

DISSERTATION

IMPACTS OF CROPPING SYSTEM AND NUTRIENT MANAGEMENT ON SOIL HEALTH
AND SOIL-BORNE PATHOGENS IN SMALLHOLDER SYSTEMS OF WESTERN KENYA

Submitted by

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ABSTRACT

IMPACTS OF CROPPING SYSTEM AND NUTRIENT MANAGEMENT ON SOIL HEALTH AND SOIL-BORNE PATHOGENS IN SMALLHOLDER SYSTEMS OF WESTERN KENYA

Crop production in smallholder farms is often limited by low soil fertility and the presence of soil-borne pathogens. Both challenges are associated with limited nutrient inputs, low rotational diversity, as well as small land holdings and the associated need for continuous cultivation in many smallholder systems. This dissertation explores the varied ways in which cropping systems and nutrient management strategies influence key soil health parameters and relationships with key soil-borne pathogens. Additionally, this research tests a suite of soil health bioassays to facilitate farmers' understanding of soil-borne pathogen status on their farms. I utilized a mix of observational research, short-term on-farm experiments, and long-term cropping system trials to understand: 1) the potential of simplified soil pathogen tests (for *Fusarium*, *Pythium*, and plant parasitic nematodes (PPN)) to provide insight on soil pathogen pressure, 2) the impact of distinct nutrient management strategies (organic vs. synthetic inputs) on key soil health parameters and associated soil-borne pathogens, and 3) effects of cropping system (mono-cropping vs. more complex systems) on key soil health parameters and soilborne pathogens.

To address these objectives, I first validated a suite of simplified soil bioassays to screen for PPN (e.g., *Meloidogyne*, *Pratylenchus*) and other key soilborne pathogens (*Pythium* and *Fusarium*) against formal laboratory methods. I collected soils across eleven on-farm trials in western Kenya (66 plots total), examining the impact organic vs. synthetic nutrient inputs on bean production. The soil nematode bioassays involved counting lesions on soybean roots and galls on lettuce roots and were strongly correlated with the abundance of gall forming, root-knot

nematodes (*Meloidogyne*) and root lesion nematodes (*Pratylenchus*) recovered in laboratory-based extractions. Effectiveness of a *Fusarium* bioassay, involving the counting of lesions on buried soybean stem, was validated via DNA sequencing to identify *Fusarium* taxa and a pathogenicity test of cultured *Fusarium* strains. Finally, a *Pythium* soil bioassay using selective media clearly showed presence of the pathogen, with seed rotting and colonies observed. When examining nutrient management impacts on nematode communities, soils amended with manure had fewer PPN and considerably more bacterivores and fungivores compared to soils amended with synthetic N and P. Similarly, *Pythium* presence was lower in soils amended with manure, and higher levels of *Fusarium* in the same plots, likely due to the ability of various *Fusarium* taxa to exist as a saprophyte. Our findings suggested that relatively simple bioassays can be used to help farmers assess soilborne pathogens with minimal costs, thus enabling them to make informed decisions on soil health and pathogen management.

In a second study, I used an exploratory approach to examine common cropping systems in western Kenya smallholders including: maize monocultures, maize-legume intercrops, maize in rotation with legumes and vegetables, and horticultural systems based on perennial crops and vegetable production. I sampled 35 farms to understand the impact of cropping system diversity and associated nutrient management on the abundance of *Fusarium* pathogens and LN. I found that organic inputs led to fewer lesion-causing nematodes compared to the inorganic inputs system, but an inverse relationship with *Fusarium* pressure was observed. Permanganate oxidizable C (POXC), particular organic matter (POM), total C, and soil pH were highly correlated with each other and negatively associated with LN pressure, while POM was positively correlated with *Fusarium* pressure.

In a third study, I leveraged a long-term (18-year) field trial in western Kenya, testing cropping

systems representative of smallholder farms. The long-term trial evaluates three cropping systems: 1) continuous maize monocrop, 2) maize in rotation with the woody legume, Tephrosia (*T. candida*), and 3) maize intercropping with soybean, and two nutrient management strategies: 1) application of farmyard manure (vs. not), and retention or removal plant residue, with all plots receiving regular fertilizer inputs. I sampled soil from 40 plots and measured soil physical (texture, POM), aggregate stability, bulk density), chemical (pH, total C, available P, POXC), and biological (*Fusarium*, *Pythium*, RKN, LN) properties. Results indicated that long-term manure significantly improved soil properties including pH, POXC, POM, total C, and soil aggregation. Moreover, manure significantly reduced *Pythium* and RKN pressure. Soil pH and POXC were associated with *Pythium* and RKN, such that plots with low pH and POXC levels had high abundance of these soilborne pathogens. *Fusarium* abundance on the other hand, was higher with manure and associated variables (aggregation, POXC, total C).

In a fourth study, I utilized a long-term trial (45 years) in Kabete, central Kenya focused on integrated soil fertility management in continuous maize-bean rotation and the resulting impacts on soil characteristics was well-suited to this goal. I examined the effects of dry manure application, maize stover management (incorporated vs. removed), and synthetic fertilizers (N and P applied vs. no application) in a full-factorial experiment on a range of soil physical, chemical, and biological properties. Results indicated that application of organic inputs, especially manure, greatly improved soil organic matter (SOM) pools, soil pH, aggregate stability, and decreased bulk density, compared to synthetic fertilizers. At the same time, manure significantly reduced *Pythium* and LN pressure, while plant residues reduced RKN and *Pythium* considerably.

In summary, the simplified soil pathogen bioassays and soil health analyses considered in this

dissertation offer a powerful set of tools to help smallholder farmers and the local research or extension organizations that they work with to monitor and anticipate soil related challenges in their fields, thus supporting agricultural livelihoods and resilience. Additionally, these findings suggest that continuous mining of nutrients and minimal returns of organic matter (i.e. removal of crop residues and no manure application) appears to drive the decline of important soil health properties (pH, POXC, POM, aggregation, and total C), with important implications for soil-borne pathogens.

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DEDICATION

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CHAPTER 1: INTRODUCTION

Crop damage by soil-borne pathogens and plant parasitic nematodes (PPN) is an escalating threat to agricultural production (Kimenju et al., 2009; Jones et al., 2013). Diseases caused by soil-borne pathogens such as *Pythium*, *Fusarium*, and important PPN (e.g. *Pratylenchus* and *Meloidogyne*) are common in sub-Saharan Africa (SSA), reducing performance of a wide variety of food crops contributing to poverty, food and nutritional insecurity. In SSA, climatic conditions such warm temperatures and humid conditions are favorable to growth and development of many soil-borne pathogens, often leading to production of multiple pathogen generations per growing season (Nicol et al., 2011; Luc et al., 2005; Coyne et al., 2018). Additionally, there are abundant weed hosts for many nematode species in the tropical regions that safeguard pathogen survival even in absence of host crops (Luc et al., 2005).

Most tropical soils have lower organic matter and nutrient levels due to continuous cultivation of crops, minimal crop diversity, and limited nutrient inputs (and associated nutrient mining), among other factors. This situation creates conducive environments for soil-borne pathogens to thrive. As such, crop production losses attributable to PPN are estimated at 14.6% in the tropical and sub-tropical climates compared with just 8.8% in developed countries (Nicol et al., 2011), while *Fusarium* and *Pythium* are estimated to cause 50 -70% of crop damage in SSA (Maina et al., 2015; Papias et al., 2016; Henry et al., 2019). This could be an underestimate since most smallholder farmers in developing nations are not aware of the existence, symptoms and/or relative damage caused by soil-borne pathogens (Jones et al., 2013; Ngoya et al., 2023). Moreover, aboveground symptoms of some soil-borne pathogens such as nematodes can present signs typical to that of drought and nutrient deficiencies (Nicol et al., 2011), which can be confusing to farmers. Additionally, even though some farmers might be aware of farm condition,

farmer to farmer learning or value of blanket recommendation is limited by the complexity and variability in management practices that create individual contexts (Giller et al., 2011). This situation is common in western Kenya, where complex biophysical circumstances indicate a strong need for context- based soil testing and evaluation of agricultural challenges (Nyamasoka-Magonziwa et al., 2020).

In western Kenya, formal laboratory analyses are often out of reach for smallholder farmers due to lack of local laboratories and high costs of transport and analyses. To better understand soil health status, there is need for developing accessible soil health testing bioassays, and viable cropping and nutrient input systems that can promote soil health and reduce damage by soil-borne pathogens.

While understanding soil-borne pathogen status using simple methods can help farmers predict future disease incidence and severity, there are uncertainties regarding nutrient inputs and cropping system options that would fit in various contexts. Integrated soil fertility management has been recommended to aid in management of the declining soil fertility and increasing soil-borne pathogens in SSA, especially use of both organic and inorganic nutrients to enhance soil physical and chemical environment, improve ecosystem nutrient cycling, and help regenerate and sustain soil health (Kamaa et al., 2011; Panwar et al., 2020). Other studies have established that changes in soil characteristics important for growth and existence of most soil biota (pH, P, N, SOM, and soil aggregation) could be induced by use of both manure and chemical fertilizer (Van Bruggen and Termorshuizen, 2003; Briar et al., 2016; Wei et al., 2017; Davey et al., 2021). While these findings are factual, mineral fertilizers use in SSA smallholder farming systems remain low due to high costs and poor accessibility (Chianu et al., 2011; Kamaa et al., 2011), and many farmers tend to rely on the accessible farmyard manure and plant residues.

Manure is widely accepted as the major contributing factor to increasing soil pH, improving soil organic matter, soil structure, and microbial activities, among other benefits (Leon et al., 2006; Kamaa et al., 2011; Noble, 2011; Bationo et al., 2013; Sun et al., 2015; Straathof et al., 2017; Hu et al., 2017). However, past studies have come up with conflicting results on the impact of manure on various soil properties. The varying results may arise from differences in the rate, frequency, or timing of application, as well as edaphic properties such as initial soil pH, although evidence still points that manure is considerably beneficial for soil health and crop performance (Rayne and Aula, 2020). Additionally, use of other organic soil amendments such as composts, cover crops, and green manure are known to improve soil physical and chemical properties and stimulate the activities of microorganisms that are antagonistic to soil-borne pathogens (Riegel and Noe, 2000; Widmer et al., 2002; Liu et al., 2021; Karuri, 2022). Recycling plant residues to soils has also been proven to supplement soil nutrients by increasing soil organic matter and enhancing soil microbial activities. However, application of plant residues in the smallholder systems result in inconsistent soil impacts due to the difference in quality and rates of application (Ayuke et al., 2011). In terms of cropping systems, rotation and intercropping of different types of crops is highly recommended to limit multiplication of soil-borne pests and diseases and reduce the reliance on specific nutrients. Practicing multiple cropping and mixed crop species have important effect on the population and diversity of nematode-trapping fungi, thus could enhance reduction and management of PPN (Sánchez-Moreno and Ferris, 2007). This dissertation seeks to better understand the relationship between soil characteristics and soil-borne pathogens in farms under various cropping and nutrient management systems. I focus on smallholder systems and long-term trials incorporating organic amendments vs. synthetic fertilizers, and multiple cropping vs. simple mono-cropping systems in western Kenya. I tested a

suite of simple soil bioassays for assessing soil-borne pathogens, comparable to standard lab analyses. I then utilize long-term trials to investigate long-term impacts of applying management practices common to the smallholder systems on soil characteristics and link these impacts to incidence of soil-borne pathogens.

This dissertation research study begins in the second chapter, assessing a suite of simplified bioassays to screen for root-knot and lesion causing nematodes and other key soil-borne pathogens (Pythium and Fusarium). The objectives of this second chapter were to: 1) validate the use of simplified soil pathogen tests (for Fusarium, Pythium, and PPN) against standard lab analyses, and 2) assess the impact of distinct nutrient inputs (organic vs. synthetic) on key soil health parameters including soil-borne disease and nematode prevalence. Simple bioassays can be used to help farmers assess soil health in a timely manner, with lower costs, thus enabling them to make informed decisions on management.

Therefore, the third chapter of this dissertation involves understanding the impact of various environmental drivers, cropping systems, and nutrient inputs practiced by the western Kenya smallholder farmers. I hypothesize that farms relying on organic nutrient inputs such as manure and compost have reduced Fusarium and PPN pressure, and experience improved soil health parameters relative to farms relying mainly on mineral fertilizers. I also postulated that diversified cropping systems experience lower pest pressure than mono-cropping systems. Results from the study illustrated the complex ways that location and management factors interact, and the need for targeted research to help disentangle the relative importance of specific factors as key drivers of soil-borne pathogens. For example, there were spatial variation in soil characteristics and climatic conditions, varied periods of time that management practices had been in place, and diverse levels of understanding and appreciation of management practices in

place. Therefore, utilizing long-term experiments to substantiate the links between management practices, key soil health metrics, and soil-borne pests and diseases, without the confounding factors of management and biophysical differences was needed.

As such, in the fourth and fifth chapters I utilize long-term researcher managed trials with known management histories typical to those of smallholder farmers to understand and quantify the impact of management practices on soil characteristics and development of soil-borne pathogens. The fourth chapter focused on the long-term impact of three cropping systems (continuous maize monocrop, maize in rotation with *Tephrosia candida*, and maize intercropping with soybean) and two nutrient management strategies (application of farmyard manure and retention or removal plant residue). Undoubtedly, use of manure considerably improved soil health and reduced soil-borne pathogens. Similarly, multiple cropping systems generally enhanced soil organic matter and enhanced soil pH, compared to monocrop systems. Then, in chapter five, I focused on comparing the 45-year impact of using organic vs. inorganic nutrient inputs, or a combination in a continuous maize-bean rotation system. Results from this chapter suggests that application of organic inputs such as manure alone, or a combination of manure and plant residues, greatly improved soil organic matter pools, soil pH, and aggregate stability, compared to synthetic fertilizers alone, or synthetic fertilizers in combination with organic amendments.

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CHAPTER 2: VALIDATION OF SIMPLIFIED BIOASSAYS FOR SOIL-BORNE

PATHOGENS IN SMALLHOLDER SYSTEMS OF WESTERN KENYA

2.1 Introduction

Soil-borne pathogens, including plant pathogenic fungi and plant parasitic nematodes (PPN), are important crop pests around the globe, damaging crops and reducing yields (George et al., 2016; Jimenez-Hernandez et al., 2021). Soil-borne pathogen infections often lead to stunting, yellowing, reduced quality and quantity of produce, and in some cases complete crop mortality (Moens et al., 2009; Jimenez-Hernandez et al., 2021). This problem is of particular concern in sub-Saharan Africa (SSA), where pathogens such as *Fusarium*, *Pythium*, root-knot nematodes (*Meloidogyne* spp.) and lesion nematodes (*Pratylenchus* spp.) pose a great threat to crop production due to continuous cultivation with minimal crop rotation and diversity, degraded soils, and limited access to pesticides, among other factors (Nicol et al., 2011).

Throughout much of East Africa, *Meloidogyne* and *Pratylenchus* species are major pests that can cause up to 50% yield decline in some fields (Kimenju et al., 2008; Chirchir et al., 2010; Atandi et al., 2017; Maina et al., 2019). Damage by these nematodes can be especially severe when conditions favor their multiple generations per growing season (i.e., low crop diversity, multiple cropping seasons per year, favorable soil characteristics; Luc et al., 2005; Sikora et al., 2018; Coyne et al., 2018). In fact, *Pratylenchus* and *Meloidogyne* generation times can be as short as 3 weeks, with both groups known to have high reproduction rates and tolerance to a wide temperature range (Jones et al., 2013; Maina et al., 2019). While *Pratylenchus* and *Meloidogyne* are among the most important and widespread pests, especially in the tropics, detailed

information on their distribution, severity and economic impact remains limited.

Similar to *Pratylenchus* and *Meloidogyne*, *Fusarium* and *Pythium* are also problematic soil pathogens across SSA, and have been reported to be abundant in agricultural soils, causing significant crop damage (Maina et al., 2015; Papias et al., 2016; Henry et al., 2019). Past research has indicated that *Fusarium* and *Pythium* abundance in agricultural soils is associated with the presence of infected plant debris and roots, use of infested seed, and soil tillage/disturbance, which leads to hyphal fragmentation, propagule dispersal and facilitates pathogen access to nutrients and oxygen (Silvestro et al., 2013; Maina et al., 2015). Continuous cultivation of soils on farms with limited land resources, as well as insufficient nutrient inputs and residue recycling have led to soil organic matter and nutrient decline, poor soil structure and decreased water holding capacity, which contribute to plant stress and susceptibility to various pests and diseases. Pathogens, such as *Fusarium*, commonly attack plants under stress, whether caused by abiotic (e.g., nutrient deficiency, alternating wetting and drying of soil, extreme temperatures, and waterlogged soils) or biotic (e.g., primary damage from other pests) factors. *Fusarium* species also create a challenge due to their widespread geographic distribution, efficient dispersal mechanisms, ability to grow in diverse substrates, and survival in soil for up to 10 years without a host (Jimenez-Hernandez et al., 2021; Pérez-Hernández et al., 2017). Meanwhile in western Kenya and much of SSA, many soils are characterized by low soil pH (Kisinyo et al., 2014), a property that can influence *Pythium* development, since acidic conditions favor formation of their oospores and sporangia, and also decrease plant nutrient availability leading to low plant vigor and greater vulnerability to pathogen infestation (Papias et al., 2016).

While numerous studies have clearly identified symptoms caused by soil-borne pathogens and

nutrient deficiencies, most smallholder farmers rarely associate these symptoms with soil-related problems (Ngoya et al., 2023), due to limited knowledge on existing soil-borne pathogens and the similarity of pathogen and nutrient stress symptoms. Nematode infections usually lead to unspecific aboveground disease symptoms akin to indicators of plant physiological stress (George et al., 2016). Additionally, when PPN damage is followed by fungal, viral or bacterial plant diseases such as *Fusarium*, *Pythium*, or *Rhizoctonia* that cause secondary infections, via nematode-inflicted wounds, disease symptoms can be more adverse and confusing (Al-Hazmi & Al-Nadary, 2015).

Researchers and extension agents often recommend use of synthetic fertilizers and organic soil amendments such as biochar, compost, manure, and plant residues to enhance soil fertility and reduce the incidence and severity of soil-borne pathogens (Roskopf et al., 2020; Hartmann et al., 2015; Hou et al., 2022). However, in our research area of western Kenya and many other smallholders farm contexts, soil and environmental conditions are highly variable so that nutrient and organic amendment additions might be expected to have varying impacts (Tittonell et al., 2005). Site specific soil testing is therefore highly relevant in these situations (Mallory et al., 2022; Nyamasoka-Magonziwa et al., 2020) to best understand soil health status and make context-based decisions on how best to improve agroecosystem productivity.

In western Kenya, common nutrient inputs include farmyard manure and synthetic fertilizers (both typically applied at well below the recommended rates) as well as plant residues and compost, if available. Plant residues are commonly used as a forage source, which implies recycling to fields as manure, with varying levels of efficiency (Castellanos-Navarrete et al., 2015), or burning of residues which represents a serious loss of soil C additions and N on some farms (Nyamasoka-Magonziwa et al., 2021). These practices can have implications for disease

and nematode pressure in these farms. Soil testing, including tests assessing pathogen prevalence in fields, can help motivate changes in nutrient inputs, removal or burning of plant residues after harvest, and improved efficiency of manure management, so that soil disease problems are not exacerbated. Soil testing might also influence crop/variety selection, as well crop rotational patterns. Therefore, accessible tools for learning about soil health, including the connections between soil health status and management practices, offer great promise for smallholder farmers and the organizations that they engage with and could greatly facilitate improved soil management decisions.

In order to help farmers better predict and manage potential soil pathogen issues in their fields, the research outlined here sought to: 1) assess the performance of previously developed, simplified soil pathogen tests (for *Fusarium*, *Pythium*, *Pratylenchus* and *Meloidogyne*) by comparing them to standard laboratory analyses, and 2) assess the impact of distinct nutrient inputs (organic vs. synthetic) on key soil health parameters, including soil-borne disease and nematode prevalence using the simplified methods. We hypothesized that the simplified soil pathogen tests would provide a reliable approximation of established laboratory methods and help provide farmers with more accessible methods for evaluating presence of major soil-borne pathogens. Additionally, we hypothesized that organic nutrient inputs would enhance soil health by increasing organic matter and soil pH, both of which in turn would suppress the major soil-borne pathogens.

2.2 Materials and Methods

2.2.1 Study site and experimental design

This research was conducted across three locations in Nandi County in western Kenya: Kapkerer

(1400-1700 m), Kapsengere (1400-1600 m), and Koibem (1700-1900 m). The locations experience mean annual temperatures of 21°C, 23°C, and 17°C, respectively, and two rainy seasons - a long-rains season occurring from February to July and a short-rains season occurring between September and November. Mean annual rainfall for Kapkerer, Kapsengere, and Koibem ranges between 1000-1800 mm, 1000-1700 mm, and 1500-2000 mm, respectively. Soils in the region are dominated by highly weathered Nitisols and Oxisols and are generally characterized by low soil pH (Jaetzold et al., 2007). Koibem is located close to Nandi Forest (0.15°N, 34.97° E), within more mountainous terrain compared to Kapkerer (0.02°N, 34.78° E) and Kapsengere (-0.01 °N, 34.75 °E), where the topography is less rugged. Farmers in all three locations typically cultivate small plots of land (0.25-5 ha), with maize and beans being the most common crops.

This research was conducted within a field experiment that was established across eleven farms in the three locations in western Kenya in March 2021 (during the long rains season) to evaluate the impact of different soil fertility amendments on soil health and the productivity of common bean (*Phaseolus vulgaris*). The experiment considered different types of organic matter input such as crop residues, farmyard manure (FYM), and biochar, as well as synthetic fertilizers, and a non-amended control. Treatments were established within 3 x 3 m plots, with each treatment present in two replicate blocks per farm, in a randomized complete block design. For this study, only a sub-set of the treatments were considered: 1) FYM applied at a rate of 5.6 Mg ha⁻¹ per season; 2) synthetic fertilizer, 36 kg N ha⁻¹ and 92 kg P₂O₅ ha⁻¹ per season applied as di-ammonium phosphate (DAP); and 3) a control treatment, with no amendment applied.

At planting, all plots were lightly tilled by hand using a hoe, and then beans (variety KK red 16) were row-planted, following the recommended spacing of 50 cm between rows and 10 cm within rows. FYM and DAP were also applied in furrows, ensuring manure and fertilizer *were* not in

direct contact with bean seeds. Weeding was done twice using a hoe, at 21 and 46 days after planting. No additional pest control practices or irrigation were applied.

2.2.2 Soil sampling and analysis

Soil samples were collected during the long rains season, in late May of 2022, shortly before bean flowering. In each plot, 10 sub-samples were taken to a depth of 15 cm using a soil auger (4 cm diameter) and mixed to form one composite sample per plot (3-5 kg field moist soil). Additional samples were collected for bulk density and aggregate stability by inserting a sharpened metal cylinder (7 cm diameter) to a depth of 5 cm at two representative points in each plot. The cylinder was inserted vertically into the soil by hand and excavated carefully. Samples from the cylinders were transported to the lab in sealed plastic bags placed within protective containers in a cooler. Upon return to the lab, the composite samples were subdivided for assessment of soil-borne pathogens and a range of physical and chemical soil properties. For the nematode assessment assays, 1 kg sub-samples per plot were taken from the composite sample, placed in sealed plastic bags and stored at 4°C until processing (within 2 days of sampling).

Soil used for assessment of *Pythium* and *Fusarium*, as well as soil physico-chemical analyses were air-dried, and passed through 2 mm sieve, and analyzed using low-cost, rapid assessment methods described by Nyamasoka-Magonziwa et al. (2020). Soil pH was measured in a 2:1 deionized water:soil suspension, evaluation of permanganate oxidizable C (POXC) was based on the oxidation of labile soil organic C by potassium permanganate (KMnO₄), available P was determined using a modified Olsen method, while particulate organic matter (POM) was determined using density flotation with deionized water (Nyamasoka-Magonziwa et al. 2020; also see <https://smallholder-sha.org>). Additionally, sub-samples were sent to a commercial laboratory in Nairobi for analysis of total soil C and soil texture, using dry combustion and

particle size analysis by hydrometer method, respectively.

The bulk density samples were weighed, and a representative sub-sample of ca. 40 g was dried at 105°C for determination of moisture content as well as calculation of oven-dry soil mass and bulk density. The remaining field moist soil was carefully passed through an 8-mm sieve and air-dried. A 70 g sub-sample of 8-mm sieved soil was used for evaluation of aggregate stability via a wet sieving method adapted from Elliott (1986). The sample was submerged for 5 minutes, for slaking, and then sieved through a 2 mm and then a 250 µm sieve by carefully lifting the sieve in and out of a pan of water, for a total of 50 oscillations over a 2-minute period. The soil remaining on each sieve was collected, dried at 105°C, and weighed to generate three aggregate size classes (>2000 µm, 250–2,000 µm, < 250 µm). Aggregate stability was calculated as the mean weight diameter (MWD), considered as the fraction of soil mass present in an aggregate size class multiplied by the mean diameter of aggregates in each size class.

2.2.3 Root lesion nematode bioassays

To evaluate the disease pressure from root lesion nematodes (*Pratylenchus* spp.) in each soil sample, we used a Cornell-developed soil bioassay modified from Gugino et al. (2006; 2009), using soybean plants as a host. Two sub-samples (lab replicates) of refrigerated soil from each plot (ca. 500 g each) were placed in separate pots (8 cm diameter, 12 cm high, with drainage holes at the bottom). Three soybean seeds were then planted in each pot, and the pots were maintained in the greenhouse and watered as needed. Soybean seeds were also planted in ca. 500 g of sterile soilless media as a control. The soybean root systems were carefully removed 3 weeks after germination and washed free of soil. Roots were visually examined for diagnostic root lesions caused by *Pratylenchus* spp., by counting and recording the total number of elongated dark brown lesions observed on the entire root system of the three plants in each pot.

The cleaned soybean roots were also weighed to evaluate fresh biomass and kept cool until subsequent extraction of nematodes. This measurement was later used to express nematode pressure on per gram of root basis.

2.2.4 Root-knot nematode bioassays

Similar to lesion nematode assessment, we evaluated the performance of a Cornell-developed root knot nematode bioassay developed by Gugino et al. (2008) using lettuce as a host plant for assessing *Meloidogyne* soil infestation. Two sub-samples (lab replicates) of refrigerated soil from each plot (ca. 500 g each) were placed in separate pots (8 cm diameter, 12 cm high, with drainage holes at the bottom). Three lettuce seeds were planted in each pot, and the pots were maintained in the greenhouse. Weeds that germinated in the pots were removed as soon as they emerged, and watering done as needed to avoid wilting. Three weeks post germination, plants were removed from the pots and roots washed free of soil prior to examination for root-galling from root-knot nematodes (RKN). The number of root galls on each of the three plants was recorded. The fresh weight of lettuce roots was also recorded. Roots were kept in plastic bags and refrigerated until extraction and assessment of endo-parasitic nematodes.

