time	<0.0001	***	<0.0001	***
	Management	<0.0001	***	<0.0001	***
	Sample Time x Management	0.2563		0.3225	
3YR	Sample Time	<0.0001	***	<0.0001	***
	Management	0.1268		0.0207	**
	Sample Time x Management	0.7978		0.3196	

Figure 1. Ordination by principal coordinates analysis (PCoA) plots of amplicon sequence variant level soil bacteria, (A) and (B), and fungi, (C) and (D), illustrating clustering of community structure (β-diversity) by treatment. Soil DNA extracted from samples taken in 2019 (3 Year rotation) and 2020 (2 Year rotation). The principal coordinates analysis plot was built based on Bray–Curtis distances. The percentage of total variance explained by each axis of the first two defined axes is shown.

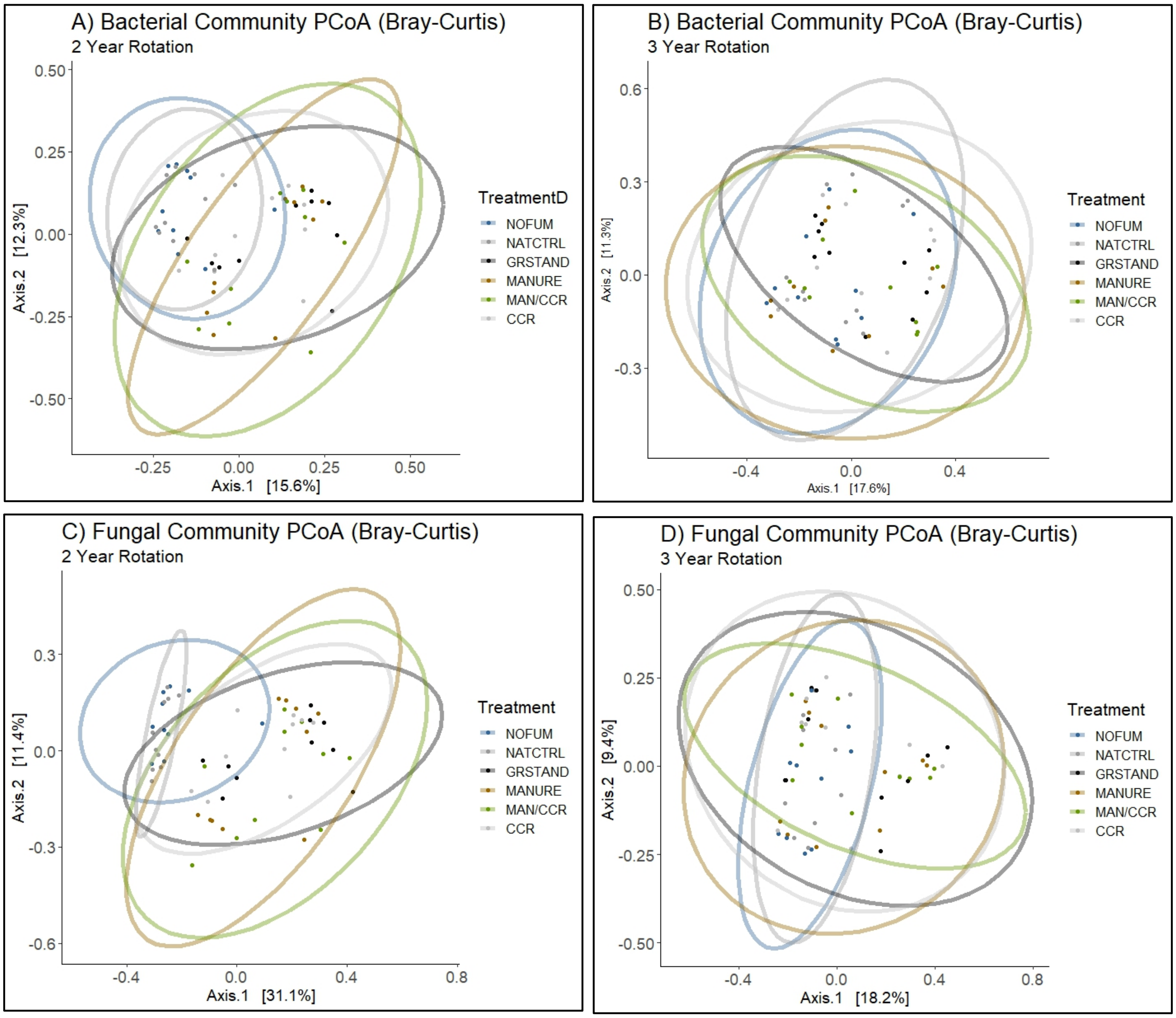

Table 17. Pairwise PERMANOVA p-values for comparison of bacterial (16S) and fungal (ITS) community structure (β-diversity) by treatment. Modeled with Bray-Curtis distances. Samples from 2020 preplant and 60DAP following fumigation in May. ns, not significantly different at α=0.05.