Although the number of lesions and number of root galls were counted in all the soybean and lettuce roots, respectively, the assumption was that severely damaged roots could have low count of nematode symptoms when roots were partially decomposed, making *Pratylenchus* and *Meloidogyne* damage less detectable. In addition, there was a need to normalize the *Pratylenchus* and *Meloidogyne* damage to the root biomass because stunted plants due to these plant parasites would have lower total number of lesions or galls. Therefore, the number of lesions and galls assessed in the bioassay were examined on a per gram of root basis.

2.2.5 Laboratory extraction of nematodes from plant roots and soil

To verify the interpretation of data collected from the bioassays described above (i.e., lesion or gall counts), which were developed by Cornell nematologists as a way of engaging growers, we evaluated nematodes in the roots of soybean and lettuce, and all nematodes (including the free-living taxa) in the soil. Within two days of root harvest, all roots from each pot were macerated in a domestic blender (at about 12000-RPM for 30 s), or chopped with a knife (when roots were too small to use a blender), for extraction of PPN using a modified Hemming Tray method (Bell & Watson, 2001). Bowls were placed on a flat surface and mesh (lined with paper towel filters) was suspended slightly above the bottom of the bowls. The chopped plant root material from each pot was placed in a clean mesh, and partially submerged in water, so that the mesh was in contact with the water. The mesh was covered with a lid and after 48 hours, live nematodes were presumed to have left the plant tissue, passed through the mesh, and then sunk to the bottom of the bowl. Nematodes were transferred into a beaker, left to settle, then passed through a 38- μ m sieve. The sieve was carefully rinsed using a spray bottle to ensure all nematodes were captured and then poured into 10 ml vials. Nematodes were then preserved in formalin prior to examination and counting on a slide under a dissecting microscope (10 to 40x magnification power). Specimens were transferred using a handling needle to a microscope slide as needed, for inspection at higher magnification with a compound microscope. All nematodes presented were identified to genus level via examination of morphology.

Nematode assessment of soils similarly relied on a modified Hemming Tray extraction method. Deep plates were placed on a flat surface, and then a colander was placed on top of each. Wet filter papers were carefully placed on the sieves ensuring full coverage of the sieves and no wrinkles or air-bubbles. Roughly 200 g of fresh soil from the pots used in the lesion nematode

bioassay (with soybean) was placed in each sieve and spread evenly before covering with a lid and placing them on plates containing ca. 200 ml tap water. A spray bottle was used to slowly add water to ensure adequate contact between the sieve and the plate, while being careful not to totally submerge soil. After 48 hours, nematodes that had migrated through the soil down to the plates were rinsed into a beaker, the screen and plate were thoroughly rinsed with a spray bottle for collection. Nematodes were allowed to settle out to the bottom of the beaker for ca. 3 hours, then each sample was passed through a 38- μm sieve and nematodes on the sieve were rinsed into 10 ml vials. Nematodes were then preserved using formalin to ensure intact specimens for identification, as described above.

2.2.6 *Pythium* assessment

A *Pythium* assessment assay was adapted from a commonly recommended procedure outlined by Ali-Shtayeh (1986). Plastic containers were filled with 125 g of soil and brought to 75% water holding capacity. Ten bean seeds were buried in the moistened soil, the container lid was sealed with parafilm, and the unit was incubated in the dark at 21 °C for 3 days. Seeds were then removed from the soil, and the number of rotten seeds and the rate of rotting were recorded. The incubated seeds were then thoroughly rinsed with deionized water, blotted dry, placed on a *Pythium*-selective medium and incubated for three more days at 21 °C. We used a standard *Pythium* selective medium PARP (pimaricin + ampicillin + rifampicin + pentachloronitrobenzene [PCNB] agar) developed by Jeffers and Martin (1986). Following incubation, seeds were evaluated for the presence/absence of mycelia growing into the media from individual seeds, and the number of observed colonies noted. Soil known to contain *Pythium* inoculum was used as the positive control, and autoclaved sand was used as a negative control. A standard compound microscope was used to examine the colonies to verify that it was

Pythium, by checking for sporangia, antheridia, oogonia, and zoospores. The efficacy of the medium, visual observation of seed rotting and the mold-colonies growing from individual seeds, and lab-microscope positive identification of *Pythium* provide high confidence for the assay, thus avoiding the need for further validation.

2.2.7 *Fusarium* relative severity assessment

Fusarium baiting assays were adapted from Furuya et al. (1999). Bean seeds were planted in a soilless medium (Cornell peat mix) and grown in the dark for 14 days at room temperature, with the aim of obtaining long white stems (ca. 25 cm) that can easily show signs of *Fusarium* infestation (Fig. 1a). Bean stems were then harvested and cut into 6 cm long segments (Fig. 2.1b). Two sub-samples (lab replicates) of the air-dried, 2-mm sieved soils (500 g per sub-sample) from each farm were placed in plastic containers with lids (11 cm diameter, 12 cm high) and water content was adjusted to 50% water holding capacity. Ten bean stem segments were then evenly spread, buried in each container, and incubated at room temperature (ca. 25°C). After 4 days, the buried segments were removed from the soil and thoroughly rinsed with clean water. The number of reddish-brown lesions longer than 1 mm and consistent with *Fusarium* damage were counted on the ten stems in each container (Fig. 2.1c). When more than two lesions joined to produce a larger lesion, lesions originating from separate infection sites were counted separately. Bean stems were also buried in autoclaved sand and the soil-free Cornell mix, which were used as controls for this assay expected to show no lesions.

2.2.8 Isolation, culturing, and identification of *Fusarium*

Fusarium species are common soil microbes, most of them being saprotrophs decomposing organic matter. However, depending on *Fusarium* genetic makeup, inoculum density, virulence

levels, and host immune system, some species become pathogenic and can cause root and stem rot, damping-off, necrosis, leaf yellowing and wilting in numerous plant species, under favorable conditions. We observed and counted the number of lesions that developed on the 6 cm long bean segments buried in each pot. To better understand the range of *Fusarium* taxa recovered and the potential pathogenicity of the different types, we cultured two replicated samples of lesions collected from each pot for further analysis. *Fusarium* were isolated by cutting off lesions from each of the two representative bean stems that had been buried in soils from the 66 plots (Fig. 2.1c). The cut segments were surface sterilized, blotted dry and then plated in fungal culture ½ PDA medium (Potato Dextrose Agar (PDA), cooled to 45°C). The plates were incubated for 7 days at room temperature and then numbers and differentiated types of fungal colonies were counted. Fungal colony types from the total of ca. 132 plates were grouped based on colony color, growth type, colony reverse color, and color of mycelia. Many of the colonies were white cottony with a dark-purple undersurface on ½ PDA and spores were oval to kidney shaped, with three septate spores, while the few other cultures were pink, reddish, and purple, and thin and colorless. Based on these characteristics, we sub-cultured 60 colonies on ½ PDA and incubated at room temperature for 14 days (Fig. 2.1d). Cultures were molecularly identified using a standard polymerase chain reaction (PCR) approach was used to extract, sequence, and identify 35 unique colonies. Extraction of DNA was done using a genomic DNA kit (Zymo Research Corporation, Irvine, CA, USA) following manufacturer's instructions. A NanoDrop Spectrophotometry ND-1000 (NanoDrop Technologies, Montchanin, DE, U.S.A.) was used to determine extracted DNA concentration and quality. Extracts were diluted to 10 ng/μl and stored at -20 °C until used. Sanger sequencing of the RNA polymerase II second largest subunit (*rpb2*) locus was conducted using methods similar to Dobbs et al., (2023).

Briefly, a 25 µl reaction was used to amplify the *rpb2* locus for each sample consisting of 2 µl each of forward (RPB2-6F: 5'-TGGGGKWTGGTYTGYCCTGC-3') and reverse (fRPB2-7cR: 5'-CCCATRGCTTGYYTTRCCCAT-3') primers (Liu et al., 1999), 12 µL of GoTaq Green Master Mix 2x (Promega, WI, USA), 4.5 µl of molecular water, and 40 ng of template DNA. The locus was amplified using a PCR cycle program of 94°C for 2 min, 40 cycles of 94°C for 40s, 58°C for 40s, and 72°C for 30s, and 72°C for 5 min. To visualize amplified PCR product using GelRed® (Biotium), the products were electrophoresed on a 1.5% agarose gel and the amplified products sequenced in both directions using Sanger sequencing at Eurofins Genomics (<https://eurofinsgenomics.com/en/home/>). Base-score quality of the sequences were visually checked using Geneious Prime v. 2022.0.1 (<https://www.geneious.com/>) and identified to putative species through BLAST analysis in the National Center for Biotechnology Information (NCBI) database (<https://www.ncbi.nlm.nih.gov/>) and Fusarium-ID.v.3.0 (<http://isolate.fusariumdb.org/blast.php>) (O'Donnell et al., 2021).

A Bayesian inference phylogeny was constructed on the *rpb2* sequences with *Fusarium* reference strains to validate identity of *Fusarium* species. Details of the GenBank numbers of the reference strains used in the phylogeny are included in the Supplementary Material (Supplemental Table 2.3). The phylogeny indicated three potential *F. oxysporum* clades and one *F. solani* clade, but the phylogeny was constructed using a single locus and did not have enough signal to separate out the *F. oxysporum formae speciales* (Supplemental Fig. 2.2). This warranted pathogenicity assays to be conducted to confirm whether the *Fusarium* isolates were able to cause disease on a susceptible common bean variety GLP2.

(Figure 2.1)

2.2.9 *Fusarium* pathogenicity test

To further validate interpretation of the visual *Fusarium* assay adapted from Furuya et al. (1999) and demonstrate the pathogenicity of the isolated strains, a common bean variety known to be susceptible to *Fusarium* (variety GLP2) was used to conduct pathogenicity assays. These assays were conducted on healthy bean seedlings 14 days after sowing in plastic pots (four plants per pot) containing ca. 500 g sterilized soil. *Fusarium* isolates grown on ½ PDA were used to make a conidial suspension that was then used to inoculate the healthy plants. To harvest the conidia, 1–5 ml of sterilized distilled water was placed onto the pure culture, which was then gently swirled and scraped. The conidial suspension was then filtered through two layers of muslin to remove mycelium. The suspension of 1×10^6 cfu g⁻¹ (colony forming unit/g) was then used as inoculum by pouring on a bruised lower stem of a healthy bean (10 days after germination), while sterile distilled water was used as an uninoculated control. Each isolate was inoculated on four replicates (pots) and these were randomly distributed on a table in a greenhouse. Pots were observed for 28 days after inoculation. Data was collected 5, 10-, 15-, 20-, and 28-days post inoculation by randomly uprooting one plant per pot at each time point and collecting the following information: plant height, wilting (Fig. 2.2a and c), vascular browning index (VBI) (Fig. 2.2b), and dissection of the stem to check the length of browning along the inner stem. The rating scale for the VBI was as follows: 0 = no vascular discoloration; 1 = discoloration restricted to base of stem only; 2 = discoloration of the ‘internode 0’ (hypocotyl) region of the stem below the cotyledons; 3 = discoloration of stem above the cotyledons; 4 = complete vascular discoloration of stem; and 5 = plant death (Becerra Lopez-Lavalle et al., 2012).

To complete the confirmation that symptoms of infections were caused by the inoculated isolates, Koch’s postulates were conducted. The diseased bean plants were sampled, ensuring

that samples were taken from the leading edge of the diseased area to avoid isolating secondary opportunistic invaders. The diseased samples yielded a range of fungi that morphologically were either *F. oxysporum*, *F. solani*, and *Rhizoctonia*. White bean stems, from beans grown in full darkness for 2 weeks and cut into 6 cm segments (as described in the *Fusarium* bioassay above) were employed to complete the Koch's postulate analysis. Conidia from the isolated cultures were harvested by placing 1-5ml of distilled water onto the cultures and gently swirling and scraping them. The suspension of ca. 1×10^6 cfu g⁻¹ was used to inoculate sterilized soil (from less disturbed area at Kenya Agricultural and Livestock Research Organization, Kisumu station) and water holding capacity adjusted to 50%. Clean bean stems were then buried in the soil and covered at room temperature for 4 days. As described above, the number of reddish-brown lesions longer than 1 mm on the stems in each container and consistent with *Fusarium* damage were then counted.

(Figure 2.2)

2.2.10 Statistical analyses

To understand the potential of bioassays to provide relative approximation of plant parasitic nematode populations compared to standard lab procedures, we used simple linear regression to assess correlations between bioassay-assessed nematode pressure and nematode communities assessed using lab extractions from roots (for *Meloidogyne*), and soil and roots (for *Pratylenchus*). We also used simple linear regression to explore relationships between the *Fusarium* bioassay and pathogenicity tests. Pairwise comparison of *Fusarium* isolate's vascular browning index means was performed using the Tukey-HSD method, and statistical significance of differences between means was determined at $P < 0.05$. Impacts of the different management treatments across the eleven fields on pathogen pressure and soil physico-chemical variables

were evaluated using ANOVA where a p-value ≤ 0.05 was considered significant. All comparisons considered treatment as a fixed variable and block as a random variable. Square root-transformations were used as needed to satisfy the ANOVA assumptions of homogeneity of variance and normality of residuals. All regression analyses and visualizations were conducted using R (version 4.0.4; and "ggplot2", "dplyr", and "ggpubr" packages), while ANOVA comparisons were done using JMP (version 15.0.0).

2.3 Results

2.3.1 Comparison of bioassay vs. lab assessment of nematodes

We identified various nematode taxa from the soils and soybean roots analyzed in the lab, including multiple PPN taxa. From soybean roots, we extracted *Pratylenchus*, *Tylenchorynchus*, *Helicotylenchus*, *Scutellonema*, and *Meloidogyne*, while in soils (of the same pots) we observed *Meloidogyne*, *Trichodorus*, *Aphelenchoides*, *Dorylaimodes*, *Pratylenchus*, *Helicotylenchus*, *Scutellonema*, and *Xiphinema*. The number of lesions found on soybean roots was positively correlated with the *Pratylenchus* extracted from the roots ($p < 0.001$; $R^2 = 0.27$). Significant relationships were also observed for the number of lesions on growing soybean roots and *Pratylenchus* abundance in soil alone ($p = 0.049$; $R^2 = 0.09$) as well as in soil and roots combined ($p = 0.026$; $R^2 = 0.12$). We note that there were a number of cases where lesions were observed on soybean roots, but no *Pratylenchus spp.* were recovered in the sample. The fact that the soil used hosted other PPN (*Trichodorus*, *Aphelenchoides*, *Dorylaimodes*, *Helicotylenchus*, *Scutellonema*, and *Xiphinema*) and soil-borne pathogens such as *Fusarium* and *Pythium*, could explain the high variation as these pathogens can work as a complex.

(Figure 2.3)

Galls assessed on lettuce roots were strongly related to the number of *Meloidogyne* nematodes (second-stage juveniles (j2s) and adult) extracted from lettuce roots in the lab ($p < 0.001$; $R^2 = 0.50$, Fig. 2.4). In addition to *Meloidogyne*, *Trichodorus*, *Pratylenchus*, *Helicotylenchus*, *Scutellonema*, *Xiphinema*, *Hoplolaimus*, and *Hemicyclophora* were found extracts from lettuce roots in the lab, indicating a wide variety of nematode species are likely causing damage on smallholder farms in our study region. In a few cases, lettuce roots were rotten or beginning to rot by the time of visual root assessment, which made it difficult to check for root galls (hence

the galls were not counted).

(Figure 2.4)

On average, the number of plant parasitic nematode taxa extracted per pot (ca.500 g of soil) was 5,576 *Scutellonema*, 3,384 *Meloidogyne*, 1,228 *Helicotylenchus*, 1,035 *Dorylaimodes*, 810 *Xiphinema*, 572 *Pratylenchus*, 455 *Aphelenchoides*, 151 *Tylenchorynchus*, 87 *Trichodorus*, 20 *Hoplolaimus*, and 10 *Hemicyclophora*.

2.3.2 *Fusarium* isolates and pathogenicity

Based on both morphological and molecular methods, nine *F. oxysporum*, two *F. solani*, and one *Waitea cincinata* isolates were identified (Table S2.1; Fig. S2.2). The pathogenicity test indicated that all nine isolates had the potential to cause wilting, vascular browning, rotting, stunting, and/or root necrosis, relative to controls inoculated with distilled sterile water (Fig. 2.2). These pathogens appeared to infect root vascular tissues, leading to browning and rotting of roots, which affected general plant health. The virulence levels of these pathogens also appeared to vary (Fig. 2.5), with *F. oxysporum* (Fo5 ($p = 0.012$) and Fo9 ($p < 0.001$) having the highest browning index and *F. solani* (Fs2) having the least, compared to control. Despite the few isolates used in pathogenicity test, lesions assessed on bean stems four days after burying them in soil were slightly correlated with select isolate's ability to cause browning in healthy bean inner stem (Fig. S2.1).

(Figure 2.5)

2.3.3 Management impacts on soil health parameters

The three soil treatments tested (i.e., manure, synthetic fertilizer, control) did not demonstrate significant effects on the measured soil health parameters (e.g., POXC, aggregate stability pH; Table 2.1). However, *Pythium*, *Fusarium*, and root-knot nematodes differed significantly among

the soil amendment treatments. *Pythium* pressure was less prevalent (35% less; $p = 0.02$) in plots receiving FYM compared to the no-input control, while *Fusarium* lesions were 23% higher ($p = 0.01$) in the FYM treatment, compared to no-input control (Table 2.1). At the same time, root galls associated with *Meloidogyne* were three times more abundant in the plots receiving synthetic fertilizer (DAP) compared to control ($p < 0.001$), which had the fewest number of galls. No significant differences were observed for *Pratylenchus* between treatments. While there were no significant differences in assessed soil health properties, it is important to note that various soil properties (e.g., POM, aggregate stability, and pH) were, on average, higher in FYM compared to DAP and the control (Table 2.1).

(Table 2.1)

2.3.4 Nematode communities in relation to nutrient input treatments

Assessment of soil nematode communities indicated large differences in the number of free-living nematodes across the three nutrient input treatments. We note that three genera of predators (*Labronema*, *Mononchus*, and *Discolaimodes*), five bacterivores (*Prismatolaimus*, *Acrobeles*, *Cephalobus*, *Eucephalobus*, and *Rhabditis*), and two fungivores (*Filenchus* and *Aphelenchus*) were identified from soybean bioassay soil samples used to assess lesion nematodes. Bacterivores and fungivores were 200% and 75% more abundant in FYM soils, respectively, compared to the no-input controls (Table 2.2). Notably, the lowest number of bacterivores and fungivores were recorded in DAP amended soils. The proportion of PPN in the whole soil nematode community (and indicator of PPN pressure) was lowest in the FYM treatment, and highest in the DAP treatment ($p = 0.003$).

(Table 2.2)

2.4 Discussion

This study sought to evaluate the performance of simplified tests for soil-borne diseases and nematodes and the ability of these tests to assess the impact of common nutrient inputs on soil root pathogens and relationships with key soil health parameters. Our results suggest that the bioassays examined here offer considerable promise to provide farmers and local technicians with more accessible techniques to evaluate levels of *Fusarium*, *Pythium*, *Meloidogyne*, and *Pratylenchus* in soil. Specifically, the bioassay indicators for PPN (i.e., lesions, galls) were found to correlate moderately well with the lab-assessed nematode abundances (Figs. 3 and 4), while the recovered *Fusaria* in the plant stem assay caused disease, highlighting the success of the *Fusarium* bioassay (Figs. 5). Bioassays using susceptible crops have historically been used to assess levels of various soil pathogens. These include the use of potato to quantify *Pythium aphanidermatum* in soil (Stanghellini & Kronland, 1985), eggplant to assess *Verticillium dahliae* (Nagtzaam et al., 1997), cotton to assess *Fusarium* wilt disease (Becerra Lopez-Lavalle et al., 2012), and spinach to predict risks of *Fusarium* wilt (Gatch & du Toit, 2015). Bioassays are useful quantitative tools that can be used to evaluate changes in a system and harmful effects of management on different factors (Terekhova, 2011). They are also valuable in that they integrate biological processes over time and provide a measure that is directly relevant to plant growth. We note that the bioassays assessed in this study are easy to follow, cost-effective, rapid, and visual, making them potentially valuable tools for smallholder systems where soil-borne pathogen pressure is high and formal laboratories are inaccessible. In addition, the assays can provide research organizations working with smallholder farmers with essential tools to train the farmers who might not have skills to identify soilborne pathogen symptoms.

2.4.1 Comparison of bioassay vs. lab assessment of nematodes

Meloidogyne and *Pratylenchus* were found in all the soils from the eleven farms, across three different locations in Nandi County. Furthermore, laboratory analyses revealed the presence of other PPN taxa (*Scutellonema*, *Helicotylenchus*, *Dorylaimodes*, *Xiphinema*, *Aphelenchoides*, *Tylenchorynchus*, *Trichodorus*, *Hoplolaimus*, and *Hemicyclophora*) in all farms. This is in agreement with other reports that, although *Meloidogyne* and *Pratylenchus* species are likely the most important nematodes in SSA, the typical crops and local conditions are associated with many PPN, which are part of a larger, complex nematode community (Coyne et al., 2018; Sikora et al., 2018). Understanding the relative importance of nematode species existing in complex communities is difficult, especially since they have varying generation periods and may thrive under different environmental conditions that vary seasonally (Luc et al., 2005).

Pratylenchus spp. are categorized as migratory endoparasites, mostly feeding and reproducing within the root system but sometimes feeding on the root surface without entering the root tissue and can also be found in soils surrounding roots. *Pratylenchus* enter and feed on plant tissue, secreting cell-wall degrading enzymes and leaving brown elongated lesions that eventually become necrotic areas (Gugino et al., 2006). While these signs can be visually observed, absolute association of brown lesions with *Pratylenchus* only, especially in soils infested with other PPNs can be challenging. We note that the bioassay used here lasted for 3 weeks and other PPN signs may not have fully developed. Coyne et al. (2018) suggests that crops grown in SSA are associated with remarkably complex nematodes communities, and it may be difficult to evaluate the relative pathogenicity of individual species. Therefore, the high variance observed ($R^2 = 0.27$) could be associated with the presence of other soil-borne pathogens that could cause necrotic lesions on soybean roots, working as a complex. However, the strong positive

relationship reported above indicates that counting lesions in the bioassay can reasonably assess the pressure of *Pratylenchus*. In previous research, methods used in this study were tested with commercial vegetable growers in New York, and results demonstrated that the number of lesions developed on the soybean roots corresponds to the relative lesion nematode infestation level in the soil (Gugino et al. 2009).

Many zero counts for *Meloidogyne* abundance in the lab assessments (Table 4) contributed significant variability and complicated the ability of our bioassay to predict *Meloidogyne* abundance. In some cases, using the bioassay, many galls were identified, but the lab extraction found zero root-knot nematodes in the j2s growth stage. This could be explained by the fact that the adult sedentary stage of *Meloidogyne* females involve feeding, swelling into pear-shape, and producing egg masses (500 to 1000; surrounded by a gelatinous matrix) on the root surface (Tapia-Vázquez et al., 2022; Vilela et al., 2023), in which these swellings could be easily counted as root galls. If root extraction is done before the eggs hatch to j2s, lab analyses would register zero *Meloidogyne*. We suspect that this relative “over counting” of reproducing females as “root knots” may be capturing future generations of root-knot nematodes, since these females will likely hatch hundreds of infective J2s, potentially making the bioassay relatively powerful as a method to assess crop risk from *Meloidogyne*. However, further research is needed to confirm this idea and provide a clearer interpretation for the gall counts.

2.4.2 Management impact on nematode communities

There were higher number of fungivores and bacterivores in the FYM plots suggesting that a greater level of labile carbon inputs in manure-treated plots may play a role in developing more balanced nematode communities in this treatment. Farmyard manure is known to improve physical and chemical properties of soil (Riegel and Noe, 2000; Liu et al., 2021; Karuri, 2022)

making them more conducive for a diverse array of soil microbes, including bacteria and fungi. By enhancing bacterial and fungal decomposers, this supports greater populations of bacterial and fungal feeding nematodes (Roskopf et al., 2020). Given the lack of differences in predatory nematodes between treatments, it appears that the high number of bacterivores and fungivores, and low number of PPN in manure-amended soils could be as result of parasitism of PPN by soil microbes. Animal manure is known to support proliferation of antagonistic fungi and bacteria, which can then reduce PPN (Oka, 2010; Bailey & Lazarovits, 2003). Although the impact that organic amendments have on soil nematodes and microbial communities can be quite complex, possible mechanisms in suppression of nematodes are release of compounds such as ammonia and fatty acids that can be nematicidal, enrichment of antagonistic organisms, change in soil physiology, or plant tolerance and resistance improvement (Oka, 2010). However, contrasting effects of manure on PPN have been reported, suggesting the need for further assessment of manure effectiveness to assist in managing PPN across variable farm field conditions in SSA (Karuri, 2022). While the recent establishment of the trials did not permit detection of significant impacts on soil health parameters (e.g., pH, POM, and MWD) in the FYM treatment, compared to DAP and control, we suspect that organic matter amendments are quite important for soil health and that the changes may be more pronounced if organic amendments are used for longer periods. This idea is supported by results from Ayuke et al., (2011) who evaluated long-term impacts of amending soil with organic inputs vs. synthetic fertilizers on soil chemistry and biology in central Kenya and found that FYM significantly improved C and N pools, as well as biological activity and diversity (i.e., macrofauna abundance).

2.4.3 Management impact on *Pythium* and *Fusarium*

The significantly lower abundance of *Pythium* under the FYM-amended soil is likely related to

the high inputs of organic matter in the FYM treatment that likely lower pathogenic *Pythium* development by promoting competition and antagonism by other organisms (Le et al., 2014). Several modes of action are involved in the activity of manure; these include the release of allelochemicals generated by consequent microbial decomposition (Bünemann et al., 2006) and increase in soil organic matter that improve water retention, create microhabitats less conducive for soil pathogens or possibly introducing biocontrol capabilities such as enhancement of organisms that are antagonists, competitors, or parasitic against soil-borne organisms (Roskopf et al., 2020).

In contrast to *Pythium*, *Fusarium* appeared to be significantly higher in plots amended with FYM. While this may seem unusual, the broad review and discussion by Alabouvette et al. (2009) indicates that the diverse species of *Fusarium* are complex, and in the FYM the diversity of *Fusarium* spp. might have been greater. Moreover, all *Fusarium* strains feed on available carbon, which was more readily available in the FYM treatment. Importantly, Alabouvette et al. (2009) discuss that both pathogenic and non-pathogenic *Fusarium* strains have the ability to colonize the root surface and to penetrate the root, in a similar manner. However, antagonism through either competition for infection sites between pathogenic and nonpathogenic strains or parasitism, are common and the main mechanisms of biocontrol by the non-pathogenic strains. In the case of our study, the many lesions that developed on bean stems were observed in soils amended with manure, suggesting a high level of *Fusarium* in those soils. This suggests that the bioassay used in our study provides an indication of possible level of infections, but actual impact on plant growth is mediated by soil health status. It is also important to note that incorporation of organic inputs such as FYM can enhance a number of soil properties (e.g., soil pH, water holding capacity, aeration, and nutrient availability), creating a more conducive

environment for soil-borne pathogens, including *Fusarium*, to survive. At the same time, these conditions often allow plants to grow more vigorously and potentially defend themselves better against pathogens, which may not be the case in nutrient poor soil since other growth factors would cause stress to crops and limit root growth (Medvecky and Ketterings, 2009), increasing crop vulnerability to pathogenic *Fusarium*. We suggest the need for additional research that examines *Fusarium* presence links to pathogenicity across a range of soil health contexts.

2.4.4 Implications and potential applications

The adapted bioassays evaluated here offer great potential for smallholder farmers, with the help of extension and field officers, to efficiently assess and manage soil-borne pathogens. Soil analysis using these bioassays could be combined with other soil health tests prior to planting, to provide farmers with valuable insight on the potential disease pressure and possible management interventions to reduce pathogen severity. Additionally, farmers and local technicians could use soil bioassays to evaluate the effectiveness of alternative or novel management practices designed to reduce pathogen pressure. The visual nature of the bioassays considered here allow for participatory root assessment to be done collaboratively between farmers and extension officers, thus helping to farmers to improve knowledge and awareness of soil-borne pathogens and facilitate the development of practices that lessen their impact. A better understanding of the problem would likely support the adoption of specific practices that show promise to lower soil-borne pathogen presence and associated threats. Despite their great promise, additional research is needed to better understand how the bioassays evaluated here relate to crop yields, and more specifically, to economic threshold levels for the management of *Fusarium*, *Pythium*, *Pratylenchus* and *Meloidogyne*. We suspect that in many cases a pathogen maybe present in a system, but that the cost of implementing alternative management practices or other pest control

measures, likely exceeds the potential cost of doing nothing; alternatively, high existing levels soil fertility and soil health generally may moderate impacts of disease on yield as discussed above.

Beyond demonstrating the potential of bioassays to inform about pest and disease issues in smallholder farming systems, our research offers some important insight about management strategies to lessen the impact of soil-borne pathogens. Specifically, our findings suggest that farmers experiencing disease symptoms linked to *Pythium* and PPN (e.g., seed and seedling rots, stem collapse, damping-off, yellowing patches, root knots, or necrotic lesions) would benefit from more frequent application of FYM. Other strategies might include growing less susceptible crops, and diversifying cropping systems through intercrops, more complex rotations, or agroforestry, all practices known to enhance general soil health status and reduce crop damage (Peiris et al., 2020; Shen et al., 2023; Yu et al., 2023). Given the heterogeneous nature of smallholder farming environments and management (e.g., Nyamasoka-Magonziwa et al. 2021), we suspect that the most effective strategies likely vary with context. As such, we suggest that the bioassays evaluated here can help researchers, field officers, and farmers to better map and understand the factors that drive soil-borne pathogen pressure and impacts on crops, and thus help guide future research to address challenges in smallholder farming systems.

2.5 Conclusions

Smallholder farming systems of SSA are often characterized by continuous cultivation and limited crop diversity, thus contributing to favorable conditions for survival and multiple generations of soil-borne pathogens. Our findings confirm the existence of major soil-borne pathogens in across the entire study area and suggest that simplified soil bioassays offer a valuable tool for smallholder farmers in collaboration with local extension agents to better evaluate and understand pathogen inoculum pressure on-farm. Furthermore, our results show that organic amendments such as FYM promote more robust soil communities and potentially suppression of PPN (as indicated by nematode community composition of free-living nematodes), likely as a result of improvements to C availability and overall soil health.

CHAPTER 2 TABLES AND FIGURES

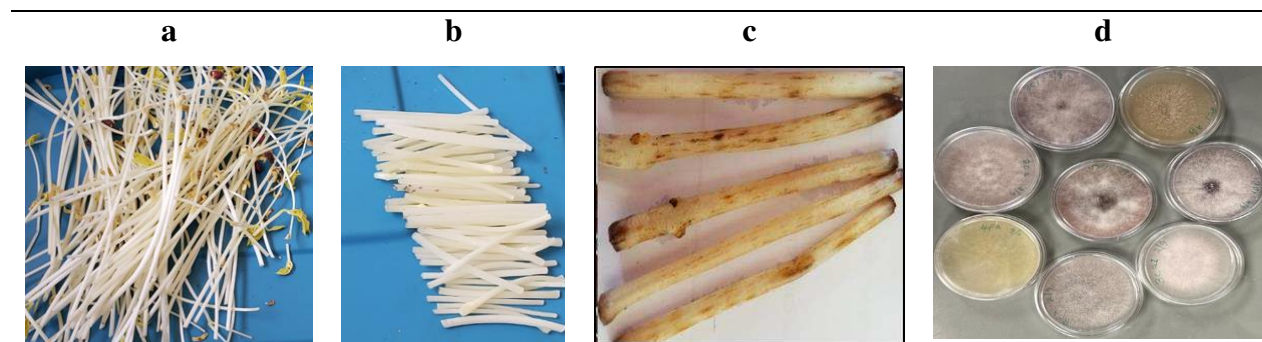


Figure 2.1 *Fusarium* assessment based on the number of lesions produced by the *Fusarium* pathogens on a 6 cm section of bean stem buried and incubated for 4 days at 25 °C in soil samples collected on-farm. Figure panels show the following: a) white stems of beans after growing in darkness for 16 days; b) ca. 6 cm long sections of bean stems (to be buried in infested soil); c) lesions development on bean stems 4 days after burying in soil; and d) a sample of cultures isolated from the lesions.

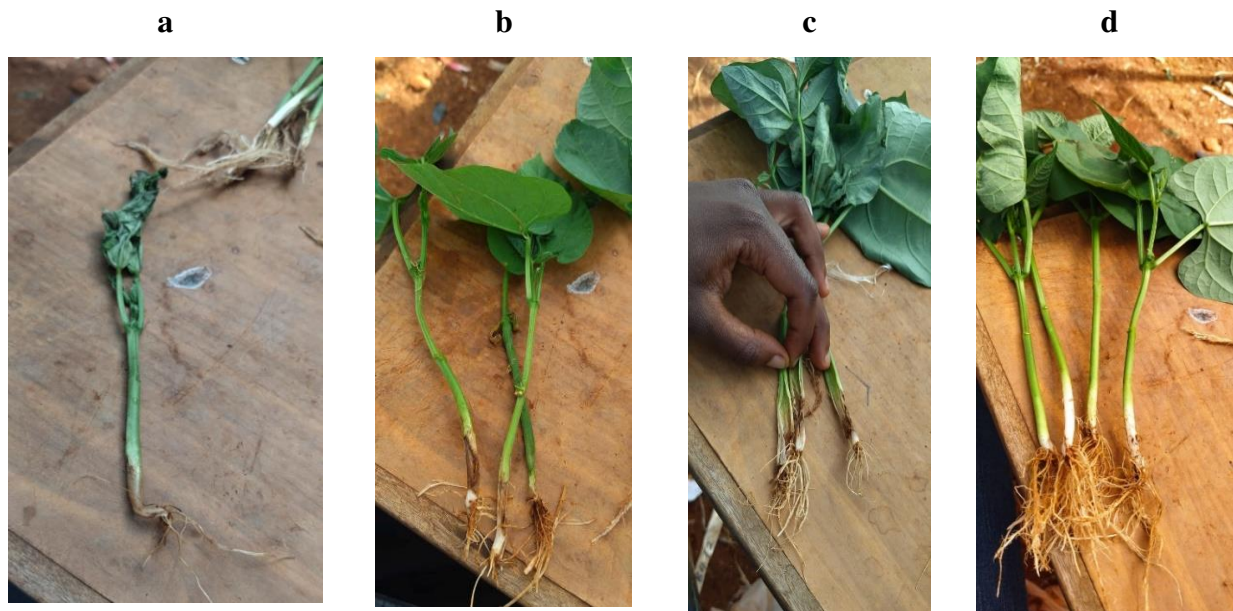


Figure 2.2 Infection of bean seedlings with *Fusarium* (the isolates from bean stems buried in soils from smallholder farms of western Kenya). Figure panels show the following: a) wilted bean seedling grown in sterilized soil 5 days after stem inoculation with a conidial suspension of *F. oxysporum* isolated from the bean stem assay; b) necrotic lesions on the lower stems of bean seedlings 10 days after inoculation with *F. solani*; c) leaf wilting and browning of the xylem tissue (vascular discoloration) 10 days after inoculation with *F. oxysporum*; and d) clean fibrous roots in the control treatment 20 days after germination.

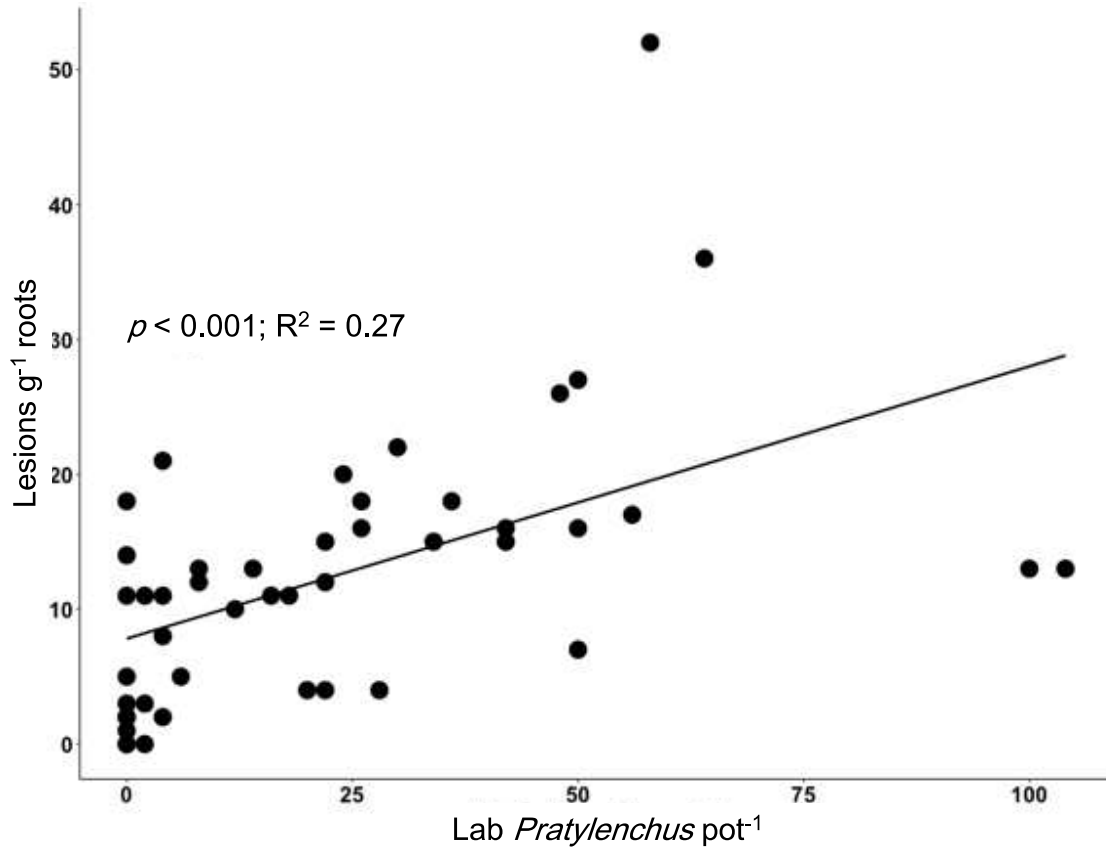
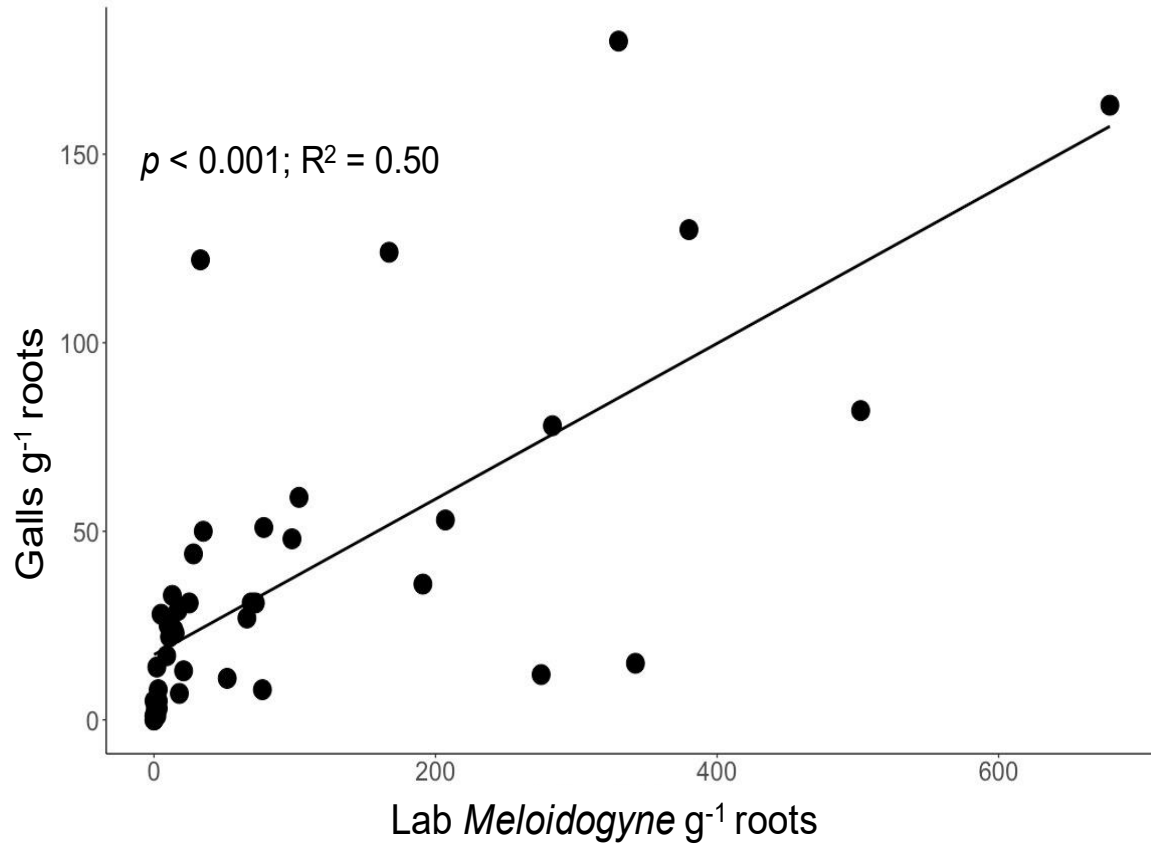


Figure 2.3 Relationship between root lesion density in the nematode bioassay using soybean as a bait plant (y-axis, lesions g⁻¹ root) and a standard laboratory assessment of *Pratylenchus* per pot (x-axis, individuals per pot in roots and soil).



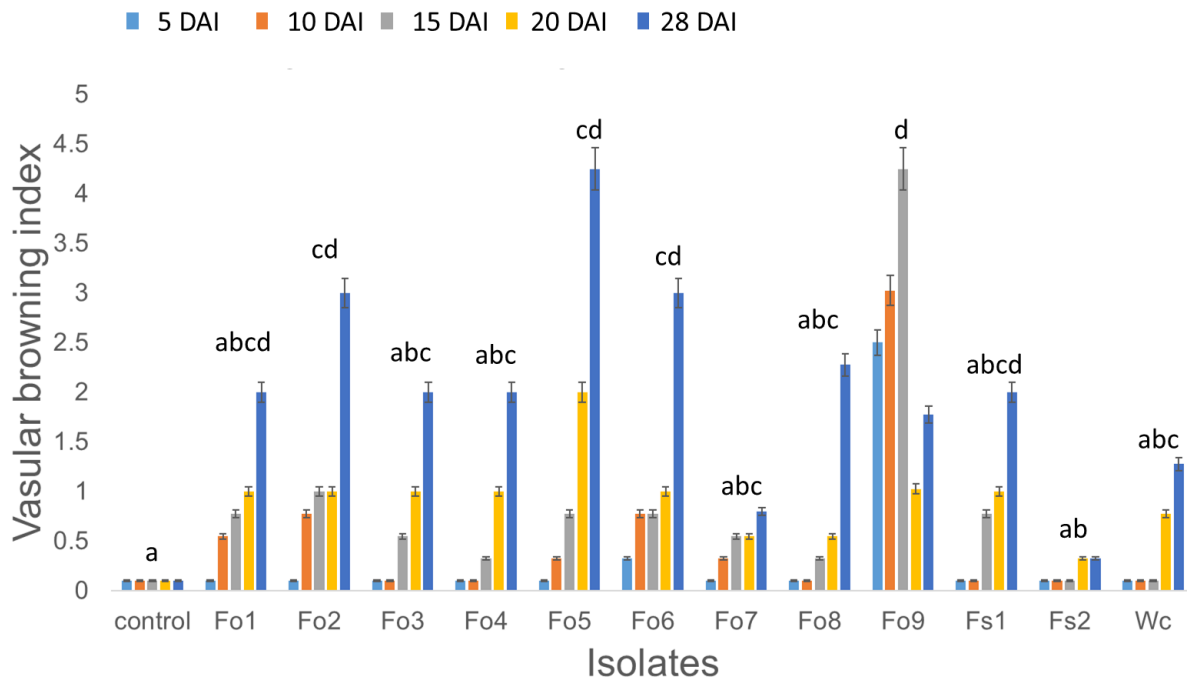


Figure 2.5 The effect of *Fusarium* inoculation on average vascular browning index of healthy bean seedlings at: 5, 10, 15, 20, and 28 days after inoculation (DAI). Measurements were taken every 5 days after beginning inoculation by randomly sampling a bean plant from each pot inoculated with individual isolates: *Fusarium oxysporum* type 1 (Fo1) to type 9 (Fo9), *Fusarium solani* 1 (Fs1) and type 2 (Fs2), and *Waitea circinata* (Wc). The isolates were identified to species level by molecular analysis (Table S1; Fig S2). Letters represent pairwise comparison of average vascular browning index, and bars with the same letter are not significantly different at $p < 0.05$.

Table 2.1 Soil health parameters and soil-borne pathogens tested across three treatments (control, di-ammonium phosphate (DAP), and farmyard manure (FYM)) applied for two long-rainy seasons in eleven farms across three locations in western Kenya. Standard errors are presented to the right of each mean in parentheses. Means followed by different letters indicate significant differences between treatments (ANOVA, $p < 0.05$).

	Control	DAP	FYM	P values
P_{avail} (mg P kg⁻¹)	4.59 (0.73)	5.55 (1.04)	4.09 (0.56)	0.41
MWD (µm)*	757.6 (38.0)	776.50 (26.5)	810.50 (40.6)	0.550
POXC (mg C kg⁻¹)	469.8 (53.7)	516.32 (46.6)	511.23 (56.5)	0.780
pH	5.70 (0.11)	5.73 (0.09)	5.80 (0.10)	0.740
POM (mg kg⁻¹)	130 (30)	120 (30)	140 (20)	0.780
Org C (g kg⁻¹)	1.98 (0.15)	2.01 (0.15)	1.97 (0.15)	0.980
<i>Pythium</i> (colonies plate⁻¹)	5.41 (0.53) a	5.18 (0.51) a	3.50 (0.49) b	0.020
<i>Fusarium</i> (lesions stem⁻¹)	17.32 (1.80) ab	13.14 (1.65) b	21.23 (1.72) a	0.010
<i>Meloidogyne</i> (knots g⁻¹ root)	27.00 (6.11) b	68.02 (13.3) a	22.21 (10.3) b	< 0.001
<i>Pratylenchus</i> (lesions g⁻¹ root)	30.80 (10.29)	9.35 (1.97)	11.93 (4.77)	0.150

*P_{avail}: available P; MWD: mean weight diameter; POXC: Permanganate oxidizable carbon; POM: Particulate organic matter; Org C: organic carbon.

Table 2.2 Average nematode abundances extracted from incubated soils across three treatments (control, di-ammonium phosphate (DAP), and farmyard manure (FYM)) applied for two long-rains seasons in 11 farms in three locations in western Kenya. Standard errors are presented to the right of each mean in parentheses. Means followed by different letters indicate significant differences between treatments (ANOVA, $p = 0.05$).

	Average			Treatments
	Control	DAP	FYM	P-values
Bacterivores	1554 (188.7) b	123 (144.7) b	3580 (484.9) a	< 0.001
Fungivores	109 (18.7) b	84 (15.6) b	186 (22.8) a	0.021
Predators	35 (6.2)	47 (7.0)	31 (4.3)	0.451
Plant parasitic nematodes (PPN)	110 (11.4) b	246 (21.6) a	85 (9.1) b	< 0.001
PPN as proportion of total*	0.12 (0.0) b	0.19 (0.04) a	0.07 (0.0) b	0.003

* the proportion of plant parasitic nematodes relative to all nematodes recovered

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CHAPTER 3: MANAGEMENT AND ENVIRONMENTAL DRIVERS OF SOIL-BORNE

PATHOGENS IN SMALLHOLDER SYSTEMS OF WESTERN KENYA

3.1 Introduction

In areas of sub-Saharan Africa (SSA) characterized by warm and wet climates, parasitic nematodes, soil-borne pathogens, and foliar diseases can cause extensive crop losses (Kimenju et al., 2009; Nicol et al., 2011). For example, yield losses due to parasitic nematodes are estimated to be roughly 15% in tropical and sub-tropical regions (Nicol et al., 2011). Despite their economic importance, smallholder farmers in the region typically know very little about plant parasitic nematodes and fungal pathogens. Thus, they often take no management actions to address these issues and the problem worsens with time (Back et al., 2002; Jones et al., 2013; Moens et al., 2009; Coyne et al., 2018).

In western Kenya, numerous factors exacerbate damage by soil-borne pathogens and pose great threat to crop production. Population density in the region, particularly in Kakamega, Vihiga, and Kisumu counties, is high, ranging between 500 and 1200 people per km² (Kenya National Bureau of Statistics 2020), thus resulting in considerable pressure on limited land suitable for rain-fed agriculture. Many farmers in the region have continuously cultivated crops on small plots of land (average of 0.6 ha) for decades with minimal diversity, further creating conditions conducive to widespread establishment of opportunistic soil-borne parasites (Tadele, 2017).

Considerable overlap in the host crops for plant parasitic nematodes and soil-borne pathogens creates additional challenges for farmers. Maize (*Zea mays*) and beans (*Phaseolus vulgaris*) are the staple food crops in western Kenya. Farmers predominantly grow maize as a monocrop or

intercropped with beans (other legumes and/or vegetables), or sometimes maize is grown in rotation with beans and other crops. Soil-borne parasites, especially root-rot causing pathogens (e.g., *Fusarium* and *Pythium* spp.) and root-feeding nematodes attack legumes and maize in different growth stages, leading to serious damage to roots and foliage, and thus low productivity and quality of yields (Broughton et al., 2003; Medvecky et al., 2007; Sinclair et al., 2012; Chirchir et al., 2010). In addition, many legume crop species and common vegetable crops (including tomato, lettuce, spinach, and carrots), as well as other crops such as sugarcane, tubers, cereals, and tea, are very susceptible to one or more nematodes, *Pythium*, and *Fusarium* species (Leslie and Summerell, 2006; Medvecky et al., 2007; Moens and Perry, 2009; Katan, 2017). Though there may be crop-free periods within smallholder systems, pathogenic fungi (Ciancio and Mukerji, 2008) and parasitic nematodes (Luc et al., 2005) have many weed hosts in the tropical and subtropical regions. Therefore, soil-borne pathogens can persist in soils even in the absence of a crop host, such as during a dry season (Leslie and Summerell, 2006).

Most soils of western Kenya have low soil organic matter (SOM) and nutrient levels due to continuous cultivation of limited land, and low inputs of fertilizer and organic matter (Kandji et al., 2001; Descheemaeker et al., 2016). Frequent tillage that is common in smallholder systems increases mineralization of SOM (Bailey and Lazarovits, 2003) and accelerates the decline of soil physical, chemical, and biological components, thus influencing long-term soil productivity and sustainability (Kanmegne et al., 2006; Sipilä et al., 2012). Crop residues are mostly fed to livestock, burned, or used as fuel, hence organic matter and associated nutrients are often not returned to the soil (Medvecky et al., 2007; Rusinamhodzi et al., 2016; Nyamasoka-Magonziwa et al., 2021).

Crop productivity is also limited by low soil pH. Although low pH is a natural property of these

highly weathered soils, it can be exacerbated by the continuous use of acidifying fertilizers (e.g., di-ammonium phosphate; DAP), and minimal incorporation of organic amendments that might otherwise help to buffer soil pH (Liu et al., 2010). Soil pH is also known to influence the abundance and severity of pathogenic fungi and parasitic nematodes (Jiang et al., 2013; Simon et al., 2018). Fungal (e.g., root rot, damping-off pathogens, ascochyta blight, angular leaf spot), viral (e.g., bean common mosaic virus), and bacterial (e.g., common bacterial blight, halo blight) diseases and pests (e.g., nematodes, aphids, and stem maggots), which tend to increase at low soil pH, have all been documented to attack crops in the smallholder farms of western Kenya (Medvecky et al., 2007; Chirchir et al., 2010; Simon et al., 2018; Mutai et al., 2019).

Recommended management strategies for soil pest and pathogens include mixed cropping, crop rotation, and use of organic inputs, e.g., amendments with high C content such as peat, composts, biochar, and farmyard manure (Noble & Coventry, 2005; Eberlein et al., 2016; Klein et al., 2012). These approaches aim to support soil health and foster diverse soil biological communities in order to suppress soil-borne diseases (Rotenberg et al., 2007; Campos et al., 2016). However, interactions between cropping system, management practices, and multiple soil health parameters in heterogeneous smallholder contexts are poorly studied and therefore not well-understood.