		NATCTRL	NOFUM	GRSTAND	MANURE	CCR	MAN/CCR
2YR	**16S**	NATCTRL	NOFUM	GRSTAND	MANURE	CCR	MAN/CCR
	NATCTRL§						
	NOFUM	ns ‡					
	GRSTAND	0.0001	0.0003				
	MANURE	0.0001	0.0001	ns			
	CCR	0.0003	0.0013	ns	ns		
	MAN/CCR	0.0001	0.0001	ns	ns	ns	
2YR	**ITS**	NATCTRL	NOFUM	GRSTAND	MANURE	CCR	MAN/CCR
	NATCTRL§						
	NOFUM	ns					
	GRSTAND	0.0001	0.0001				
	MANURE	0.0001	0.0002	ns			
	CCR	0.0002	0.0002	ns	ns		
	MAN/CCR	0.0001	0.0001	ns	ns	ns	
3YR	**16S**	NATCTRL	NOFUM	GRSTAND	MANURE	CCR	MAN/CCR
	NATCTRL§						
	NOFUM	ns ‡					
	GRSTAND	ns	ns				
	MANURE	ns	ns	ns			
	CCR	ns	ns	ns	ns		
	MAN/CCR	ns	ns	ns	ns	ns	
3YR	**ITS**	NATCTRL	NOFUM	GRSTAND	MANURE	CCR	MAN/CCR
	NATCTRL§						
	NOFUM	ns					
	GRSTAND	0.0382	0.0216				
	MANURE	ns	ns	ns			
	CCR	ns	ns	ns	ns		
	MAN/CCR	0.0200	0.0092	ns	ns	ns	

§ NOFUM, 'Superior' potatoes planted without fumigation and with mineral fertilizer only. GRSTAND, 'Superior' potatoes planted after spring chloropicrin (CP) fumigation and with mineral fertilizer only. MANURE, 'Superior' potatoes planted after spring CP fumigation and with combination mineral fertilizers and annual application of poultry litter. MAN/CCR, 'Superior' potatoes planted after spring CP fumigation and with combination mineral fertilizer and annual poultry litter, winter rye cover crop planted after corn harvest and incorporated in the spring. CCR, 'Snowden' potatoes planted after spring CP fumigation and with mineral fertilizer only, winter rye cover crop planted after corn harvest and incorporated in the spring. NATCTRL, 'Russet Burbank' potatoes planted after spring metam sodium fumigation and with mineral fertilizer only.
‡ ns, not significant at α=0.05

Table 18. *Verticillium dahliae* and *Pratylenchus penetrans* (root lesion nematode) populations from soil samples taken during potato years at planting and mid-season.

Site		Sample Time	GRSTAND §	NOFUM	MANURE	MAN/CCR	CCR	NATCTRL	Pr>F	Fum [a]
2YR	**V. dahliae Propagules** per gram soil	Preplant 2020	0	1	0	0	1	3	ns ‡	ns
		60DAP 2020	1	3	1	0	1	0	ns	ns
		Preplant 2022	20 b †	12 a	21 b	20 b	13 a	17 ab	0.0287	+8*
		60DAP 2022	1	12	12	8	15	16	ns	ns
	P. penetrans per 100 cc soil	Preplant 2020	50	74	79	47	79	68	ns	ns
		60DAP 2020	37	68	84	26	68	49	ns	ns
		Preplant 2022	999 b	670 a	772 a	682 a	610 a	580 a	0.0280	ns
		60DAP 2022	676c	389ab	531bc	342a	465ab	477ab	0.0150	ns
3YR	**V. dahliae Propagules** per gram soil	Preplant 2019	0	0	0	0	1	0	ns	ns
		60DAP 2019	-	-	-	-	-	-	-	-
		Preplant 2022	0 a	1 ab	1 abc	2 bc	3 c	2 bc	0.0374	ns
		60DAP 2022	0	4	1	0	0	1	ns	ns
	P. penetrans per 100 cc soil	Preplant 2019	68	76	51	59	83	72	ns	ns
		60DAP 2019	-	-	-	-	-	-	-	-
		Preplant 2022	54	101	99	139	54	99	ns	ns
		60DAP 2022	565	250	441	390	423	522	ns	ns

§ GRSTAND, 'Superior' potatoes planted after spring chloropicrin (CP) fumigation and with mineral fertilizer only. NOFUM, 'Superior' potatoes planted without fumigation and with mineral fertilizer only. MANURE, 'Superior' potatoes planted after spring CP fumigation and with combination mineral fertilizers and annual application of poultry litter. MAN/CCR, 'Superior' potatoes planted after spring CP fumigation and with combination mineral fertilizer and annual poultry litter, winter rye cover crop planted after corn harvest and incorporated in the spring. CCR, 'Snowden' potatoes planted after spring CP fumigation and with mineral fertilizer only, winter rye cover crop planted after corn harvest and incorporated in the spring. NATCTRL, 'Russet Burbank' potatoes planted after spring metam sodium fumigation and with mineral fertilizer only.

¶ *Pratylenchus penetrans* populations modeled with a normal distribution (Pr>F). *Verticillium dahliae* propagule count modeled with Poisson distribution and compared using nonparametric hypothesis testing (Pr> χ^2).

† Values within a row followed by the same lowercase letter are not significantly different at α=0.05. ‡ ns, not significant at α=0.05

[a] Multiple degree of freedom contrast GRSTAND, MANURE & MAN/CCR vs NOFUM. Compares treatments that were fumigated prior to 2019/2020 planting to that which was planted without fumigation. Asterisks indicate thresholds of significance (ns, P > 0.05; *, P < 0.05; **, P < 0.01; ***, P < 0.001).

Figure 2. Water stable macroaggregates, A, and microaggregates, B. Error bars represent one standard error, values within the same study followed by the same letter are not significantly different at α=0.05.