Our research sought to understand management and environmental drivers of key soil-borne crop pests and diseases in western Kenya (Fig. 3.1). We collected soils from smallholder farms utilizing different cropping systems and nutrient management strategies across five sites in western Kenya. For each soil sample we evaluated a suite of physical and chemical properties and assessed these against the results of simple visual assays that allowed us to measure potential infestation by *Fusarium* pathogens and lesion nematodes. We hypothesized that: 1) farms relying

on organic nutrient inputs (e.g., manure, compost) have reduced *Fusarium* and lesion nematode pressure (incidence & severity) and improved soil health parameters relative to farms relying mainly on mineral fertilizers; 2), diversified cropping systems experience lower pest pressure than simpler cropping systems, based on just one or two crops; and 3), beyond management, soil and environmental variables unique to each site explain variability in *Fusarium* and lesion nematode pressure.

(FIGURE 3.1)

3.2 Materials and methods

3.2.1 Study area

The study was carried out at five sites: Kapkerer, Kiptaruswo, and Koibem locations in Nandi County, and Waitaluk and Kinyoro in Trans-Nzoia County. Western Kenya is highly heterogeneous, and these sites represent a range of environmental conditions in terms of precipitation, temperature, and elevation (Table 3.1). Precipitation in Trans-Nzoia is unimodal, with 7–8-months of rainy season (March/April – October) and 4–5-months dry season (November – February/March), whereas sites in Nandi have bimodal rainfall (long rains from March to July, and short rains from September to December).

(TABLE 3.1)

Kenyan smallholder farmers practice rain-fed mixed farming, with crops and livestock coexisting on most farms. Many Nandi farmers are also involved in farming of cash crops (mainly tea) to supplement household income. Most of the communities in Trans-Nzoia County comprise a mix of large- and small-scale farmers, with maize being the dominant crop, and, on the larger farms,

mostly grown for sale. Farmers in this region are also involved in dairy and horticultural enterprises. Farmers in Nandi County typically cultivate smaller plots of land, with maize and beans being the most common crops. Soils are dominated by humic Ferralsols in Trans-Nzoia, and Acrisols in Nandi County and typically have low inherent fertility and low pH (Ojiem, 2018; Medvecky & Ketterings, 2009).

3.2.2 Site selection and soil sampling

Thirty-five farms, representative of the biophysical and management characteristics prevalent in the region, were selected for this study. Information on cropping system and nutrient input management (going back at least 5 years) was collected for each farm via farmer interviews. These farms practiced one of four cropping systems: 1) maize monocrop (n=3), 2) maize-legume intercrop (n=12), 3) maize-legume/vegetables rotations (hereinafter 'rotation system'; n=10), and 4) horticultural systems (n=10). Nutrient management strategies included 1) organic nutrient inputs (compost and manure, n=9), 2) mineral fertilizers alone (n=20), and 3) combination of organic and inorganic nutrient inputs (n=6).

At the beginning of long rains growing season in March 2019, soil samples were collected using a soil corer (2.5 cm diameter) to a depth of 15 cm. Fifteen cores (sub-samples) were taken directly beneath the plants of the dominant crop within each farm, excluding 1 m buffer zones at both ends of each plot. The sub-samples of bulk soil were then pooled and homogenized resulting in roughly 3 kg of soil per composite sample for each farm. From this composite sample a representative sub-sample (~ 1 kg) was collected for nematode bioassays (see below) in a separate plastic bag and stored in a cooler with ice packs for transport to the laboratory at the Kenya Agricultural and Livestock Research Organization near Kisumu, Kenya. Upon arrival at

the lab, the nematode samples were stored at 4 °C until the bioassays were conducted (within 5 days of sampling). The rest of each composite sample was air-dried and passed through an 8 mm sieve in preparation for analysis of *Fusarium*, as well as for measurement of soil physicochemical properties.

3.2.3 Soil physicochemical properties

Soil physicochemical properties, including soil pH, permanganate oxidizable C (POXC), available P, and particular organic matter (POM) were evaluated using a soil tool kit developed for low-cost, rapid assessment of soil on smallholder farms, according to methods described by Nyamasoka-Magonziwa et al. (2020). pH was measured in a 2:1 deionized water:soil suspension, evaluation of POXC was based on the oxidation of labile soil total C by potassium permanganate (KMnO₄), available P was determined using a modified Olsen method, while POM was determined using density flotation with deionized water (Nyamasoka-Magonziwa et al. 2020). Additionally, sub-samples were sent to the Crop Nutrition Laboratory Services Ltd. (Nairobi, Kenya) for analysis of total soil C and soil texture, using dry combustion, and particle size analysis, respectively.

3.2.4 Assessment of *Fusarium* pathogens

Fusarium baiting assays were conducted according to the protocol of Furuya et al., (1999). Bean (*Phaseolus vulgaris*; variety GLP2) seeds were planted in a soil-less media (Cornell peat mix) (Boodley and Sheldrake, 1977) and grown in the dark for 14 days at room temperature, with the aim of obtaining long white stems (≥ 25 cm) that can easily show signs of *Fusarium* infestation. Bean stems were then harvested and cut into 6 cm long segments. Two sub-samples (lab replicates) of the air-dried, 8 mm sieved soils (500 g per sample) from each farm were placed in

sealed tubes (8 cm diameter, 12 cm high) and water content adjusted to 50% water holding capacity. Ten bean stem segments were then buried in each tube and incubated at room temperature. After 4 days, the buried segments were removed from the soil, rinsed with water, and the number of reddish-brown lesions longer than 1 mm and consistent with *Fusarium* damage were counted on the ten stems in each tube. When more than two lesions joined to produce a larger lesion, lesions originating from separate infection sites were counted separately. Bean stems were also buried in autoclaved sand and the soil-free Cornell mix, which were used as controls for this assay. The assay was further validated by Mutai et al. (*submitted*) using molecular analysis to identify the organisms isolated from the lesions as well as pathogenicity tests to assess whether the isolated strains were pathogenic the *P. vulgaris* cultivar used for the stem pieces.

3.2.5 Evaluation of root lesion nematodes

To assess the abundance of lesion nematodes (*Pratylenchus* spp.), we used a soil bioassay developed at Cornell University by Gugino et al. (2006; 2009) and further validated by Mutai et al. (*submitted*). Two sub-samples (lab replicates) of refrigerated soil (~ 500 g each) were placed in separate pots (8 cm diameter, 12 cm high) with drainage holes. Three soybean seeds were then planted in each pot without adding any fertilizer. The plants were maintained in the green house on a workbench, watered as needed for 2-3 weeks. Soybean seeds were also planted in soilless media (~ 500 g) as a control. After three weeks, the soybean plants were carefully removed, and the roots washed free of soil. The number of elongated dark brown lesions observed on the main root of each plant were then counted and recorded.

3.2.6 Statistical analyses

Lesion counts from the two lab replicates of each bioassay were averaged for all analyses. Pearson's correlations were used to assess bivariate relationships between the soil parameters, elevation, *Fusarium* pathogens and lesion nematode abundance. To compare *Fusarium* and nematode incidence, and key soil parameters between the different management approaches, we used multiple linear regression, considering cropping system (four levels) and nutrient input strategy (three levels) as categorical explanatory variables, and elevation, sand content and total C as covariates to account for variability associated with site-level environmental drivers. The multivariate model allows for stratified analysis and elimination of confounding effects of cropping system and nutrient inputs. Interactions between factors were not included in the model due to insufficient sample size and because the principal aim was to establish the major drivers of root disease. Response variables included number of lesions caused by *Fusarium* and lesion nematodes, as well as soil variables we felt could be more sensitive to management and thus act as mediating factors relating management to more proximal potential causes and deterrents of root disease (POM, available P, POXC, soil pH, and total C). Log-transformations were applied for *Fusarium* lesions, nematode lesions, and POM to homogenize variances of the results. To explore multivariate differences between cropping systems and nutrient management strategies, we conducted a Between-Class Analysis (BCA) including soil physico-chemical parameters as well as the number of lesions caused by *Fusarium* and lesion nematodes. This approach relies on permutation tests to compare an observed test statistic against random permutations of the data (Monte-Carlo test). All data was analyzed using JMP Pro 14 or R software version 3.6.1 (R Core Team, 2019).

3.3 Results

The bioassays indicated the presence of *Fusarium* pathogens and lesion nematodes in soils from all study locations. Soil physical and chemical properties varied widely across sites (Table 3.2).

(TABLE 3.2)

3.3.1 Bivariate relationships between soil parameters and pathogens

Pearson's correlations revealed significant bivariate relationships between lesion nematode pressure and multiple soil variables (Table 3.3). For example, soil pH and POM, POXC and total C demonstrated significant negative correlations with lesion nematode pressure, while % sand was positively related to lesion nematodes. Only POM showed a significant positive correlation with *Fusarium* pressure. Significant positive relationships were also observed between POM and soil pH, POXC and total C. Elevation was positively correlated with available P and total soil C across sites. Total C was positively correlated with POXC, and both variables were negatively correlated with % sand.

(TABLE 3.3)

3.3.2 Cropping system and nutrient management effects

Multiple linear regression analyses indicated that both lesion nematode pressure and *Fusarium* differed significantly with the type of nutrient input used ($p = 0.003$ and $p = 0.034$, respectively; Table 3.4). Lesion nematodes were lowest in soil receiving only organic nutrient inputs (5-7 lesions sample⁻¹) and highest in soil with only mineral fertilizers (10-13 lesions sample⁻¹), while soils receiving a mix of organic and inorganic inputs presented intermediate values (Fig. 3.2a). *Fusarium* showed the opposite trend, with highest numbers in the organically fertilized plots (12-

14 *Fusarium* lesions sample⁻¹) and lowest numbers in the soil receiving only inorganic nutrient inputs (7-9 lesions sample⁻¹; Fig. 3.2b).

(FIGURE 3.2)

(TABLE 3.4)

At the same time, available P and POXC showed significant differences across cropping systems ($p = 0.002$ and $p = 0.001$, respectively). Horticultural systems had the highest available P (18-25 mg kg⁻¹) while the lowest levels occurred under maize-legume intercrop (4-10 mg kg⁻¹; Fig. 3.3a). The rotation system had the highest amounts of POXC (750-900 mg kg⁻¹), while the lowest values were observed under maize-legume intercrop systems, which had 360-520 mg kg⁻¹ (Fig. 3.3b).

Total C showed a nearly significant difference between cropping systems ($p = 0.068$; Table 3.4): Horticultural and rotation systems had significantly higher amounts of total C than maize monocrop and maize-legume intercrop systems. Soil pH also showed a nearly significant difference among nutrient management strategies ($p = 0.096$; Table 3.4). Generally, cropping system and nutrient management appear to be associated with soil organic matter, which seem to influence both lesion nematode and *Fusarium* pressure. For example, the high amounts of POXC observed in the rotation system appear to be negatively associated with lesion nematodes (Table 3.3).

(FIGURE 3.3)

3.3.3 Multivariate difference between management systems

BCA revealed clear multivariate differences according to nutrient input strategy ($p = 0.026$, based on Monte-Carlo permutations test; Fig. 3.4). The three input strategies are largely

separated based on the first principal component (PC 1, explaining 94.1% of the overall variability), with organically fertilized systems on one side, associated with higher levels of available P, pH, and *Fusarium* pressure, while inorganic systems projected on the other side, associated with higher abundance of lesion nematodes. The second principal component (PC 2, explaining 5.1 % of the variability) was associated with elevation, total C, POM and POXC, but did not explain differences between nutrient input types.

(FIGURE 3.4)

The BCA also revealed clear multivariate separation based on the four cropping systems considered here ($p < 0.001$, based on Monte-Carlo permutations test; Fig. 3.5). The first principal component (PC 1, explaining 58.0% of the overall variability) mostly separates the four cropping systems, with the more diversified systems (horticulture and rotation systems) on the right, and the more simplified practices (maize-legume intercrop and maize monocrop) on the left. The horticulture system was associated with higher levels of available P, while the rotation system was associated with higher levels of POXC, POM and total C. Meanwhile the maize-legume intercrop and maize monocrop systems were associated with higher abundance of lesion nematodes and sand content. The second principal component (PC 2, explaining 32.3% of the variability) was most associated with elevation and available P and mostly differentiated the rotation from the horticultural system.

(FIGURE 3.5)

3.4 Discussion

3.4.1 Associations between nutrient management, soil properties and plant pathogens

In the present study organic input use tended to have lower abundance of lesion nematodes but higher abundance of *Fusarium*, compared to inorganically fertilized farms.

Past research has indicated that use of organic soil amendments (e.g. compost, animal manure, peat mixes, green manure, farmyard manure, nematocidal plants, cover crops, and proteinaceous wastes) can suppress soil-borne diseases, as well as plant parasitic nematodes (Widmer et al., 2002; Oka, 2010; Forge et al., 2016). A number of possible mechanisms contributing to nematode suppression by organic amendments were reviewed by Oka (2010). These mechanisms include introduction or enrichment of antagonistic microorganisms, improved plant tolerance and resistance due to improved nutrition and vigorous growth, and changes in soil characteristics to conditions unfavorable to nematode activity and survival (Oka, 2010). Similarly, a study carried out in central Kenya comparing organic to conventional farming practices reported that use of organic inputs (such as compost, *Tithonia diversifolia* residues, and neem cake) are likely to suppress plant parasitic nematodes, compared to conventional inputs (i.e., inorganic fertilizer and nematicides), or no input applied (Atandi et al., 2017). In other studies, incorporation of organic amendments have been shown to enhance soil physical, chemical, and biological properties (Leon et al., 2006; Pérez-Piqueres et al., 2006; Bailey & Lazarovits, 2003), serving as a source of carbon and energy to soil microbes and leading to increased microbial diversity including beneficial fungi, such as nematode trapping fungi (Hu et al., 2017).

In this study, continuous use of inorganic fertilizers was associated with lower pH, compared to farms receiving only organic inputs. Diammonium phosphate (DAP) is a widely used fertilizer in western Kenya (Medvecký et al., 2007) and has been shown to lower pH over time, due to the

nitrification of ammonium by soil bacteria (Chen et al., 2020). This decrease in pH associated with some mineral fertilizers may in part explain the observed differences in lesion nematode pressure, and accords well with Simon et al. (2018) who found that lesion nematodes to decrease by 63% for every unit increase in soil pH. It has been reported that populations of beneficial nematodes such as fungivores, microbivores and omnivores are less stable as soil becomes acidic, whereas the opposite is true for plant parasitic nematodes due to reduced predation (Jiang et al., 2013; Liang et al., 2020). Also, the common practice of crop residue removal reduces SOM and nutrient availability and lowers the natural buffering capacity of soils, contributing to changes in soil chemical properties such as pH (Medvecky et al., 2007), creating a condition that affects the rhizosphere communities directly or indirectly by modifying root growth and exudates (Watt et al., 2006).

While lesion nematodes were reduced with organic inputs, *Fusarium* pressure appeared to increase in these systems. This finding is inconsistent with several studies that have found that organic amendments can improve soil biological, physical and chemical properties, and reduce existence of pathogenic organisms (Campos et al., 2016; Sipilä et al., 2012). Numerous studies, reviewed by Noble and Coventry (2005) suggest that application of high rates of organic inputs can have a suppressive effect on soil-borne disease-causing pathogens, including pathogenic *Fusarium* species. Some of the disease control mechanisms suggested in this review include beneficial microorganisms being able to successfully parasitize pathogens, produce antibiotics, effectively compete for nutrients, and activate disease-resistance genes in the plant (Noble & Coventry, 2005).

The fungal genus *Fusarium* is one of the largest, most complex and agriculturally damaging in the world. Among these, the soilborne pathogen *F. oxysporum* is one of the most ubiquitous and

damaging members. Though it lives primarily as a parasite in the vascular system of its crop host, it is also able to decompose dead plant material. Mutai et al. (*submitted*) used molecular analysis to identify *F. oxysporum* as the dominant *Fusarium* recovered from farmers' soils in the region using the bean stem baiting assay. Moreover, they were able to complete Koch's postulates with the isolates recovered, confirming that they were pathogenic on beans, though some isolates were observed to be more virulent (cause greater damage) than others. Inconsistencies in the literature on the effects of organic matter management may arise from differences in the rate, frequency, or timing of application, as well as edaphic properties such as initial soil pH. For instance, previous research by Noble (2011) shows that a high rate of compost application (at least 15 Mg ha⁻¹) is required to attain disease suppression. However, such large amounts are typically unavailable on smallholder farms. In this study, farmers reported using amounts that translate to 0-5 Mg ha⁻¹ of dry organic manure. Moreover, amending with only compost often results in inconsistent levels of disease control (Noble & Coventry, 2005), resulting in effective management of one crop disease and ineffective against another (Noble, 2011). Others have reported that, initially, both harmful and beneficial soil organisms can grow in response to application of organic amendments, and that suppressive organisms tend to flourish once decomposition processes are further along (Hoitink & Boehm, 1999; Klein et al., 2012). Bonanomi et al. (2013) observed that amending soil with labile C-rich substrate triggered pathogen spores to germinate and hyphal growth immediately after application, which stopped after about 3 weeks due to intense soil microbial activity that lead to depletion of labile substrates. The timing of our sampling could also further explain our observation of increased *Fusarium* pressure with use of organic inputs, since sampling was done a few weeks after incorporation of compost and/or manure, which could have triggered *Fusarium* germination.

3.4.2 Associations between cropping system impacts and soil parameters

We observed large differences in available P, POXC, and total C among the cropping systems studied. We found that the horticultural and rotation systems had relatively high levels of P, POXC, and total C, compared to maize monocrop and maize-legume intercrop (Fig. 3.2; Table 3.4). Similarly, our BCA analysis suggests that multiple soil properties vary with cropping system: soil pH, POM, POXC, and total C were all associated with the rotation system, while available P was more associated with horticultural systems (Fig. 3.5). These differences may be related to cropping system diversity since both the rotation and horticultural systems tend to incorporate a richer diversity of crops than the maize-monoculture or maize-bean intercrop. It is also important to note that the horticultural and rotation systems tended to rely more on organic inputs, and previous studies have found that the combination of mixed cropping with organic inputs can significantly increase total soil C and crop productivity (Adamtey et al., 2016). However, given that our multiple linear regression model at least partly accounted for variability associated with nutrient input strategies, we suggest that the cropping system differences observed here are likely due to factors beyond the types of soil inputs applied.

While we did not detect a clear effect of cropping system in our regression model, we note that the BCA analysis indicated that nematode pressure was more associated with maize-legume intercrop. The reservoir of plant pathogenic inoculum in a field is known to be influenced by the most frequently grown crops, as well as pathogen persistence in the absence of hosts (Straathof et al., 2017). Continuously rotating susceptible crops of the same family increases fungal mycelium in soil, leading to growing disease problems (Taylor et al., 2018). At the same time, crop diversity and organic inputs can influence the activity and diversity of soil biological communities, including antagonists and predators of plant feeding nematodes, that eventually

contribute to soil suppressiveness and lower pressure from pathogens, compared to a monocrop farming system (Eberlein et al., 2016; Atandi et al., 2017). Growing a mixture of crops and crop rotation can reduce most soil-borne or residue-borne diseases (Moussart et al., 2013). In this respect, the negative association between nematode lesions and crop rotation system could be explained by the fact that this system had relatively high levels of available P, total C, POM, and POXC, and may support a more diverse microbial community and diversity of root exudates that potentially influences pest performance. Several studies have reported that diversified cropping, rotations and high amounts of SOM increase microbial activity and diversity contributing to suppression of soil-borne fungi (Klein et al., 2011; Postma & Schilder, 2015; Campos et al., 2016; Eberlein et al., 2016). A diverse microbial community can contribute to soil suppressiveness against plant parasitic nematodes, either by increasing antagonistic and predator microorganisms, such as nematode-trapping fungi, predatory nematodes and microarthropods, or by changing soil properties that are less conducive for nematode persistence, or a combination of the two mechanisms (Oka, 2010; Forge et al., 2016; Atandi et al., 2017). While we did not observe strong impacts of cropping system *per se* in this study, where cropping system was co-varying with other types of management predictors, others have shown rotations and diversified cropping systems to be important and to be associated with the suppression of plant parasitic nematodes compared to monocrop systems (Kimenju et al., 2008). In our study, rotational cropping and horticulture system improved soil health characteristics such as pH, POM, and POXC, especially when organic amendments were used simultaneously. It is also important to note that influence of management and soil characteristics on soil-borne parasites can be direct or indirect (Forge et al., 2016). In our study, indirect influence may be seen by the way cropping system and nutrient inputs influence soil properties that have direct effect on the occurrence of

lesion nematodes and *Fusarium* pathogens (Fig. 3.1). For instance, high amounts of available P and POM, which was associated with horticulture system and continuous use of organic inputs, seemed to be linked to reduction in lesion nematodes.

3.4.3 Relationships between soil health parameters

Our results indicate a complex network of associations between environmental characteristics, cropping system, nutrient input, *Fusarium*, and lesion nematodes. We found a positive association between root lesions caused by lesion nematodes and soil sand content, along with negative correlations between nematode lesions and pH, POM, and POXC. Although nematodes are found in a large range of textures, severe nematode infestations have been shown to be more common on sandier soils (Koenning et al., 1996; Simon et al., 2018). Since nematodes live in water films, soil texture is an important factor that influences abundance and distribution of plant parasitic nematodes. Therefore, the highly porous and aerated sandy soils favor nematode mobility, especially in wetter climates (Chirchir et al., 2010; Costa et al., 2011).

Total C and available P had a positive association with elevation. The western Kenya high altitude areas tend to be less depleted of SOM and phosphorus compared to the lowlands. The possible explanation for this trend is the increased stream water losses, decline in SOM, and high mineralization rates in the warm lowlands that lead to rapid release and losses of N and P, compared to recent conversion of natural forests to agricultural land in the high altitude areas (Recha et al., 2013). Moreover, higher elevation farms typically get more rainfall which supports higher productivity and, thus, contributes to more soil C. Repeated cultivation over many years in tropical lowlands, coupled with increased mineralization rates, can lead to declining soil available P and C levels (Caulfield et al., 2020; Mobius-Clune et al., 2011; Nyberg et al., 2012). The positive relationship observed between soil pH and POM may be related to the fact that the

farms with high POM were mostly those that applied organic inputs on their farms or those that have been recently converted from forest to agricultural land. Additionally, soil acidity results in poor biomass production by crops, leading to reduced inputs of crop residues back to the soil, and continued low amounts of POM. Burning and removal of maize stovers in most maize fields in Trans-Nzoia County may also contribute to the low POM and pH levels observed here (Medvecky et al., 2007).

3.5 Implications for soil management and future research

Smallholder farms in Western Kenya encompass a diverse range of biophysical and socioeconomic contexts, which in turn influence the types of agroecological challenges that farmers experience, as well as the remedial measures that will help to ameliorate them. Our findings suggest that cropping system, nutrient input strategy, and environmental variables all influence soil characteristics, and indirectly determine abundance and impact of soil-borne pathogens and plant parasitic nematodes. However, our research also illustrates the complex ways that site and management factors interact and highlights the need for targeted research to help disentangle the relative importance of specific factors as key drivers within these systems. For instance, our results suggest that increased soil pH, higher crop diversity, reduced use of inorganic fertilizers, and increased SOM content lead to reduced lesion nematode pressure. However, it is important to further unravel these associations in isolation, to understand the extent to which each variable is of importance in terms of reducing nematode damage to crops in these systems. This will allow farmers and policymakers to better target innovations and incentives for changed management to improve crop performance and food security in the region.

Of special note, the methods used in this paper were selected based on their relative accessibility to resource-poor smallholder farmers and small research-oriented non-governmental organizations (NGOs). As such, these methods offer great promise for on-farm assessments and a means for farmers to work together with local technicians to quantify pest and disease pressure within a relatively short timeframe. This would allow farmers to respond more rapidly to disease outbreaks and facilitate more effective research on management strategies to address these issues. However, we suggest further testing of these assays across a wider range of agroecological conditions would help to validate these methods and understand the applicability for local practitioners. Overall, the methods applied here offer great promise in allowing farmers to better manage their soils and associated crop pests with important implications for improving productivity and food security in western Kenya and beyond.

CHAPTER 3 TABLES AND FIGURES

Table 3.1 Location and environmental characteristics of the five study locations in Nandi and Trans-Nzoia counties, Kenya.

Site	GPS coordinates	Mean annual rainfall (mm)	Average annual Temperature	Altitude Range (m)
Waitaluk	0.96°N, 35.02° E	1000- 1200	18 °C	1700-1900
Kinyoro	1.01°N, 34.93 °E	1000- 1200	18 °C	1840-1850
Kapkerer	0.02°N, 34.78° E	1000- 1800	21 °C	1400- 1700
Kiptaruswo	0.04°N, 34.56° E	1500- 2200	17 °C	1500- 2200
Koibem	0.15°N, 34.97° E	1500- 2000	17 °C	1500- 2100

GPS coordinates were taken from the center of each study area

Table 3.2 Mean values and observed ranges of soil parameters in the five study locations in Nandi and Trans Nzoia counties, Kenya.

Site	Sand (%)	Clay (%)	Total C (%)	pH	P _{Avail} (mg kg ⁻¹)	POXC (mg kg ⁻¹)	POM (mg g ⁻¹)
Kapkerer	63 (16-82)	26 (10-66)	1.8 (1.1-2.3)	5.8 (4.9-7.5)	6.8 (2.3-16.5)	484 (221-896)	0.66 (0.2-1.7)
Kinyoro	49 (44-54)	43 (34-50)	2.9 (2.6-3.4)	6.3 (5.9-6.6)	25.8 (20.4-31.5)	536 (460-593)	1.53 (1.3-1.7)
Kiptaruswo	49 (32-68)	34 (18-50)	3.2 (2.1-4.0)	6.0 (5.0-7.3)	10.9 (4.5-19.7)	836 (497-1000)	1.23 (0.6-2.1)
Koibem	41 (39-42)	44 (42-48)	4.2 (4.0-4.5)	5.7 (5.2-6.0)	5.1 (3.0-6.9)	935 (835-1037)	1.57 (0.8-2.2)
Waitaluk	59 (38-70)	30 (18-50)	2.1 (1.3-2.3)	5.3 (4.7-5.9)	14.9 (6.5-22.0)	525 (423-637)	0.28 (0.1-0.4)

POXC - permanganate oxidizable carbon, POM - particulate organic matter, P_{Avail} - available phosphorus

Table 3.3 Pearson correlation coefficients for relationships between soil parameters, elevation and Fusarium and lesion nematode pressure in soils. Correlations are based on soils collected in March 2019, from 35 smallholder farms in Nandi and Trans Nzoia counties, Kenya.

	Lesion	<i>Fusarium</i>	Altitude	pH	POM	POXC	P _{Avail}	Sand	TotalC
Lesion	1.00								
<i>Fusarium</i>	-0.29	1.00							
Altitude	-0.23	0.21	1.00						
pH	-0.39*	0.14	-0.11	1.00					
POM	-0.53	0.34*	0.11	0.49**	1.00				
POXC	-0.39*	-0.01	0.24	0.14	0.37*	1.00			
P _{Avail}	-0.24	0.24	0.52**	0.21	0.14	-0.10	1.00		
Sand	0.34*	0.14	-0.26	0.03	-0.29	-0.60***	-0.04	1.00	
Total C	-0.48**	0.10	0.43*	0.18	0.63***	0.69***	0.03	-0.63***	1.00

Values in bold represent significant coefficients. *are treatment with significant correlation at p value < 0.05, ** at p value < 0.01, *** at p value < 0.00; POXC - permanganate oxidizable carbon, POM - particulate organic matter, P_{Avail} - available phosphorus

Table 3.4 The p-values for the coefficients of multiple linear regression analyses considering management and site factors. Numbers in bold numbers indicate statistical significance ($p \leq 0.05$).

Response variables	Explanatory variables				
	Nutrient input	Cropping system	Elevation	Sand	Total C
Fusarium (Log)	0.034	0.225	0.147	0.232	0.177
Lesion nematode (Log)	0.003	0.586	0.353	0.178	0.059
POM (Log)	0.148	0.545	0.653	0.274	<0.001
P _{Avail}	0.145	0.002	0.158	0.827	0.835
POXC	0.405	0.001	0.554	0.052	0.074
pH	0.096	0.891	0.207	0.654	0.103
Total C*	0.797	0.068	0.032	0.002	--

*Total C removed from the model when included as response variable; POXC - permanganate oxidizable carbon, POM - particulate organic matter, P_{Avail} - available phosphorus

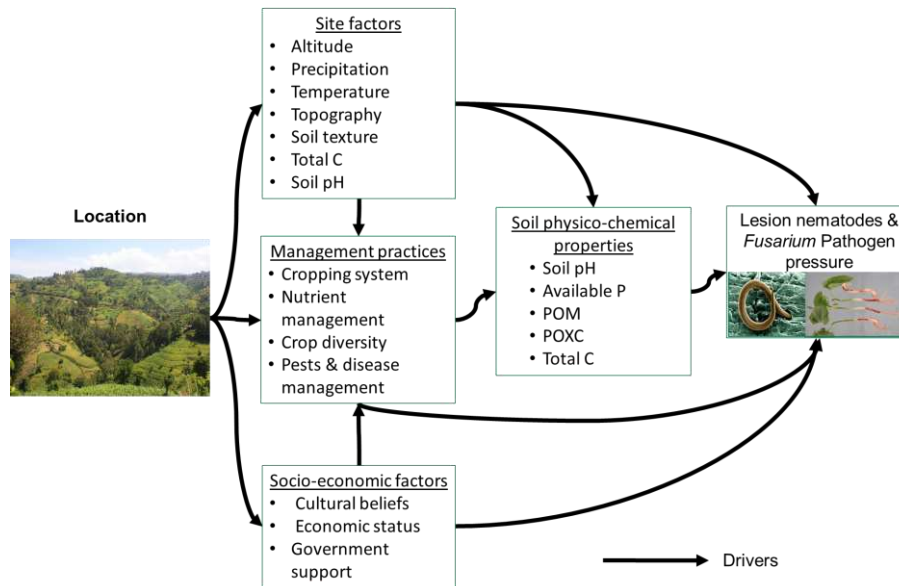


Figure 3.1 Conceptualization of the biophysical and socio-economic interactions that influence *Fusarium* and lesion nematode pressure within the smallholder systems of western Kenya.

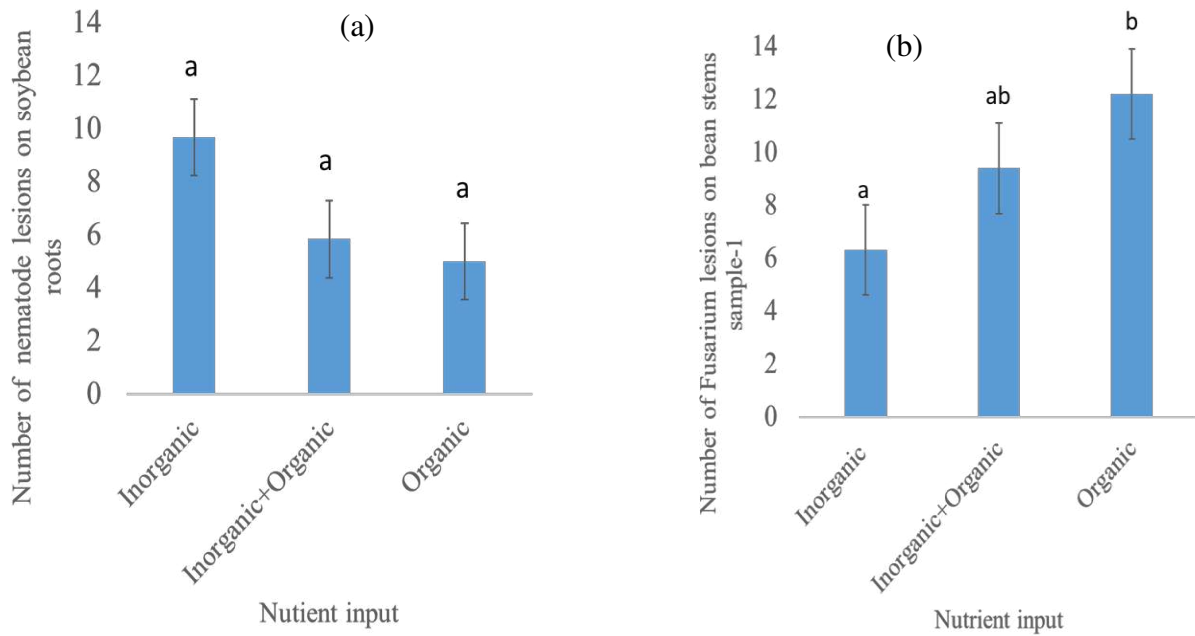


Figure 3.2a and b. Least square means for: a) lesion nematode pressure and b) Fusarium pressure from soils collected under different nutrient management strategies on smallholder farms in western Kenya. Bars represent the standard errors, whereas letters represent Tukey's test comparison, such that means with the same letter indicate no significant difference between strategies at 95% confidence interval.

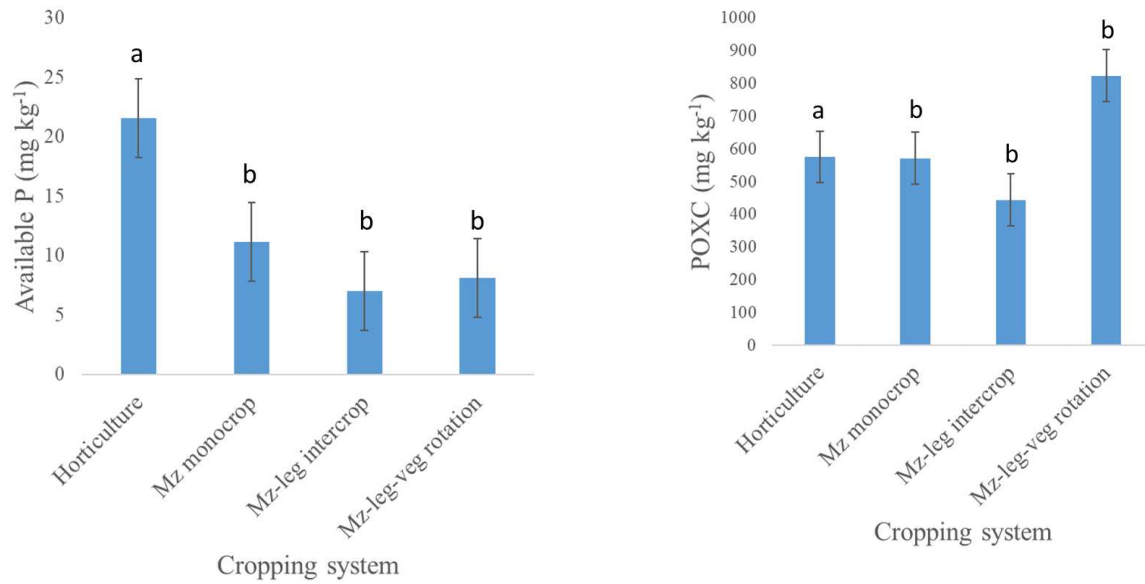


Figure 3.3a and b. Least square means for a) available P and b) Permanganate oxidizable C (POXC) from soils collected under different cropping systems on smallholder farms in western Kenya. Bars represent the standard errors, whereas letters represent Tukey's test comparison, such that means with the same letter indicate no significant difference between systems at 95% confidence interval.

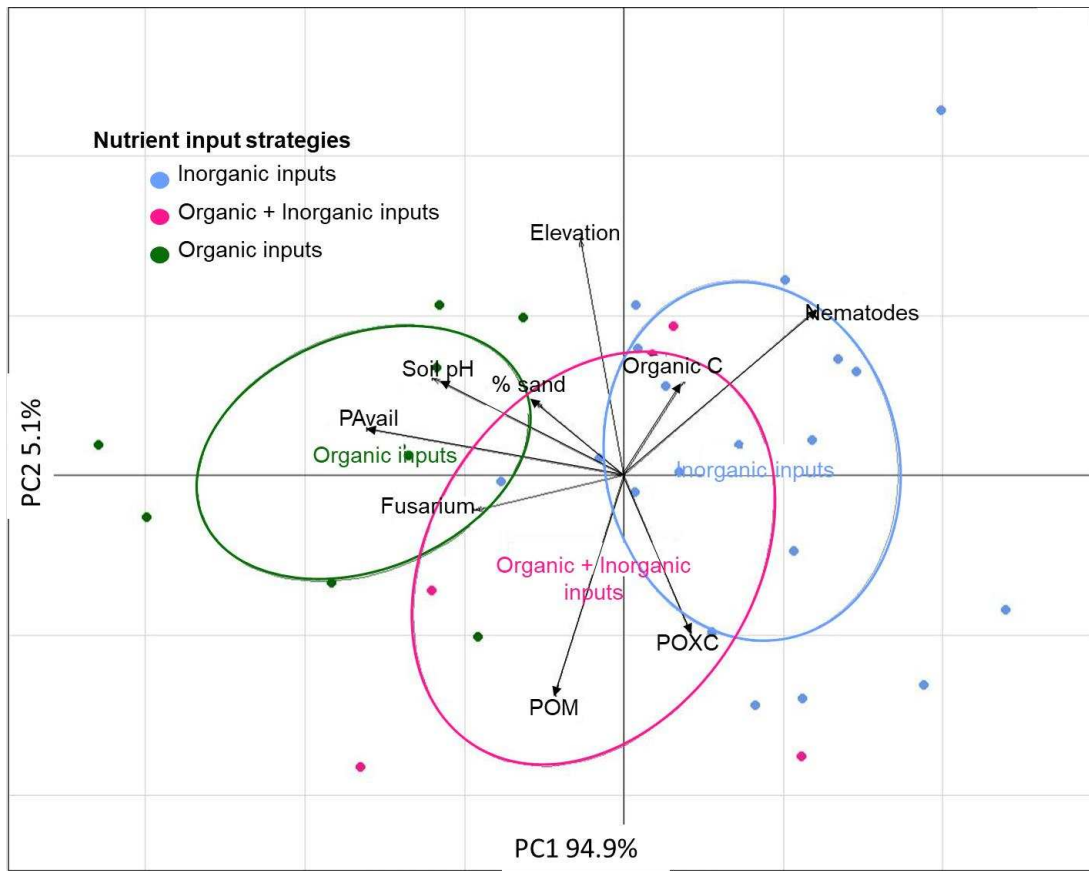


Figure 3.4 Between-Class Analysis (BCA) of studied variables based on principal components analysis grouped by the nutrient management (organic, organic + inorganic, and inorganic input use) applied in the smallholder farming systems of western Kenya. Points represent farms and ellipses represent nutrient input type. POXC - permanganate oxidizable carbon, POM - particulate organic matter, P_{Avail} - available phosphorus

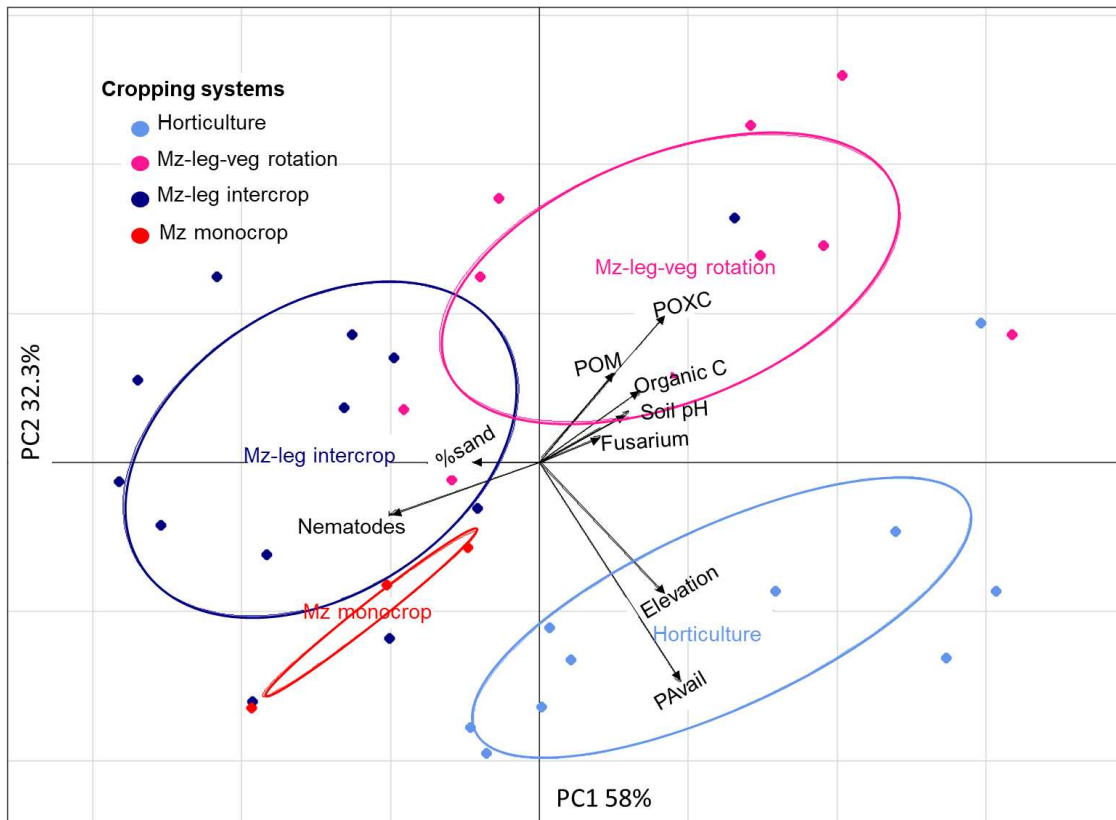


Figure 3.5 Between-Class Analysis (BCA) of studied variables based on principal components analysis grouped by the management system (Horticulture, maize-legume intercrop, maize monocrop, and maize-legume-vegetable rotation) practiced in the smallholder farming systems of western Kenya. Points represent farms and ellipses represent cropping system. POXC - permanganate oxidizable carbon, POM - particulate organic matter, P_{Avail} - available phosphorus

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CHAPTER 4: CROPPING SYSTEM AND MANURE EFFECTS ON SOIL HEALTH

PARAMETERS AND SOIL-BORNE PATHOGENS

4.1 Introduction

Sustaining soil health and productivity is a growing concern for smallholder farmers around the world, and this is especially true in sub-Saharan Africa (SSA), where soil degradation along with high pest and disease pressure often limit crop performance and lead to food insecurity (Tadele, 2017; Nafi et al., 2020). Smallholder farming systems in SSA are typically rain-fed and often associated with frequent tillage, minimal use of external inputs, continuous cultivation of just a few crops. These agricultural practices have led to continued declines in soil health and contributed to increased prevalence of soil-borne pathogens in smallholder systems (Ojiem et al., 2014; Castellanos-Navarrete et al., 2015; Rusinamhodzi et al., 2016; Ochieng et al., 2019). Common soil-borne pathogens in the region include *Fusarium* spp., *Pythium* spp., and plant parasitic nematodes (PPN, e.g., *Meloidogyne* and *Pratylenchus* species). Damage caused by these organisms is evidenced through root rot, damping off, wilt, stem rot, stunting, loss of foliage, and yellowing of leaves, and causes major crop losses in the region (Kimenju et al., 2008; Medvecky & Ketterings, 2009; Atandi et al., 2017). These issues are expected to worsen in the face of climate change, as increasing temperature and more erratic rainfall further stress crops and contribute to pest and disease incidence and severity (Pires et al., 2023).

Soil degradation in the region is often reflected by low pH, depleted soil organic matter (SOM), and poor aggregation, which are important drivers of soil microbial communities, and are likely linked to soil suppressiveness, or the ability of a soil to prevent disease in the presence of a plant

pathogen (Bonanomi et al., 2018; Roskopf et al., 2020). Practices such as the application of organic amendments and reduced tillage, that improve soil organic matter levels and enhance biological activities, have been shown to increase suppressiveness against common soil-borne diseases such as those caused by pathogenic soil fungi and plant parasitic nematodes (Stirling et al., 2012; Kurm et al., 2023). Soil chemical properties such as pH also play an important role in determining this potential. For example, ammonia, which is generated from decomposing organic inputs, plays an important role in suppressing soil-borne pathogens by interfering with their attraction to plant roots and reducing root invasion. However, there is still need for further research to understand mechanisms of soil suppressiveness in different soil types.

Cropping system diversification, crop residue retention, and application of organic nutrient inputs are known to enhance soil health and improve crop performance (Odhiambo, 2011; Castellanos-Navarrete et al., 2015; Vanlauwe et al., 2015; Liu et al., 2021), and these technologies have been promoted widely (Bayu et al., 2005; Mhango et al., 2012; Traill et al., 2018). Despite the promise of improved soil management practices, the biophysical and socioeconomic contexts of smallholder farms in western Kenya are highly heterogeneous, such that effect of different cropping systems and fertilization regimes can vary greatly from farm to farm (Tittonell et al., 2013; Nyamasoka-Magonziwa et al., 2021). Such differential impacts of management are likely due to variation in agroecological zones, soil type, rates of inputs applied, mode and timing of application, ability of farmers to sustain practices, and crops used in the rotation/intercrop systems (Okalebo et al., 2006; Ojiem et al., 2014). We also note that much of this work has involved short-term experiments, conducted for just 1 to 3 years, and thus impacts of alternative management practices on soil health may not have had sufficient time to fully develop. Long-term studies are therefore essential, to understand the potential effects of

management on soil health and overall agroecosystem performance and to better elucidate linkages between management, key soil health metrics, and soil-borne pests and diseases.

Like most smallholders in SSA, farmers in western Kenya do not have access to effective tools for managing risks to their production from biotic and abiotic stresses, including a lack of knowledge of soil-borne pathogens. These challenges, along with limited access to formal lab assessments of soil-borne pathogens prevent farmers from making informed management decisions to effectively address soil-borne pathogen issues. To further complicate matters, soil degradation and low fertility in the region often result in nutrient deficiencies that can mask the symptoms of root pathogens and/or further exacerbate their impact on crop growth (Talwana et al., 2016).

To evaluate impact of management practices on soil health, we used an existing long-term trial in western Kenya with distinct cropping systems and organic input management strategies to understand impacts on soil health and relationships with major soil-borne pathogens. The trial compares two representative cropping systems in the region (continuous maize monocrop and maize intercropped with soybean), with the inclusion of a high-residue legume (maize in rotation with *Tephrosia*) and nutrient management strategies (application of manure vs. not, and retention or removal plant residues). Utilization of *Tephrosia* (leguminous woody plant) as an agroforestry plant rotated with maize in this trial, offered a valuable opportunity to assess the importance of cropping system diversification and organic matter inputs in managing soil-borne diseases. We used existing simple and visual bioassay methods validated by Mutai et al. (*submitted*) to assess soilborne pathogens, methods that farmers could potentially use with the help of extension officers to assess incidence and severity of soil-borne pathogens on their farms. We hypothesized that: i) long-term organic matter inputs (manure, crop residues, and *Tephrosia*) leads to improved

soil health, as indicated by greater soil aggregation, SOM pools, and nutrient availability (avail P, pH), and reduced *Fusarium*, *Pythium*, and PPN pressure; and ii) continuous mono-cropping practices (continuous maize) increase *Fusarium*, *Pythium*, and PPN abundance, compared to crop rotation and intercropping. This study sought to elucidate the impacts of long-term rotations and organic matter management on soil characteristics and soil-borne pathogens, to clarify the effectiveness of strategies to improve soil health, reduce soil-borne pathogen pressure, and increase crop productivity and resilience in smallholder agriculture in Africa.

4.2 Materials and methods

4.2.1 Study area

This research was conducted in a long-term field trial established by the International Center for Tropical Agriculture (CIAT) in 2003, in Siaya County, Western Kenya (34° 24' 13.7' E 00° 08' 38.3" N). The trial focuses on nutrient management strategies and cropping rotation with the aim of improving soil health and cropping system productivity. At an altitude of 1330 m above sea level, the site experiences two rainy seasons (long rainy season from March to July and the short rainy season from September until January) with total precipitation of roughly 1800 mm yr⁻¹ and a mean annual temperature of 23 °C. Maize (*Zea mays*) is the dominant staple crop in this region and is often grown intercropped with legumes such as common bean (*Phaseolus vulgaris*) or soybean (*Glycine max*). Soils are highly weathered with low pH, low CEC, and high aluminum saturation and are dominantly acric ferralsols (Sommer et al., 2018).

4.2.2 Experimental design

For this research we selected a sub-set of treatments within the larger study (see Sommer et al.

2018 for a full experiment description). The study utilizes a randomized, split-split plot design with all treatments present in four replicate blocks. The main plot consists of manure application treatments (4 Mg dry manure ha⁻¹ per season vs. no manure application). The manure treatment main plots are then sub-divided into two residue management treatments: 1) plant residue retention (2 Mg ha⁻¹ maize stover retained each season) vs. all stover removed. These plots are then further divided into randomly allocated crop rotations: 1) continuous maize (M-M) and 2) *Tephrosia*-maize rotation (T-M: *Tephrosia* (*T. candida* species) grown in the long-rains season and maize grown in the short rains season), with each plot measuring 4.5 × 6 m. Additionally, we considered a third treatment (maize-soybean intercropping (M-S) grown in short and long rains), but only considered the plots without residue retention (farmer practice), for a total of 40 plots considered. All plots received 60 kg elemental K ha⁻¹ season⁻¹ in the form of muriate of potash, and 60 kg elemental P ha⁻¹ season⁻¹ as triple super phosphate. Under the T-M rotation, all *Tephrosia* biomass was chopped and spread across the plot before maize planting. Additional details on the experimental setup and management are provided by Sommer et al. (2018).

4.2.3 Soil sampling and analysis

Soil samples (0–15 cm) were collected in June 2021 using a soil auger (4 cm dia.), with the aim of assessing soilborne pathogens and key soil health parameters in the surface layer. Fifteen cores were taken from each plot following a zig-zag pattern (and avoiding edge effects) and combined into one composite sample and placed in sealed plastic bags. A separate sample (0-5 cm) was collected using a sharpened metal cylinder (7 cm dia.) for analysis of bulk density and wet-aggregate stability. With this second sample, two representative samples were collected per plot and carefully placed in air-tight plastic bags for transport. All soil samples were kept cool and returned to the laboratory for assessment of lesion nematodes (LN), root-knot nematodes

(RKN), *Pythium*, *Fusarium*, and key soil health parameters. Soils meant for the nematode assessment were kept on ice (at ~4 °C) during transport and processed within 24 hours of sampling. The rest of the bulk soil samples were air-dried and passed through a 2-mm sieve for subsequent assays of *Pythium* and *Fusarium*, as well as for evaluation of soil physical and chemical properties. Soils collected for the bulk density and aggregate stability were first weighed, and then a sub-sample (~40 g) was oven-dried at 105°C to determine moisture content. The remaining field moist soil was carefully passed through an 8-mm sieve before air-drying.

4.2.4 Soil physicochemical properties

Soil pH, available P, permanganate oxidizable C (POXC), and particulate organic matter (POM) were assessed using methods described by Nyamasoka-Magonziwa et al. (2020; also see <https://smallholder-sha.org>). Soil pH was assessed in a 2:1 deionised water: soil suspension, POXC was evaluated based on the oxidation of labile soil organic C by KMnO₄ (adapted from Weil et al. 2003), available P was determined using a modified Olsen method, while POM (250-2000 µm) was evaluated by aggregate disruption and density flotation in water (Nyamasoka-Magonziwa et al. 2020). Soil texture (percentages of sand, silt, and clay) and total soil C were analyzed in a commercial lab in Nairobi (Crop Nutrition Laboratory Services Ltd.), using the hydrometer method and dry combustion, respectively. Aggregate stability was evaluated using a wet-sieving method adapted from Elliott (1986; also see Nyamasoka-Magonziwa et al., 2020;) that involved slaking a sub-sample (~ 70 g) of the 8-mm sieved, air-dried soil by submerging it in deionized water for 5 minutes, then sieving through a 2 mm and then a 250 µm sieve by carefully lifting the sieve in and out of a pan of water, for a total of 50 oscillations over a 2-minute period. The soil that remained on each sieve was collected, dried at 105°C, and weighed to generate three aggregate size classes (>2,000 µm, 250–2,000 µm, <250 µm). Aggregate

stability was then calculated as the mean weight diameter (MWD) by multiplying the proportion of soil present in an aggregate size class by the mean diameter of aggregates in each size class (van Bavel et al. 1950).

4.2.5 Root-knot and root lesion nematode bioassays

Lesion nematodes (*Pratylenchus penetrans*) were evaluated using a soil bioassay developed by Gugino et al., (2008) and further validated in western Kenya by Mutai et al., (*submitted*). Briefly, two sub-samples (lab replicates) of refrigerated soil (~500 g each) were placed in separate pots (8 cm dia., 12 cm high) with drainage holes. Three soybean seeds were then planted in each pot. The plants were maintained in a greenhouse and watered as needed (no fertilizer added) for 3 weeks. Soybean seeds were also planted in soil-less media (Cornell peat mix: Boodley and Sheldrake, 1982) as a control. After three weeks, the soybean root systems were carefully removed and washed free of soil. Diagnostic root lesions were then examined on the main root by counting and recording the total number of elongated dark brown lesions observed, and the total root biomass was recorded. The number of lesions developed on the soybean roots serves as a proxy for the LN infestation level in each soil.

To estimate RKN (*Meloidogyne*) abundance, lettuce was grown according to a soil bioassay developed by Gugino et al, (2009) and validated by Mutai et al., (*submitted*). Soil processing, planting, and management of lettuce plants (three per pot) was done as described for soybean in the LN assessment above, then 3 weeks after germination, plants were removed from the pots and roots washed free of soil before the examination for root-galling. Number of galls per pot (galls on roots) were recorded and fresh lettuce roots weighed for later data calculations. Again, the number of galls per gram of lettuce roots is interpreted as an indicator of RKN infestation in a particular soil.

4.2.6 *Fusarium* *Pythium* assessment

Fusarium baiting assays were adapted from the protocol of Furuya et al. (1999), which was further evaluated by Mutai et al., (*submitted*). White bean stems, planted in a soil-less media and grown in the dark for 14 days at room temperature, were used to evaluate *Fusarium* incidence. Two sub-samples of the air-dried, 2-mm sieved soils (500 g per sample) from each farm were placed in plastic containers with lids (11 cm dia., 12 cm high) and water content adjusted to 50% field capacity. Ten white bean stem segments were then buried in each container and incubated at room temperature. After 4 days, the buried segments were removed from the soil, rinsed with clean running water, and the number of reddish-brown lesions longer than 1 mm and consistent with *Fusarium* damage were counted on the ten stems in each container. Bean stems were also buried in autoclaved sand and the soil-free Cornell mix, which were used as controls for this assay. To confirm that this *Fusarium* baiting method can be used to assess the presence of pathogenic *Fusarium* taxa, lesions were isolated, and molecular analysis and pathogenicity tests were done in a previous study (Mutai et al.; *submitted*).

For the *Pythium* assay, plastic containers (11 cm dia., 12 cm high) were filled with 125 g soil and brought to 75% field capacity. Ten bean seeds were buried in the moistened soil in sealed containers, incubated at 21 °C for 3 days. Seeds were then removed from the soil, thoroughly rinsed with de-ionized water, blotted dry, placed on a *Pythium*-selective medium (Ali-Shtayeh, 1986), and incubated for 2.5 days. Following incubation, seeds were evaluated for the presence/absence of mycelia growing into the media from individual seeds, and number of observed colonies noted. Soil known to contain *Pythium* inoculum of the (positive control) and an autoclaved sand (negative control) were included in the assay.

4.2.7 Data analysis

A three-way ANOVA was conducted to evaluate management impacts on studied soil properties and soil-borne pests and disease. The model included farmyard manure, cropping system, residue management, and all possible interactions as fixed effects, while block (replicates), sub-plots, and sub-sub plots were included in the model as random effects to address the split-split plot design of the experiment. Since we did not assess the residue retention treatments for the maize intercropped with soybean rotation, we conducted analyses using two separate ANOVA models: one omitting maize-soybean intercrop system in the model ($n = 32$), and the other including all the cropping systems but leaving out plant residue retention treatments ($n = 24$). The significance level for all statistical tests was set at $p < 0.05$. A square root transformation was applied to *Fusarium*, *Pythium*, and nematode abundances as well as POM in both models, to meet ANOVA assumptions related to homogeneity of variance and normality of the residuals. To understand the relationship between soil properties, *Fusarium*, *Pythium*, LN and RKN, Pearson's correlation analysis was performed. All analyses were conducted in JMP Pro version 16.2.0.

4.3 Results

4.3.1 Changes in soil health properties with addition of manure, plant residues, and distinct cropping systems

Cropping system and manure application were the most important drivers of the observed differences in soil properties. For example, manure additions significantly increased pH by 7% and total C by 9% across all treatments, while cropping system influenced both pH and bulk density, such that T-M consistently had lower pH and bulk density than M-M (Table 4.1). Numerous significant interactions, however, suggest that effects were not uniform across treatments for many of the soil variables (Table 4.1). For example, both manure and the T-M rotation generally increased POXC, but a significant two-way interaction between manure and cropping system reveals that T-M increased POXC the most (relative to M-M) in plots receiving manure. A similar interaction between manure and residue management suggested that residue retention only increased POXC in the presence of manure. While POM was generally higher in T-M vs. M-M, a significant three-way interaction suggested that the effect was strongest with both manure and residue application. Similarly, aggregate stability was generally increased T-M, but the magnitude of the effect was not consistent across all residue and manure treatments (Table 4.1). Although plant residue did not show any simple effects on soil properties, significant interactions with manure suggest that plant residue management does have some effect on both POXC and available P.

When the M-S intercrop system was included in the model (and the residue retention treatments ignored), manure significantly enhanced soil POXC, MWD, and pH, while the T-M rotation system consistently increased total C and available P and reduced soil pH (Table 4.1).

(TABLE 4.1)

(FIGURE 4.1)

Our findings reveal multiple effects of cropping system and organic matter application on soil-borne pathogens, but significant interactions (Table 4.2) for all pathogens suggest many dependencies between management drivers. Perhaps the clearest effect was observed for RKN, where manure addition reduced RKN presence by 92%. An interaction with cropping system suggested that T-M generally increased RKN in the absence of manure (Fig. 4.1a), but not in its presence. Manure also increased *Fusarium* by 54% (Fig. 4.1c) and had a marginally significant ($p = 0.066$) reduction of *Pythium* colonies (Fig. 4.1d), relative to no manure application. Significant interactions also suggests that T-M rotation appeared to increase *Fusarium* much more in the presence of manure than in its absence. *Pythium* shows a largely opposite trend, whereby T-M decreased *Pythium* in the absence of manure, but less so when manure was added. For LN, a significant interaction between residue management and manure indicates that residue retention increased the number of lesions per gram of root in the absence of manure, but not when manure was applied.

Similar cropping system by manure interactions were observed in the analyses including the M-S treatment (and with residue retention treatments ignored). For example, M-S increased the number of lesions from both the *Fusarium* and LN assays in the absence of manure, but not when it was applied.

(TABLE 4.2)

4.3.2 Relationships between soil properties and soilborne pathogens

Bivariate correlations revealed a number of significant relationships among soil properties and disease incidence. For example, pH was positively correlated with POXC and bulk density and negatively related to POM, while POM was negatively correlated with aggregate stability (Table

4.3). All soil-borne pathogens assessed, except LN, were significantly correlated with at least one soil health parameter across management treatments (Table 4.3). Both *Pythium* and RKN were negatively correlated with pH and POXC, while *Fusarium* was positively correlated with POXC, Total C and % sand (Table 4.3).

(TABLE 4.3)

4.4 Discussion

4.4.1 Impacts of organic matter inputs on key soil health parameters

Our study tested the hypothesis that long-term organic matter inputs, specifically manure and plant residue return, would improve SOM, soil aggregation, and nutrient availability, thereby reducing the presence of soil pathogens. Our findings showed that manure application appeared to have had a stronger effect on SOM, compared to plant residue return. For instance, while plant residue applied alone did not have any significant effect on the assessed SOM pools or any other soil properties, manure applied alone significantly increased POXC and total C, and increased POM when applied in combination with plant residue. This differential impact likely stems from the quantity and quality of organic inputs, with manure applied at a higher rate of 4 Mg ha⁻¹ per season compared to plant residue retention of just 2 Mg ha⁻¹ maize stover per season. These results are consistent with previous findings in the same long-term experiment which found that manure application slowed down losses of soil organic carbon overtime, compared to not applying manure (Sommer et al., 2018). Ye et al. (2019) in a different study also found animal manure increases SOM more than plant residue and suggested that manure enhanced SOM better through increased recalcitrant organic C input, soil aggregate protection, and somewhat through fungal necromass enhancement.

Literature extensively corroborates that organic inputs such as manure, especially when combined with appropriate crop rotation and application of crop residues, result in enhanced levels of SOM, water retention, diversified soil biological communities (Mubarak et al., 2003; Liu et al., 2016; Soonvald et al., 2019; Nafi et al., 2020). Interestingly, we found that manure increased soil pH, but there was no effect from maize residue return, suggesting that manure could have resulted in improved soil buffering capacity, adjusting soil pH, as explained by Shi et

al. (2019). Sommer et al. (2016) suggested that manure can enhance macro and micro-nutrients in soil, which are essential for plant growth. Such improvements to soil health parameters are likely to facilitate crop growth and rooting ability and the capacity to take-up necessary nutrients, which in turn increases potential crop residue return (both above and below ground) and can be an important factor in further contributing to SOM pools (Liu et al., 2016; De Bauw et al., 2021). However, in this study residue return was maintained at a constant rate (2 Mg yr^{-1}) across residue retention treatments, which may have limited these sorts of positive feedback effects from soil health and nutrient increase and resulting increases in residue return.

4.4.2 Impacts of cropping systems on key soil health parameters

In this study, we found T-M rotation had the highest SOM levels over the years relative to continuous M-M and M-S system. Specifically, there were significant increases of POXC, POM, in T-M rotation indicating that rotation system with leguminous trees (*Tephrosia*) increases soil organic matter due to large inputs of high quality litter (Munthali., 2014), compared to M-S and M-M systems. Additionally, we observed an increase in soil aggregation and available P, and a reduction in bulk density in the T-M rotation system. In a separate study conducted in the same experimental site, the highest seasonal maize yields were observed in T-M treatments compared to maize monocrop and maize intercropped with soybean - considering treatments with equal levels of nutrient inputs - (Sommer et al., 2016), implying that enhancement in soil health parameters associated with T-M contribute to improved crop performance. *Tephrosia* is one of the leguminous plants recommended for rehabilitating degraded soil due to its ability to increase SOM through above- and below-ground biomass (Akinnifesi et al., 2010; Munthali., 2014). The enhancement of soil parameters in the T-M system supports the suggestion by Akinnifesi et al. (2010) that *Tephrosia* integration in smallholder farming systems experiencing continuous

nutrient mining would deliver a range of other benefits including high N inputs and biomass production, leading to enhanced soil physical and chemical properties, and subsequent crop performance.

Despite soils in most parts of western Kenya being innately acidic, agricultural activities that exhaust SOM often further accelerate soil acidification leading to base cations leaching, reduction in cation exchange capacity, poor soil structure, and general lowering of soil productivity (Nyambo et al., 2018). Contrarily, although T-M rotation led to SOM enhancement in our study, it seemed to lead to lower soil pH compared to continuous M-M system. Past research also suggest that legume inputs can acidify soil overtime through excretion of H⁺ to maintain internal plant electroneutrality during N-fixation (Maltais-Landry, 2015), results were different from the findings in the degraded tropical soils of Ethiopia, where *Tephrosia* enhanced soil organic carbon, pH, and total N (Mamuye et al., 2020). As the present study has shown, however, despite *Tephrosia*'s potential to lower soil pH, its cumulative impact on soil is likely more beneficial given its positive influence on numerous other drivers of soil health characteristics such as SOM, aggregation, and bulk density.

While many smallholder farmers in western Kenya intercrop maize with soybean or common bean, results from this study suggest that soybean integration in the system without residue return to soil may not alter SOM and nutrients availability, compared to integration of N-rich legumes such as *Tephrosia*.

4.4.3 Impacts of organic matter inputs and cropping system on soil-borne pathogens

We hypothesized that monocropping and lack of organic matter inputs use would increase *Fusarium*, *Pythium*, and PPN abundance, compared crop rotation and intercropping. Results indicate that some soil-borne pathogens can be reduced significantly by management practices

that increase SOM and associated C pools (i.e., POXC, POM), especially manure addition. Organic inputs additions improve plant growth (reduce stress) through increased soil nutrients, boosting their ability to resist attacks by soilborne pathogens (Vida et al., 2020). Organic amendments may also support antagonistic microbes that control and/or compete with soil-borne pathogens and thus suppress them (Bonanomi et al., 2018).

While the T-M rotation system appeared to generally enhance soil health parameters, it also seemed to increase RKN relative to both M-M and M-S systems. *Tephrosia*, particularly *T.candida* and *T. vogelii* species, can be beneficial leguminous cover crops improve soil fertility and also manage crop insect pests due to their production of insecticidal rotenoids rotenone (Koono & Dorn, 2005; Kayange et al., 2019), in addition to their compatibility with maize. However, studies done by Desaegeer & Rao, (2001) in western Kenya revealed that *T. Vogelii* , which has similar pesticidal properties to *T. candida* used in this study, can be a good RKN host, and increased RKN and the nematode damage on the subsequent crop.

Whilst T-M increased RKN compared to M-S, the SOM increase with T-M appeared to have played a beneficial role. For instance, POXC, which had significant negative correlations with *Pythium* and RKN, was enhanced the most by the T-M system. Organic matter additions represent a source of new energy resources that can help support soil food webs and have been reported to reduce pathogenic *Pythium* abundance by supporting antagonistic activities (Nzungize et al., 2012). This association points out the possible significance of integrating *Tephrosia* or other high-residue legumes into smallholder cropping systems in efforts to improve soil fertility. However, in the case of *Tephrosia* there is a trade-off between its beneficial effect on SOM and the multiplication of RKN associated with it, which could potentially lower subsequent crop performance.

In this study, *Fusarium* incidence increased with manure addition, T-M rotation, and enhanced total C, POXC, and soil aggregation. Similar results were established by Wakelin, et al. (2008), where incorporation of maize residue enhanced soil organic C and *Fusarium* populations. In another study which compared the effect of cropping rotations on cellulolytic Fungi, they reported high incidence and variety of *Fusarium* spp. in a more complex rotation system (corn followed by wheat-soybean, then followed by soybean), compared to a simplified system of wheat-soybean rotation (Luque et al., 2005). *Fusarium* species are widely distributed in soil, associated with plants, and most of them harmless organic matter decomposers (Alabouvette et al., 2009). Moreover, it has been described that *Fusarium* strains (both pathogenic and non-pathogenic) are able to colonize plant root surface and can penetrate the roots following a similar pattern (Alabouvette et al., 2009). However, under favorable conditions (good host, favorable temperature and moisture, insufficient SOM levels to support their energy needs, and high virulence spp.), pathogenic *Fusarium* spp. can cause important diseases such as *Fusarium* wilt, root rot, blight, and damping off in a large number of plant species (Summerell et al., 2010). In our previous study, we sought to validate methods used in this study (including *Fusarium* assessment bioassay) and after isolation, DNA sequencing and pathogenicity tests, we found that most *Fusarium* spp. that caused lesions in the stem assay were pathogenic, although some of these had very low virulence levels suggesting they could be saprophytic (Mutai et al., *submitted*). Therefore, it is not surprising that our results suggest that *Fusarium* abundance increases with manure addition and multiple cropping systems that enhanced SOM, which points out the need for further research to understand the relationship between high carbon source and the extent to which *Fusarium* population that may result from high SOM conditions actually causes plant disease and negative impacts on yield.

4.5 Conclusions

Smallholder agricultural systems are predominantly characterized by low crop diversity, constrained nutrient inputs, and continuous cultivation, which predispose them to significant soil-borne pathogen damage. This challenge is anticipated to intensify with the dual pressures of population growth and climate change, as outlined by Pires et al. (2023). Evidence from the long-term trial analyzed here, including cropping systems and nutrient management, reveals that the application of farmyard manure and the rotation with leguminous woody plants, such as *Tephrosia*, substantially enhance SOM levels, soil aggregation, and bulk density. Furthermore, these practices have been shown to markedly reduce the prevalence of some key soil-borne pathogens, with some key exceptions that show the need for further research and caution about beneficial crops and their potential roles as alternate hosts in the case of *Tephrosia* and RKN. Nevertheless, the adoption and consistent application of many of these strategies could significantly improve soil productivity, diminish pathogen-induced damage, and elevate crop productivity, underscoring their importance in the sustainability and resilience of smallholder farming operations.

CHAPTER 4 TABLES AND FIGURES

Table 4.1 Mean values for key soil health properties evaluated across three cropping systems, two residue management and two manure application treatments in a long-term trial in Siaya County, western Kenya. Sampling was conducted in June 2021, 18 years after long-term trial establishment. Values to the right of each mean, in parentheses, represent the standard error of the mean. ANOVA results for soil properties and all possible treatment interactions are presented below, with significant effects ($p < 0.05$) in bold. The first ANOVA table includes all the plots, except those with soybean ($n = 32$), while the section at the bottom includes soybean treatments but excludes those with residue retention ($n = 24$). Means and SEs are represented for raw data, while p-values are presented for transformed data.

Manure	Plant	Cropping	pH	POM	POXC	BD	MWD	Total C	P_{Avail}
	residue	system		(mg kg ⁻¹)	(mg C kg ⁻¹)	g cm ⁻³	(μm)	(g kg ⁻¹)	(mg P kg ⁻¹)
No	No	M-M	4.97 (0.03)	100 (20)	414 (14.0)	1.43 (0.04)	531 (39.3)	1.70 (0.04)	24.28 (1.27)
No	No	T-M	4.73 (0.05)	160 (10)	399 (34.6)	1.33 (0.04)	591 (45.1)	1.72 (0.06)	28.41 (0.36)
No	No	M-S	4.96 (0.04)	140 (10)	388 (29.4)	1.34 (0.08)	572 (44.1)	1.56 (0.10)	25.14 (1.99)
No	Yes	M-M	4.97 (0.06)	150 (10)	338 (21.9)	1.48 (0.07)	612 (23.4)	1.77 (0.07)	29.20 (1.58)
No	Yes	T-M	4.77 (0.04)	170 (0)	380 (14.9)	1.35 (0.04)	566 (10.5)	1.76 (0.11)	27.87 (1.72)
Yes	No	M-M	5.26 (0.05)	140 (20)	543 (19.4)	1.51 (0.08)	577 (40.1)	1.86 (0.10)	24.38 (1.40)
Yes	No	T-M	5.05 (0.02)	150 (20)	650 (30.0)	1.45 (0.06)	601 (48.5)	2.00 (0.07)	27.80 (1.38)
Yes	No	M-S	5.22 (0.08)	140 (20)	563 (29.0)	1.37 (0.07)	660 (40.5)	1.72 (0.08)	22.08 (3.52)
Yes	Yes	M-M	5.33 (0.01)	100 (0)	653 (26.7)	1.64 (0.09)	520 (19.2)	1.64 (0.09)	23.30 (1.13)
Yes	Yes	T-M	5.15 (0.06)	160 (10)	728 (16.1)	1.39 (0.05)	656 (31.3)	2.06 (0.11)	27.80 (1.61)

ANOVA results	p-value	p-value	p-value	p-value	p-value	p-value	p-value
Manure	0.004	0.477	<0.001	0.339	0.384	0.050	0.232
Plant residue	0.125	0.377	0.121	0.613	0.622	0.817	0.176
Cropping system	<0.001	0.003	0.004	0.009	0.046	0.061	0.004
Manure x plant residue	0.323	0.075	0.002	0.629	0.607	0.256	0.044
Manure x cropping system	0.619	0.914	0.022	0.710	0.093	0.074	0.121
Plant residue x cropping system	0.499	0.888	0.681	0.339	0.946	0.402	0.178
Manure x plant residue x cropping system	0.856	0.040	0.153	0.401	0.018	0.300	0.054
ANOVA results soybean included							
Manure	0.011	0.729	0.006	0.465	0.046	0.092	0.614
Cropping system	<0.001	0.105	0.160	0.355	0.355	0.048	0.049
Manure x cropping system	0.744	0.160	0.105	0.656	0.656	0.686	0.642
<hr/> POM- Particulate organic matter; POXC- Permanganate oxidizable C; BD- bulk density; and MWD- Mean weight diameter. Treatments are designated as: Continuous maize monocrop, M-M; Tephrosia grown in rotation with maize, T-M; Maize intercropped with soybean, M-S; plant residue retention, RES, and farmyard manure application, FYM. <hr/>							

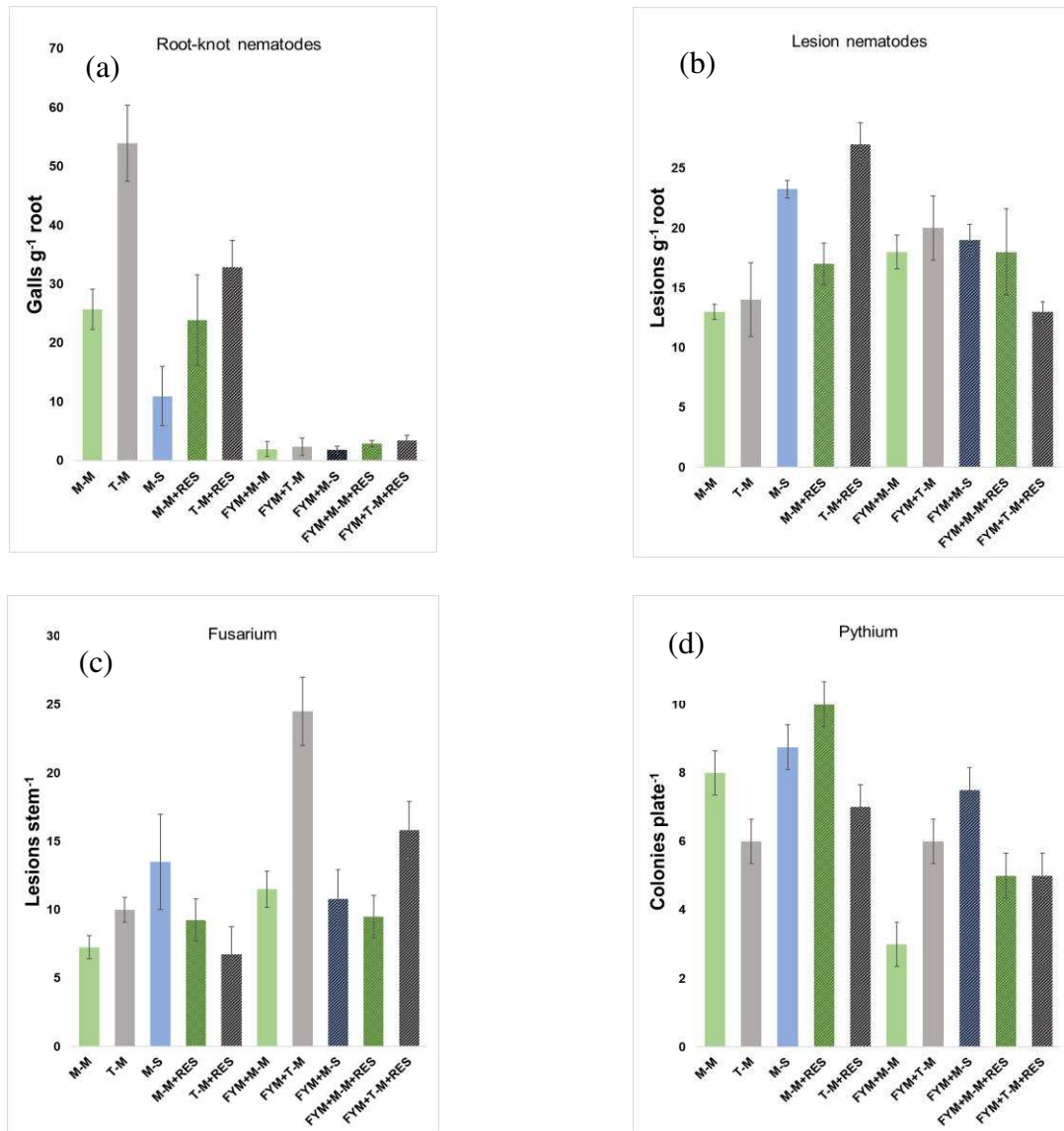


Figure 4.1 Mean soil-borne pathogen pressure for: a) root-knot nematodes, b) lesion nematodes, c) *Fusarium*, and d) *Pythium* assessed across three cropping systems, two residue management and two manure application treatments in a long-term trial in Siaya County, western Kenya. The error bars presented represent the individual standard errors from each treatment. Treatments are designated as: Continuous maize monocrop, M-M; Tephrosia grown in rotation with maize, T-M; Maize intercropped with soybean, M-S; plant residue retention, RES, and farmyard manure application, FYM.

Table 4.2 ANOVA results (p-values) for organic matter management and cropping system treatment effects (and all possibly interactions) on soil-borne pathogens in soils collected from a long-term field trial in Siaya, Kenya in June 2021, 18 years after trial establishment. The first ANOVA table includes all the plots, except those with soybean (n = 32), while the table at the bottom includes soybean treatments but excludes those with residue retention (n = 24).

	<i>Fusarium</i> (lesions stem ⁻¹)	<i>Pythium</i> (colonies plate ⁻¹)	RKN (knots g ⁻¹ root)	LN (lesions g ⁻¹ root)
ANOVA results	p-value	p-value	p-value	p-value
Manure	<0.001	0.066	0.001	0.482
Plant residue	0.050	0.264	0.328	0.415
Cropping system	0.010	0.758	0.043	0.213
Manure x plant residue	0.223	0.663	0.017	0.050
Manure x cropping system	0.007	0.011	0.098	0.013
Plant residue x cropping system	0.050	0.200	0.432	0.732
Manure x plant residue x cropping system	0.647	0.745	0.512	0.006
ANOVA results with soybean included	p-value	p-value	p-value	p-value
Manure	0.025	0.234	0.004	0.291
Cropping system	0.022	0.008	0.003	0.028
Manure x cropping system	0.018	0.036	0.004	0.039

The (x) symbol refers to the interaction between different treatments. M-M- maize monocrop; T-M- Tephrosia grown in rotation with maize; and M-S- maize intercrop with soya beans; RKN- root knot nematodes; LN- lesion nematodes

Table 4.3 Pearson correlation coefficients for relationship between soil physical, chemical and soil-borne pathogens in soils collected from a long-term trial in western Kenya, in June 2021

	<i>Fusarium</i>	<i>Pythium</i>	RKN	LN	pH	POM	POXC	P _{Avail.}	BD	MWD	Total C	% Sand
Fusarium	1.00											
Pythium	-0.08	1.00										
RKN	-0.39	0.23	1.00									
LN	-0.08	0.15	0.01	1.00								
pH	0.07	-0.41**	-0.79***	-0.11	1.00							
POM	0.10	0.13	0.25	0.05	-0.46**	1.00						
POXC	0.47**	-0.55**	-0.66**	-0.20	0.68***	-0.20	1.00					
P _{Avail.}	0.12	0.19	0.19	0.00	-0.45**	0.38*	-0.15	1.00				
BD	-0.05	-0.12	-0.23	0.00	0.35*	-0.39*	0.22	0.02	1.00			
MWD	0.29▪	0.25	-0.08	-0.20	-0.09	0.39*	0.11	0.00	-0.05	1.00		
Total C	0.46**	-0.22	-0.29	-0.12	0.13	0.17	0.45**	0.30*	0.11	0.19	1.00	
% Sand	0.42**	-0.21	-0.13	0.06	0.07	0.05	0.27▪	-0.05	0.04	-0.02	0.21	1.00

* Significant correlations at p value < 0.05, ** at p value < 0.01, *** at p value < 0.001; ▪marginal significance (p < 0.10). RKN: Root-knot nematodes; LN: lesion nematodes; POM: Particulate organic matter; POXC: Permanganate oxidizable C; P_{Avail.}: Available P; BD: Bulk density; MWD: Mean weight diameter

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CHAPTER 5: LONG-TERM ORGANIC MATTER INPUTS ENHANCE SOIL HEALTH AND REDUCE SOIL-BORNE PATHOGEN PRESSURE IN MAIZE-BEAN ROTATION SYSTEMS

5.1 Introduction

Poor soil health and insufficient nutrient inputs combined with increasing soil-borne pest and disease pressure constrain crop productivity in most smallholder farming systems in sub-Saharan Africa (SSA) (Medvecky et al., 2007; Bayu et al., 2005; Amusan et al., 2011; Mhango et al., 2012). Crops rely on healthy soils that support good tilth, water holding capacity, nutrient supply and retention, and beneficial soil organisms, as well as the regulation of plant pathogens and pests (Larkin, 2015). Soil organic matter (SOM) is a key element of soil health, as it regulates important soil properties such as soil structure and porosity, water infiltration and drainage, nutrient availability, as well as soil organismal diversity and activity (Liu et al., 2021; Olayemi et al., 2022). In Kenya, as in much of SSA, soil degradation has resulted in reduced SOM, soil structure and pH that ultimately contribute to poor and unstable crop yields (Githongo et al., 2023). Soil-borne pathogens further reduce crop performance and are increasingly being widespread especially in intensively cultivated soils of the region (Mwang'ombe et al., 2008; Atandi et al., 2017).

A variety of pathogens can cause extensive damage to maize and grain legumes, the staple crops in Kenya (Henry et al., 2019). For example, plant parasitic nematodes (PPN) cause damage by disrupting the vascular system and interfering with physiological processes, leading to poor growth, wilting, and sometimes crop death. Root knot nematodes (*Meloidogyne* spp.) and lesion nematodes (*Pratylenchus* spp.) are among the most damaging PPN reported in association with

continuous cultivation, low soil nutrients, and sub-optimal agronomic practices in Kenya (Villinger et al., 2016; Atandi et al., 2017; Mutai et al., *submitted*). Similarly, *Pythium* and *Fusarium* spp. are important soil-borne microbial pathogens (oomycetes and fungi, respectively) of considerable economic importance and are associated with up to 70% of crop losses in some farms of the region (Nzungize et al., 2012). Effects of these soil-borne pathogens includes inhibition of germination, poor emergence, or rotting and collapse of seedlings at the soil level, generally described as damping off (Lamichhane et al., 2017; Henry et al., 2019). Additionally, in established crops, *Fusarium*, *Pythium* and PPN can still cause symptoms such as root and stem browning, rots of roots, stunting, root lesions, crown necrosis, loss of root density, wilting, decay and stem collapse, reducing crop performance (Ayala-Doñas et al., 2020). We also note that soil borne pathogens (fungi, oomycetes, PPN) often share the same habitat in the rhizosphere (Ayala-Doñas et al., 2020), and can interact to cause significant losses to farmers.

Increasing reliance on organic nutrient inputs represents a key principle of regenerative agriculture that can improve soil health while supporting crop productivity (Lundgren et al., 2021; Prairie et al., 2023). Organic amendments typically support soil biodiversity and more robust soil communities that are important for healthy soils (Bruggen and Termorshuizen, 2003; Wei et al., 2017; Bonanomi et al. 2018; Olayemi et al., 2022). Livestock manure and crop residues are the main organic inputs applied in smallholder systems of Kenya (Castellanos-Navarrete et al., 2015), while use of mineral fertilizers remains low due to high costs and limited accessibility (Chianu et al., 2011; Kamaa et al., 2011; Nyamasoka-Magonziwa et al., 2021). Animal waste and crop residue recycled to soils by resource-poor farmers to supplement soil nutrients often varies widely in quality and application rates, resulting in inconsistent impacts on yield (Ayuke et al., 2011). Regardless of the short-term impacts on crop growth, organic inputs

are known to enhance soil physical, chemical and biological properties (Ghorbani et al., 2008), and thus support crop productivity in the long-term. In the smallholder systems where quality and rate of organic resources are variable, integrated soil fertility management (combining organic inputs and inorganic fertilizers) has been widely promoted as a means to support soil health while ensuring that crops have adequate nutrition (Vanlauwe et al., 2010).

While organic amendments are known to support soil health, knowledge of the relationship between soil-borne pathogens, soil management practices, and soil properties remains limited in most smallholder farming systems (Ngoya et al., 2023). The high pressure of pests and diseases, in addition to soil nutrient deficiencies can produce symptoms that are often challenging to distinguish. In some cases, pathogen to pathogen interaction between common pathogens such as *Fusarium*, *Pythium*, *Macrophomina*, *Rhizoctonia*, *Phytophthora*, and plant parasitic nematodes can lead to more confusing disease complexes. Thus, there is a great need for improved assessment of major soil-borne pathogens and to relate them to soil conditions and management practices (e.g., organic vs inorganic inputs) so that farmers can better manage pathogens, enhance overall soil health, and support crop yields.

To address these knowledge gaps and support improved management of soil-borne pathogens in the region, we leveraged a long-term (45-yr) trial in Kabete, central Kenya focused on integrated soil fertility management within a maize-bean rotation, the dominant cropping system for many smallholder farmers in the region. Long-term experiments are critical for evaluating the impact of management on soil health, as many soil parameters, especially those linked to SOM, are slow to respond and may require more than a decade to result in measurable differences (Peterson et al., 2012). The aim of this study was therefore to assess the long-term impact of distinct nutrient management strategies on key soil health properties and major soil-borne pathogens. We

hypothesized that increased reliance on organic amendments such as farmyard manure and crop residues would enhance multiple soil health parameters and reduce soil-borne pathogen pressure.

5.2 Materials and methods

5.2.1 Study area and experimental design

This study was conducted within a long-term trial based at Kenya Agricultural and Livestock Research Organization (KALRO) in Kabete, near Nairobi, Kenya (Lat. 2574 S, Long. 36.774 E). At 1740 m above sea level, the site receives a mean annual rainfall of 940 mm distributed over two rainy seasons: the long rains from March to May and short rains from October to December. The mean annual minimum and maximum temperature is 13 °C and 24 °C. The area corresponds to a dry-winter subtropical highland climate (Cwb), according to Köppen climate classification. Soils in the area are classified as humic nitisols and are typically clay loam texture. The soils are well drained, deeply weathered, dark reddish brown to dark red, with friable structure (Ayuke et al., 2011), with existence of a slight incline on the slope of the plots.

The experiment was established in 1976 to compare impacts of organic and synthetic inputs alone and in combination. Since the start of the experiment, all plots (7 m by 4.5 m) have been typically tilled by hand hoeing at the beginning of each season and weeded twice within each season. For this study, we selected treatments based on three input factors: 1) application of synthetic fertilizers (or not), 2) application of manure (or not), and 3) incorporation vs. removal of maize stover, in all possible combinations, for a total of eight treatments. The cropping system in this trial entailed growing maize (*Zea mays*; Hybrid 512) in the long rains season and common bean (*Phaseolus vulgaris*; variety Rosecoco) in the short rains season. Synthetic fertilizer is typically applied at rate of 52.6 kg P₂O₅ ha⁻¹ yr⁻¹ as triple superphosphate (TSP) at maize

planting for P, and 120 kg N ha⁻¹ yr⁻¹ as calcium ammonium nitrate (CAN) top-dressed at seven weeks after planting maize, for N. Manure application treatments receive 10 Mg ha⁻¹ yr⁻¹ dry cattle manure that is incorporated into the soil prior to planting at the start of the long-rains season (maize). For the treatments receiving plant residues, all maize stover from the previous crop in each plot is incorporated into the soil (20 cm). Bean residues are always removed from all plots, as is the common practice for harvest. The experimental design consisted of a split-split-plot design, with four replicate blocks, and all treatments randomly applied to plots within each block. The first split consisted of synthetic fertilizers applied vs not, the second split entailed farmyard manure addition vs not, and last split maize stover incorporated into the soil vs not. We note that a compound fertilizer 20:20:0 was used as a source of N and P in the early years of the experiment (from 1976 to 1980), but was replaced in 1981 by CAN and TSP at the rates stated above (Kamoni et al., 2007).

5.2.2 Soil sampling and analysis

Soil was sampled from all 32 plots in June 2021, by collecting 15 sub-samples per plot in a zig-zag pattern to a depth of 15 cm using a soil auger (4 cm dia.). Sub-samples were then pooled, homogenized, and a representative composite sample of 3 kg fresh soil was collected from every plot. The soil samples were used in four bioassays: 1) assessment of lesion causing nematodes, 2) root knot nematodes, 3) *Pythium*, and 4) *Fusarium* pathogens, as well as for the analysis of soil physical and chemical properties. Upon arrival in the lab, samples were air-dried and passed through a 2 mm sieve prior to analysis. Soil collected in the field for nematode bioassays (1 kg of fresh composite sample) was kept cool and processed within 1 day of arriving at the lab.

Soil chemical parameters measured on the 2 mm sieved air-dried soil include permanganate oxidizable C (POXC), particular organic matter (POM), available P, and pH. These analyses

were conducted using a soil tool kit developed for low-cost, rapid assessment of soil on smallholder farms, according to methods described by Nyamasoka-Magonziwa et al., (2020; also see <https://smallholder-sha.org>). Soil pH was measured in a 2:1 deionised water:soil suspension. Evaluation of POXC was based on the oxidation of labile soil C by potassium permanganate (KMnO₄), available P was determined using a modified Olsen P (Olsen extraction and ascorbic acid colorimetric method), while POM (250-2000 µm in size) was determined via sieving and density flotation with water to separate POM from sand (Nyamasoka-Magonziwa et al. 2020). A separate set of soil samples were collected to a depth of 5 cm from two representative points per plot, using a sharpened metal cylinder (7 cm dia.) for evaluation of bulk density (BD) and aggregate stability. The cylinder was inserted vertically into the soil by hand and excavated carefully, placing the soil from within the cylinder in a plastic bag and transporting them to the lab in a cool plastic container (to avoid compaction). Upon arrival in the lab, samples were weighed, and a subsample (40 g) was oven-dried at 105 °C for determination of gravimetric water content and BD. The two cores were then pooled, gently broken apart by hand along natural planes of weakness to pass through an 8-mm sieve, and air-dried. A 70 g subsample of the 8-mm sieved, air-dried soil was evaluated for aggregate stability via a wet sieving method (Nyamasoka-Magonziwa et al., 2020). Briefly, a 2 mm sieve was placed in a small basin and soil was sprinkled onto the sieve and submerged for 5 min. of slaking. The sieve was then repeatedly submerged 50 times over a 2-min period. Soil passing through the 2 mm sieve was then transferred to a 250-µm sieve and the process was repeated. Soil remaining on each sieve was collected, oven-dried at 105 °C, and weighed to generate three aggregate size classes (>2000 µm, 250–2000 µm, <250 µm). Aggregate stability was calculated as the mean weight diameter (MWD), considered as the fraction of soil mass present in an aggregate size class multiplied by

the mean diameter of aggregates per size class.

5.2.3 Fusarium and Pythium assessment

Fusarium baiting assays were conducted according to the protocol adapted from Furuya, et al., (1999), and further validated by Mutai et al., (*submitted*). Briefly, bean seeds were planted in a soil-less media and grown in the dark for 14 days at room temperature to obtain long white stems (25 cm) that can easily show signs of *Fusarium* infestation. Bean stems were then harvested and cut into 6 cm long segments. Two sub-samples (lab replicates) of the air-dried, 2-mm sieved soils (500 g per sample) from each farm were placed in sealed containers (8 cm dia., 12 cm high) and water content adjusted to 50% water holding capacity. Ten bean stem segments were then buried in each container and incubated at room temperature (25 °C). After 4 days, the buried segments were removed from the soil, rinsed with clean water, and the number of reddish-brown lesions longer than 1 mm and consistent with *Fusarium* damage were counted on the ten stems in each container. When more than two lesions joined to produce a larger lesion, lesions originating from separate infection sites were counted separately. Bean stems were also buried in autoclaved sand and the soil-free Cornell mix, which were used as controls for this assay.

For the *Pythium* assay, plastic containers (8 cm diameter, 12 cm high) were filled with 125 g soil and brought to 75% water holding capacity. Ten bean seeds were buried in the moistened soil, the container lid was sealed with parafilm, and the unit was incubated at 21 °C for 72 hr. Seeds were then removed from the soil, thoroughly rinsed with de-ionized water, blotted dry, placed on a *Pythium*-selective medium (Ali-Shtayeh et al., 1986), and incubated for 60 hrs. on a *Pythium* selective media. Following incubation, seeds were evaluated for the presence/absence of mycelia growing into the media from individual seeds, and number of observed colonies noted. Soil known to contain *Pythium* inoculum of the (positive control) and an autoclaved soil (negative

control) were included in the assay.

5.2.4 Lesion and root knot nematode bioassays

To evaluate the abundance of lesion causing nematodes (LN; such as *Pratylenchus*), we used a modification of the soil bioassay developed by Gugino et al. (2008) and validated by Mutai et al., (*submitted*). In summary, lesion-causing nematodes were assessed using soybean as a bait crop, grown in pots (8 cm dia., 12 cm high) and maintained for 3 weeks in the greenhouse. Three soybean seeds were planted in pots containing air-dried soil from each plot, with two lab replicates per soil each containing ~500 g soil. After three weeks, the soybean plants were carefully removed from the pots and washed free of soil for careful examination of diagnostic root lesions. Elongated dark brown lesions on the main root consistent with lesion nematodes signs were then counted and recorded. Soybean roots of plants grown in soilless media (~500 g) as a control were also observed. Fresh root mass in each pot was recorded for estimation of lesions on a per gram of root basis.

To estimate root knot nematode (RKN; *Meloidogyne*) abundance, we grew lettuce according to a modified soil bioassay developed by Gugino et al. (2009) and validated by Mutai et al., (*submitted*). Soil processing and management of lettuce was done as for soybean in lesion nematodes assessment, then after 6 weeks, plants were removed from the pots and roots washed free of soil, before the examination for root galling. Number of galls per root were recorded, and roots weighed for estimation on a per gram of root basis.

5.2.5 Statistical analyses

Lab replicates for all the pathogen bioassays (number of galls, lesions, and colonies) were averaged and mean values used for all statistical analyses. To understand treatment differences

and main effects, we used three-way analysis of variance (ANOVA), with synthetic fertilizers, manure, plant residue, and all possible interactions considered as fixed effects, including block (replicates), sub-plots, and sub-sub plots in the model as random effects to address the split-split plot design of the experiment. Square root transformations were applied as needed (for POM, *Fusarium* lesions, *Pythium* colonies, nematode galls, and nematodes lesions) to meet assumptions of homogeneity of variance. Pearson's correlations were used to explore the link between key soil health characteristics and the soil-borne pathogens examined here. The significance level was set at 95% confidence interval. The above statistical analyses were performed using JMP (version 15.0.0). Between-class principal component analysis (BCA) was also conducted using R version 4.0.4 to extract the main axes of variation and visualize clusters of closely related variables. BCA analysis calculates the barycenter of data for each grouping category of interest then computes principal component analysis of the set of barycenter.

5.3 Results

5.3.1 Treatments impacts on soil physical and chemical properties

Amendments and residues added to soil over the previous 45 years had significant impacts on a range of soil health parameters, including organic matter fractions (POM and POXC), soil pH, and soil aggregate stability (MWD; Fig. 5.1; Table 5.1). Long-term manure application significantly increased soil POM by 37%, POXC by 114%, pH by 1.24 units (24%), and MWD by 74%, and reduced bulk density by 6%, compared to treatments without manure application. Similar to manure, maize stover retention significantly enhanced POM (47% increase), POXC (11% increase), pH by 0.27 (5% increase), and MWD (28% increase), compared to no residue. Use of synthetic fertilizers on the other hand showed a significantly increased POXC by 5% and marginally significant increase of POM by 47%, compared to no synthetic fertilizer (Table 5.1). Additionally, manure and synthetic fertilizers significantly enhanced P availability by 41% and 67%, respectively. An interaction between residue management and fertilizer application suggested that residue retention was more effective at increasing POXC in the absence of synthetic fertilizer. Similarly, an interaction between manure and fertilizer for available P suggested that fertilizers increase P availability more in the absence of manure than when manure is applied.

(FIGURE 5.1)

(TABLE 5.1)

5.3.2 Treatments impacts on soil-borne pathogens

Fusarium, *Pythium*, and plant parasitic nematodes were found in all plots in the long-term trial in Kabete. However, the different treatments had varied impacts on soil-borne pathogen levels. For

instance, manure application significantly reduced RKN by 68%, *Pythium* colonies by 39%, LN by 28%, but increased *Fusarium* by 205% (Fig. 5.2 and Table 5.2). Additionally, synthetic fertilizers significantly reduced *Pythium* by 41% (Fig. 5.2 and Table 5.2). There were no simple effects of residue management on the soil-borne pathogens assessed, but there were several significant interactions. For example, an interaction between residue management and fertilizer application suggested that synthetic fertilizer reduced *Fusarium* more in the absence of plant residue. Additionally, an interaction between manure and fertilizer application suggested manure reduced *Pythium* colonies more in the presence of synthetic fertilizers.

(FIGURE 5.2)

(TABLE 5.2)

5.3.3 Relationships between soil pathogens and soil health parameters

Soil-borne pathogens were significantly correlated with a number of soil chemical and physical parameters. Pearson's correlations indicated that *pythium* was negatively correlated with POM, POXC, pH, aggregate stability, and available P, and positively related to bulk density (Table 5.3). Similarly, both RKN and LN were negatively associated with pH, POXC, and aggregate stability. Meanwhile, *Fusarium* showed the opposite trend, and was positively correlated with pH, POM, POXC, aggregate stability, and P availability and negatively correlated with bulk density (Table 5.3).

(TABLE 5.3)

When examining the PCA results, PC1 axis explained the 71.6% of the variation and clearly separated treatments with and without manure (Fig. 5.3). On the left side of PC1, *Fusarium* was associated with pH, POXC, MWD and the treatments receiving manure, while bulk density projected in the opposite direction, towards the treatments not receiving manure. POM and

available P projected more strongly on PC2 (explaining just 14.3 % of the variability) and were associated with treatments receiving synthetic fertilizer, while *Pythium* projected in the opposite direction, associated with the absence of fertilizer inputs (Fig. 5.3).

(FIGURE 5.3)

5.4 Discussion

This study sought to assess the long-term effects of different nutrient management strategies on soil health parameters and soil-borne pathogens after 45-years in a maize-bean rotation in Kenya. Findings from this study indicate that use of organic amendments such as manure and plant residues improve soil health and reduce incidence of important soil-borne pathogens.

5.4.1 Long-term nutrient management effects on soil C variables

The application of farmyard manure and plant residues appeared to play a significant role in improving soil POM and POXC. Organic soil amendments are a key source of SOM, and contribute greatly to soil structure and support soil biological activity (Kamaa et al., 2011; Sun et al., 2015; Liu et al., 2021). The mechanism by which organic amendments have such beneficial impacts relates to their ability to feed and provide habitat for soil microbial communities and fauna. These organisms, in turn, facilitate decomposition, nutrient transformation, and formation of stable aggregates that improve soil structure and can further protect SOM from decay (Six, et al., 2004; Lavelle et al., 2020). Although effects of addition of synthetic fertilizers on the measured soil health parameters was much less than those of manure and crop residues, and marginally significant effect suggested that fertilizers increase in POM by 47%, which was comparable to the observed effect of manure additions and crop residue retention. The relatively large effect of fertilizer and residue retention POM vs. other soil C parameter is probably due to enhanced plant growth resulting in more residues, suggesting that POM is more plant-derived than other SOM fractions (e.g., POXC) that are better supported by manure inputs. Previous research at the site suggests that maize yields were roughly four times higher in the treatments with manure and fertilizer ($6.0 \text{ t ha}^{-1} \text{ yr}^{-1}$) compared to just the treatment where maize stover

alone was applied ($1.4 \text{ t ha}^{-1} \text{ yr}^{-1}$) (Kapkiyai et al., 1999). Since all maize stover from the previous crop in each plot was incorporated back into the soil, yield performance enhanced by manure and synthetic fertilizers would mean that crop residues incorporated into the soil in these plots also increased overtime, which can explain the associated increase in POM. Although use of synthetic fertilizers can enhance SOM, amending soil with organic inputs such as manure and plant residues is likely to be more effective through provision of a more balanced input of nutrients and other benefits such as enhanced microbial activity, that are not likely to occur with synthetic fertilizers (Ghorbani et al., 2008). Additionally, even if our results show high POM with added mineral fertilizers, likely due to increasing biomass returns to soil from larger plants, N fertilizer has been suggested to reduce stabilization of SOM in soil aggregates (Fonte et al., 2009), which corresponds well to the lack of a fertilizer effects on soil aggregation in our study.

5.4.2 Long-term nutrient management effects on soil chemical and physical parameters

Our study revealed stronger positive effects of manure than crop residues on soil pH. On average, soil pH in plots with long-term addition of manure, plant residue, and synthetic fertilizers in this study was 6.5, 5.9, and 5.8, respectively. While synthetic fertilizers had no significant effect on pH in our study, other studies done in the same experimental site reported a drop in soil pH from 5.5 to 4.3 units with continuous application of synthetic fertilizers (Kibunja et al., 2010). Organic soil amendments on the other hand can increase pH (Hu et al., 2017). Significant manure effects on soil pH most likely improved plant growth and performance, which in turn, increased crop residue return and further contributed to SOM and available P enhancement. Sun et al., (2015) compared the impact of livestock manure and wheat straw on bacterial diversity and soil nutritional status and found that manure had greater effects on bacterial community, which was positively correlated with soil nutritional status. In this study,

the stronger effect of synthetic fertilizer on available P can be explained by the application of TSP at maize planting, which readily enhances P availability.

The greatest MWD was obtained with farmyard manure application, followed by plant residue addition. This is probably associated with the increase in SOM in plots where manure and plant residues were applied. Meanwhile, farmyard manure application significantly lowered bulk density, which fits with the improved aggregation, since larger aggregates likely translate to a greater volume of macropores and overall porosity. Our results also indicated a slight increase in bulk density (by 1.2%) with use of synthetic fertilizers, which is consistent with Kibunja et al. (2011) who found synthetic fertilizers to increase bulk density significantly in the same trial.

4.3 Management and soil health implications for soil-borne pathogens

We observed a number of strong treatment effects on soil-borne pathogens and suspect that these may, at least in part, be governed by the observed changes in soil physical and chemical properties. For instance, *Pythium* was negatively related to soil pH, POM, POXC, available P, and MWD, and positively related to BD (Table 3). We also note that soils from the no-amendment plot produced the highest number of *Pythium* colonies, compared to all the other treatments. Manure and synthetic fertilizers appeared to have created significant reductions in *Pythium* through increases in SOM and overall plant health, which are likely associated with improved plant growth and performance. These factors not only increase plant resistance to pathogens but may also provide an energy source for beneficial microbes that can introduce biocontrol (Dubey et al., 2023). SOM is thought to serve as source of energy and nutrients to many microorganisms that may compete or antagonize pathogenic *Pythium* spp. (Broders et al., 2009). Schmidt et al. (2004) found that the antagonist (*P. fluorescens* B5) was more effective in controlling damping-off caused by *Pythium* in organic carbon rich soils, compared to low

organic content soil. In another study, Gravel et al. (2009), found that manure significantly lowered root colonization by *Pythium* spp., compared to conventional fertilization. A study by Medvecky et al., (2007) found that soil water holding capacity was reduced by removal of maize stover, leading to enhanced *Pythium* infection of incubated bean seeds. The significant negative association between soil pH and *Pythium* observed in this study may also be related to the biocontrol performance of *Pythium* antagonists with changes in soil pH. Schmidt et al. (2004) reported an increase of biocontrol performance by *P. fluorescens* B5 in controlling *Pythium* damping-off at pH higher than 6, while Holmes et al. (1998) reported the same for *Pythium oligandrum* antagonism performance against *Pythium* damping-off at pH between 7.0 and 7.5, thus supporting our observation of significant negative relationship between *Pythium* and soil pH.

Our results also suggest that manure can significantly reduce incidence of LN and RKN, via increases in SOM and related parameters. Several studies conducted to assess the capacity of organic amendments to control soil-borne pathogens including PPN, suggest that compost, manure, and crop residues are the most suppressive organic amendments. The mechanisms to which these organic amendments can control PPN is directly by making microhabitats less conducive to reproduction and survival through increase in mineral SOM, or indirectly through mechanisms such as antagonism, competition for nutrients, predation, and parasitism (Roskopf et al., 2020). Atandi et al. (2017) found significant reduction in the abundance of PPN including *Pratylenchus* and *Meloidogyne* in the organic farming system, compared to conventional management practices. Similar results were also established by Mutai et al. (*submitted*), where they report potential of short-term application of manure to suppress PPN in western Kenya. Organic soil amendments, particularly those that increase the active fraction of SOM (e.g.

compost, rotational and cover crop debris, and green manures) have been found to affect total soil nematode communities and suppress PPN, through improvement of the physical, chemical, and biological properties of soil (Widmer et al., 2002). Forge et al. (2016) found that poultry manure and compost could suppress root lesion nematodes to the same extent as fumigation. They suspected the compost-induced suppression of PPN was related to richness and activity enhancement of nematode trapping fungi (antagonists), as well as microarthropods and predacious nematodes (Forge et al., 2016). Similar results were also established in our previous study, where we found more bacterivorous and fungivorous nematodes and fewer PPN in soils amended with farmyard manure, compared to mineral fertilizers (Mutai et al., *submitted*). Our findings also demonstrate a negative correlation of soil health properties (i.e., pH, POXC, POM, aggregation) with the assessed PPN. RKN and LN were more abundant in acidic soils; however, it is difficult to distinguish the effects of higher pH soils from that of increased SOM, as both were associate with manure additions. Our results are comparable to findings by Wang et al., (2009), who found *Meloidogyne hapla* to increase in low pH of between pH 4.5 and 5.4, and they suggest that nematodes, including RKN, are attracted to low pH soils. They further speculate that this could be related to the fact that actively growing roots tend to be more acidic at the root surface and even more at the elongation zone, target areas for PPN. Future studies should pay more attention to how nutrient management is linked to soil pH and the related soil nutrient modifications that can trigger incidence of important soil-borne pathogens.

Our results for *Fusarium* were less straightforward than for *Pythium* and PPN, in that *Fusarium* were positively correlated with SOM pools, soil pH, and aggregation, especially with manure addition. This manure impact on *Fusarium* could be related to differential provision of food sources with manure addition vs. not, and the general soil chemical changes, i.e. pH, which could

be influencing *Fusarium* germination and reproduction. This finding corroborates past research by Kamaa et al. (2011), who worked in the same long-term trial and found enriched soil fungal and bacterial communities with long-term combination of organic and inorganic amendments. Similar results were also reported by Henry et al. (2020), who found significant increase in *Fusarium oxysporum f. sp. fragariae* in soils that received organic amendments or incorporated plant residues, and they argue that both pathogenic and non-pathogenic *Fusarium* are competitive soil saprophytes, and can colonize applied plant residues, leading to increased overall *Fusarium* inoculum. Additionally, results from our previous study (Mutai et al., *submitted*) demonstrated that the *Fusarium* species isolated from bean stems had varying levels of pathogenicity, suggesting that soil context can shape species pathogenicity, and that *Fusarium* may not always play a harmful role in these cropping systems.

5.5 Conclusions

The present study utilized a 45-year long-term experiment with a cropping system (continuous maize-bean rotation) relevant to many smallholder systems in Kenya. The experiment provided us with valuable data for understanding and assessing soil health and sustainability of nutrient management strategies. We evaluated impacts of organic vs. inorganic inputs on soil pH, SOM, aggregation, and soil-borne pathogens, impacts which most of the time become evident only after many years of agricultural practice. The results generated from this research shows that long-term organic inputs are useful to enhance a range of soil properties such as pH, SOM, aggregation, and bulk density, and that soil health improvements, in turn, may play an important role in reducing the incidence of soil-borne pathogens, either through improved crop nutrition and resistance, or providing a food base for enhanced soil biological diversity that acts to suppress pathogens. In particular, continuous addition of farmyard manure appears to significantly improve soil health parameters and reduce *Pythium*, root knot and lesion nematodes. For smallholder farmers practicing simultaneous and continuous cultivation of maize and beans, results from this study will help to gauge potential soil-borne pathogen pressure and contribute to the development of appropriate and sustainable nutrient strategies. Our work highlights the possible soil health improvements in the smallholder systems in Kenya and those in related environments, with utilization of manure and plant residues. Our work also raises some unexpected soil quality improvement (enhanced SOM, pH, aggregation and bulk density) impacts on *Fusarium*, and there is need to more fully understand the kind of *Fusarium* species that are enhanced with improved soil health.

CHAPTER 5 TABLES AND FIGURES

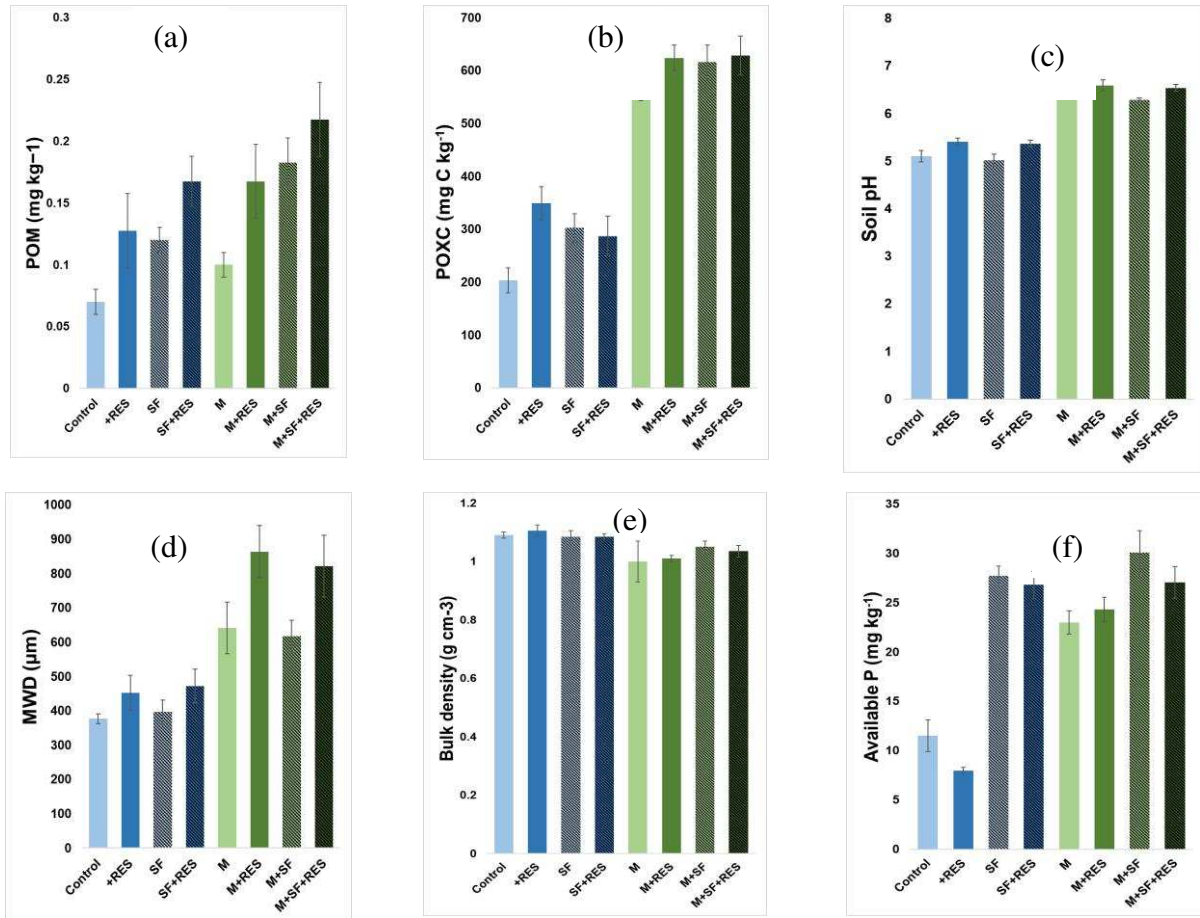


Figure 5.1 Treatment mean effects of different organic and inorganic nutrient input combinations on: soil particulate organic matter (a), permanganate oxidizable C (b), pH (c), aggregate stability (mean weight diameter) (d), bulk density (e), and available P (f) in soils sampled from a long-term trial in Kabete, Kenya in May 2021. The treatment names are abbreviated as follows: Control - no amendment, +RES - maize residue retention, SF - synthetic fertilizers application, and M - farmyard manure addition.

Table 5.1 ANOVA results (p-values) for the impact of long-term nutrient management strategies (synthetic fertilizers, farmyard manure and plant residue) and all possible interactions on select soil properties, with significant impacts at $p \leq 0.05$ marked in bold. Sampling was conducted in Kabete, Kenya in May 2021, 45 years after the implementation of the long-term experiment.

Treatments	POM (mg kg ⁻¹)	POXC (mg C kg ⁻¹)	pH	MWD (μm)	Bulk density (g cm ⁻³)	Available P (mg P kg ⁻¹)
Plant residue	0.002	0.016	<0.002	0.004	0.243	0.237
Synthetic fertilizers	0.067	0.041	0.133	0.912	0.399	<0.001
Manure	0.023	<0.001	<0.001	<0.001	<0.001	<0.001
Plant residue * synthetic fertilizers	0.307	0.014	0.566	0.905	0.699	0.732
Manure * plant residue	0.746	0.341	0.375	0.117	0.169	0.587
Manure * synthetic fertilizers	0.725	0.932	0.769	0.378	0.228	<0.001
Manure * synthetic fertilizers * plant residue	0.684	0.097	0.843	0.910	0.177	0.181

POM- particulate organic matter; POXC- permanganate oxidizable carbon; MWD- mean weight diameter of soil stable aggregates.

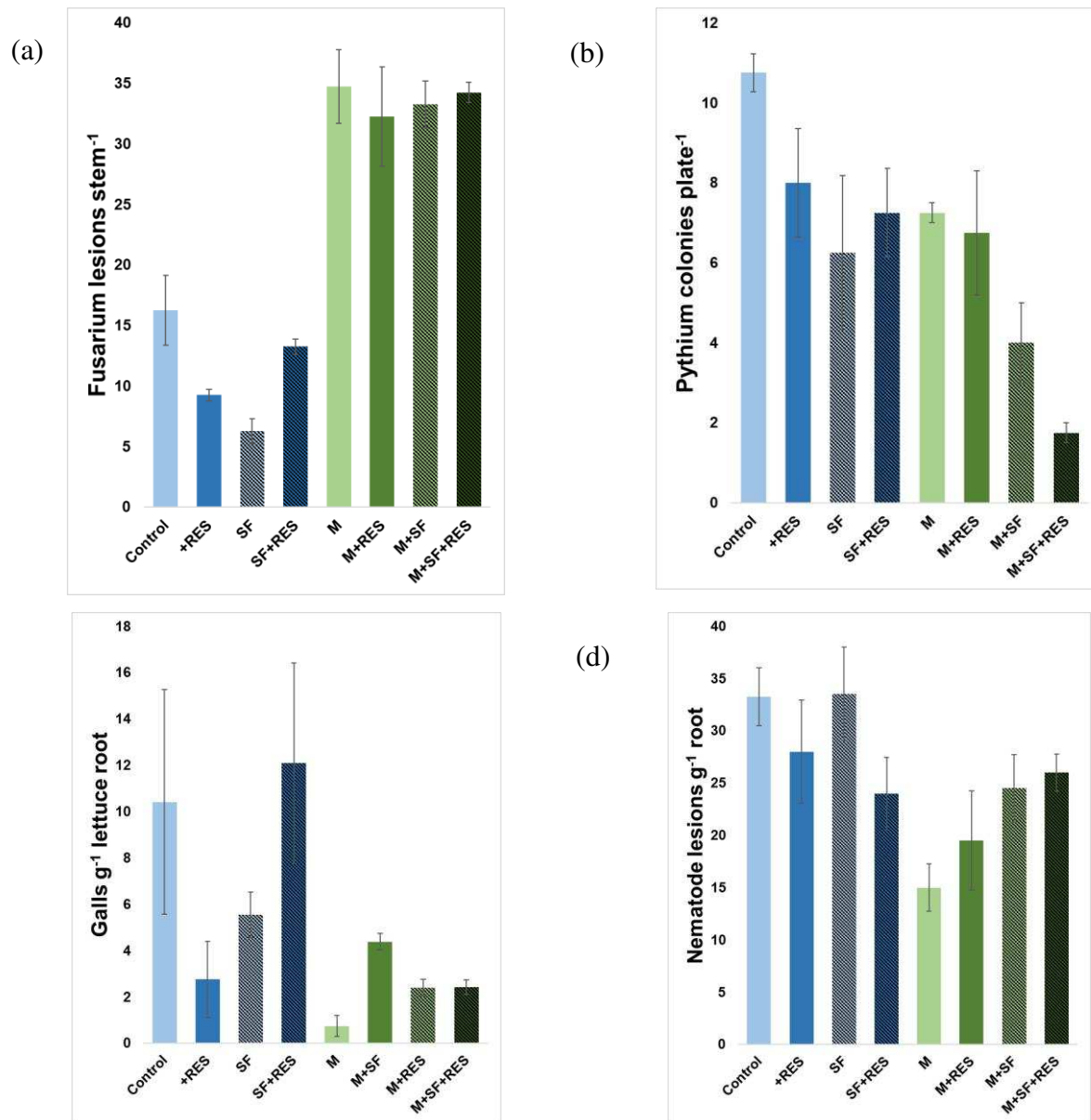


Figure 5.2 Average nutrient input (synthetic fertilizers, farmyard manure and plant residue and their combinations) effects on incidence of *Fusarium* (a), *Pythium* (b), root knot nematodes (RKN) (c) and lesion-causing nematodes (LN) (d) in soils sampled from a long-term trial in Kabete, Kenya in May 2021. The treatment names are abbreviated as follows: Control- no amendment, +RES- plant residue addition, SF- synthetic fertilizers application, and M- farmyard manure addition.

Table 5.2 ANOVA results for the impact of long-term nutrient management strategies (synthetic fertilizer, farmyard manure and plant residue) and all possible interactions on major soil-borne pathogens, with significant impacts at $p \leq 0.05$ marked in bold. Sampling was conducted in Kabete, Kenya in May 2021, 45 years after the implementation of the long-term experiment.

Treatments	<i>Fusarium</i> (lesions stem ⁻¹)	<i>Pythium</i> (colonies plate ⁻¹)	RKN (knots g root ⁻¹)	LN (lesions g root ⁻¹)
Plant residue	0.925	0.224	0.763	0.370
Synthetic fertilizers	0.257	0.003	0.206	0.190
Manure	<0.001	<0.001	0.016	0.044
Plant residue * synthetic fertilizers	0.006	0.829	0.145	0.430
Manure * plant residue	0.629	0.498	0.206	0.026
Manure * synthetic fertilizers	0.127	0.030	0.918	0.151
Manure * synthetic fertilizers * plant residue	0.033	0.148	<0.001	0.948

RKN- root knot nematodes; LN- lesion-causing nematodes

Table 5.3 Pearson correlation coefficients for relationships between select soil health parameters and soil-borne pathogens for soils collected in May 2021, from a long-term trial in Kabete, Kenya

	POM	POXC	pH	MWD	BD	P _{Avail}	<i>Fusarium</i>	<i>Pythium</i>	RKN	LN
POM	1									
POXC	0.41*	1								
pH	0.42*	0.92***								
MWD	0.48**	0.77***	0.77***	1						
BD	-0.31*	-0.76***	-0.72***	-0.67***	1					
P _{Avail}	0.52**	0.45**	0.39*	0.37*	-0.52**	1				
<i>Fusarium</i>	0.31*	0.78***	0.90***	0.60***	-0.71***	0.37*	1			
<i>Pythium</i>	-0.46**	-0.52**	-0.43*	-0.38*	0.43*	-0.48**	-0.36*	1		
RKN	-0.15	-0.47**	-0.48**	-0.39*	0.23	-0.08	-0.37*	0.23	1	
LN	-0.05	-0.47**	-0.59***	-0.31*	0.32*	-0.13	-0.53**	0.19	0.19	1

*denotes variables with significant correlation at p value < 0.05, ** at p value < 0.01, *** at p value < 0.00; POM- particulate organic matter; POXC- permanganate oxidizable carbon; BD-bulk density; MWD- mean weight diameter; P_{Avail}- Available P; LN- lesion causing nematodes; RKN- root knot nematodes

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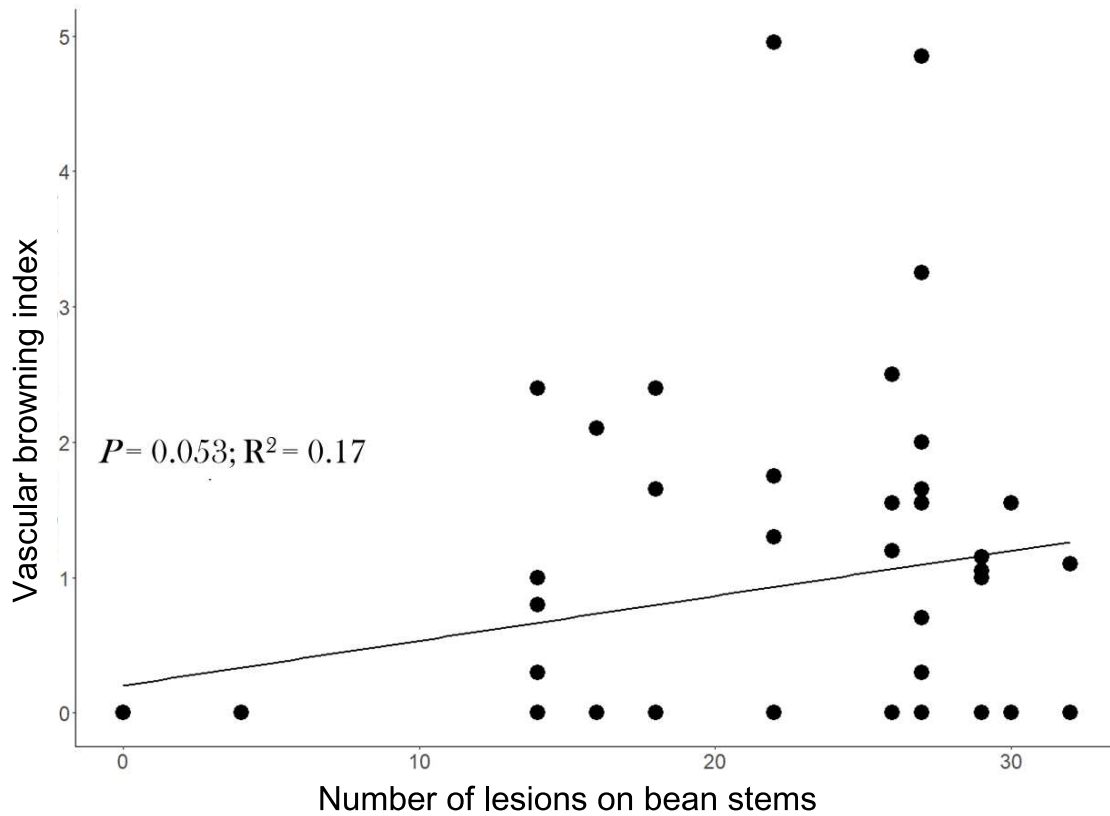
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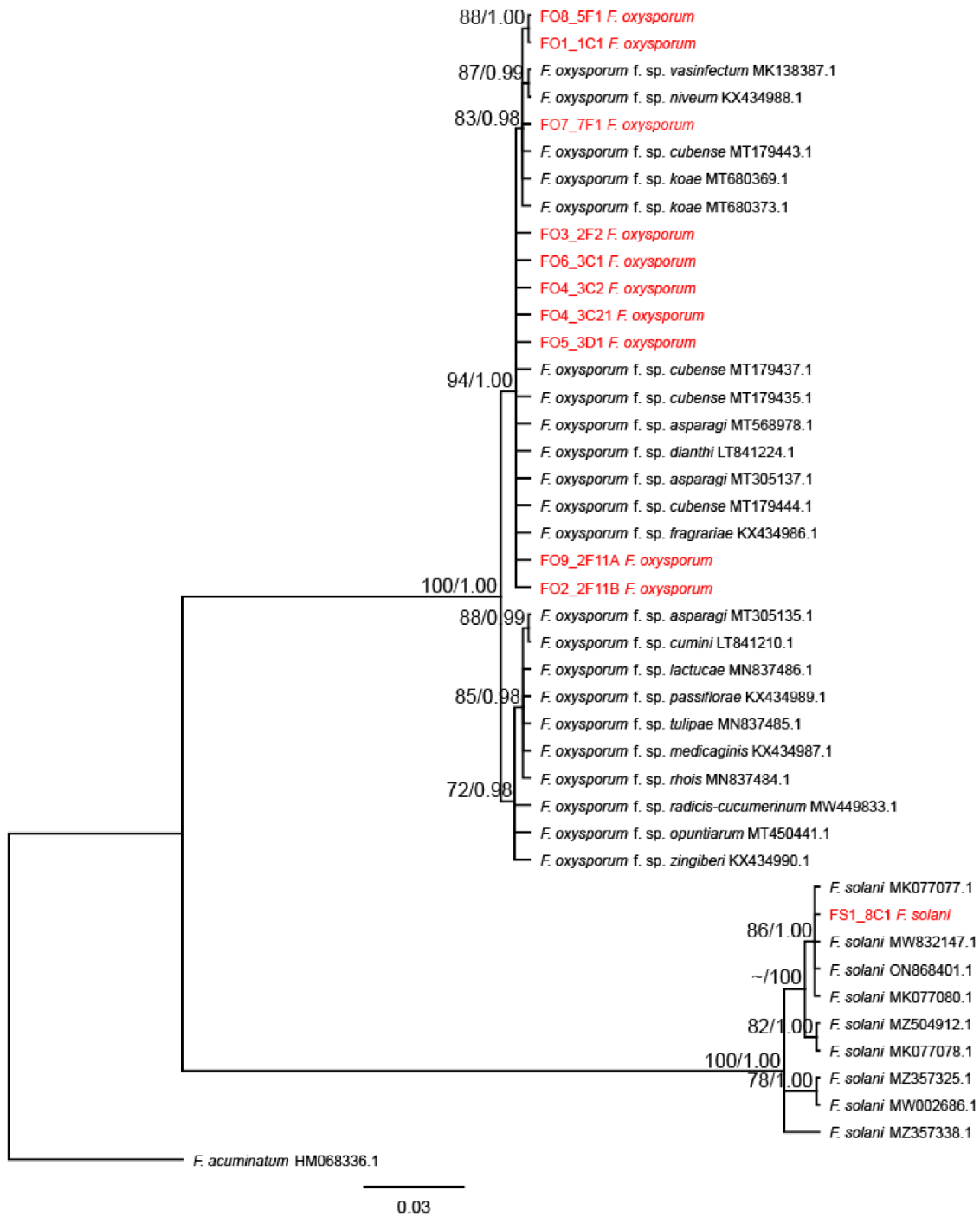
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APPENDIX A. SUPPLEMENTARY MATERIAL



Supplemental Figure 2.1 Assessment of *Fusarium* bioassay, with x-axis being visual assessment and counting the number lesions on bean stems; and y-axis being the pathogenicity test of select isolates isolated from bean stem lesions.



Supplemental Figure 2.2 Bayesian phylogeny of the *Fusarium* isolates sequences from farm soils in western Kenya, sampled in May 2022 (red colored), and the National Center for Biotechnology Information (NCBI) sequences with their accession numbers retrieved from NCBI (black colored).

Supplemental Table 2.1 Accession numbers for *Fusarium* and *Waitea* isolates from this study and reference strains from National Center for Biotechnology Information (NCBI) used in phylogenetic analysis.

Isolate_ID	Organism	<i>Forma specialis</i>	GenBank number	accession	Isolate_Origin
FO7_7F1	<i>Fusarium oxysporum</i>		OR355479		This study
FO1_1C1	<i>Fusarium oxysporum</i>		OR355470		This study
FO3_2F2	<i>Fusarium oxysporum</i>		OR355471		This study
FO8_5F1	<i>Fusarium oxysporum</i>		OR355478		This study
FO2_2F11B	<i>Fusarium oxysporum</i>		OR355473		This study
FO4_3C2	<i>Fusarium oxysporum</i>		OR355475		This study
FO5_3D1	<i>Fusarium oxysporum</i>		OR355477		This study
FO4_3C21	<i>Fusarium oxysporum</i>		OR355476		This study
FO6_3C1	<i>Fusarium oxysporum</i>		OR355474		This study
FO9_2F11A	<i>Fusarium oxysporum</i>		OR355472		This study
FS1_8C1	<i>Fusarium solani</i>		OR355480		This study
Wc_4F2A	<i>Waitea circinata</i>		OR355481		This study
Wc_4F2B	<i>Waitea circinata</i>		OR355482		This study
NRRL 54218	<i>Fusarium</i> <i>acuminatum</i>		HM068336.1		Reference
BRIP39299	<i>Fusarium oxysporum</i>	<i>zingiberi</i>	KX434990.1		Reference
DB18AGO01	<i>Fusarium oxysporum</i>	<i>opuntiarum</i>	MT450441.1		Reference
BRIP28044	<i>Fusarium oxysporum</i>	<i>passiflorae</i>	KX434989.1		Reference
BRIP5189	<i>Fusarium oxysporum</i>	<i>medicaginis</i>	KX434987.1		Reference
PD0150475089	<i>Fusarium oxysporum</i>	<i>lactucae</i>	MN837486.1		Reference

CBS 220.49	<i>Fusarium oxysporum</i>	<i>rhois</i>	MN837484.1	Reference
CBS 242.59	<i>Fusarium oxysporum</i>	<i>tulipae</i>	MN837485.1	Reference
F11	<i>Fusarium oxysporum</i>	<i>cumini</i>	LT841210.1	Reference
GR_FOA168	<i>Fusarium oxysporum</i>	<i>asparagi</i>	MT305135.1	Reference
FRCKSU17	<i>Fusarium oxysporum</i>	<i>radicis-</i> <i>cucmerinum</i>	MW449833.1	Reference
CAV3484	<i>Fusarium oxysporum</i>	<i>cubense</i>	MT179437.1	Reference
RBH3	<i>Fusarium oxysporum</i>	<i>vasinfectum</i>	MK138387.1	Reference
NTP-Dc36955	<i>Fusarium oxysporum</i>	<i>niveum</i>	KX434988.1	Reference
CAV3130	<i>Fusarium oxysporum</i>	<i>cubense</i>	MT179443.1	Reference
44	<i>Fusarium oxysporum</i>	<i>koae</i>	MT680373.1	Reference
166	<i>Fusarium oxysporum</i>	<i>koae</i>	MT680369.1	Reference
CAV3128	<i>Fusarium oxysporum</i>	<i>cubense</i>	MT179444.1	Reference
GR_FOA230	<i>Fusarium oxysporum</i>	<i>asparagi</i>	MT305137.1	Reference
	<i>Fusarium oxysporum</i>	<i>dianthi</i>	LT841224.1	Reference
BRIP53860	<i>Fusarium oxysporum</i>	<i>fragariae</i>	KX434986.1	Reference
CAV	<i>Fusarium oxysporum</i>	<i>cubense</i>	MT179435.1	Reference
MA_FOA24	<i>Fusarium oxysporum</i>	<i>asparagi</i>	MT568978.1	Reference
KARE233	<i>Fusarium solani</i>		MK077077.1	Reference
KARE221	<i>Fusarium solani</i>		MK077080.1	Reference
BMU 03270	<i>Fusarium solani</i>		MW832147.1	Reference
JZB3110248	<i>Fusarium solani</i>		ON868401.1	Reference
KARE398	<i>Fusarium solani</i>		MK077078.1	Reference
XG1 bai	<i>Fusarium solani</i>		MZ504912.1	Reference
NsPed1	<i>Fusarium solani</i>		MW002686.1	Reference

CHS8	<i>Fusarium solani</i>	MZ357325.1	Reference
TNM31R	<i>Fusarium solani</i>	MZ357338.1	Reference
