PLANT DIVERSITY AS A LEVER FOR INTEGRATED NUTRIENT MANAGEMENT AND SOIL BIODIVERSITY IN AGROECOSYSTEMS

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ABSTRACT

In the modern age, soil fertility in agriculture is managed through the use of highly soluble fertilizers. The sourcing, manufacture, and application of these fertilizers have large environmental footprints, however. To overcome reliance on finite raw materials and lessen the deterioration of the planet's natural resources, more ecologically-sound nutrient management is needed. Simultaneously, simplified cropping systems with high disturbance contribute to degradation of soil biological diversity. Such decline in diversity carries the risk of losing functional capacity of soils to support life above ground into the future. Plant diversity management could be the key to turning the tide of these trends in agriculture. Plants are active agents in the agroecosystem with potential to stack many functions. This thesis explores the role of plant diversity in providing ecosystem services to crop production as introduced in the literature review chapter. The next chapter looks at the phosphorus cycling potential of cover crops in soils from Michigan corn-soy-wheat rotations. The objective of this experiment was to compare the relative contributions of cover crop species, arbuscular mycorrhizal fungi (AMF) colonization, and soil phosphorus distribution to cover crop phosphorus uptake. Hairy vetch (*Vicia villosa*) was more highly colonized by AMF than rye (*Secale cereale*). When grown in soil from an organically managed agronomic treatment, vetch responded with significantly higher biomass and phosphorus acquisition when AMF was present than without colonization. Such results can inform selection of cover crops for phosphorus cycling in agriculture, accounting for the context of soil nutrient and biological status that could influence plant performance. The final chapter utilizes a dataset from smallholder farms in Central Malawi to determine how environment and farmer practices shape differences in soil microbial communities. DNA sequence data revealed differences in sample fungal and prokaryote diversity driven not only by environment but also farm management factors. In particular, intercrop diversification and crop residue retention show promise for promoting soil microbial diversity on smallholder farms in Central Malawi. Further investigation could uncover whether such shifts in microbial diversity have functional implications for soil and plant health in these settings. These chapters illustrate how managing plant diversity in agriculture recouples essential cycles that contribute to thriving agroecosystems.

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LIST OF ABBREVIATIONS

16S	16S ribosomal RNA gene
AMF	Arbuscular Mycorrhizal Fungi
ANOVA	Analysis of Variance
ASV	Amplicon Sequence Variant
C:P	Carbon to Phosphorus ratio
CRD	Completely Randomized Design
ENM	Ecological Nutrient Management
EPA	Extension Planning Area
ITS	Internal Transcribed Spacer
KBS	Kellogg Biological Station
LTER	Long Term Ecological Research
MAP	Mean Annual Precipitation
MAT	Mean Annual Temperature
MCSE	Main Cropping System Experiment
NDVI	Normalized Difference Vegetation Index
PERMANOVA	Permutational Analysis of Variance
PCoA	Principal Coordinate Analysis
POM	Particulate Organic Matter
RDA	Redundancy Analysis
SOC	Soil Organic Carbon
T1	Treatment 1 (Conventional Agronomic Treatment)
T4	Treatment 4 (Biologically-Based Agronomic Treatment)

CHAPTER 1:
Reviewing the Potential of Plant Diversity Management for Nutrient Cycling and Promoting Soil Biodiversity

The Modern Fertilizer Paradigm

Certain elemental nutrients are essential for all life forms. Some of these nutrients are used in complex biochemical cascades, while others are foundational to the macromolecules forming the basic building blocks of organisms. Our genetic code, passed down in the form of DNA, is comprised of long strands of nucleotide polymers interconnected with phosphorus. Nitrogen shapes the proteins that do the work in our bodies. Along with carbon, these two nutrients are rooted in the very core of how biology functions. As such, nitrogen and phosphorus are necessary for all organisms. Much complexity and diversity in biology is dedicated to finding ways to access these nutrients in usable forms. In a way, our own pursuit of these nutrients as humans leading up to the present is a magnification of what it takes to grow: find new ways to remove limitations. This is the model that guides the current trends in anthropogenic nutrient cycling globally. Whether intentionally or as a matter of course, humanity has set its sights on eliminating the limitations of food production by getting nutrients from all around the world into our growing populations.

Many innovations have contributed to the relatively recent rise in human food production, but perhaps none more so than the modern discovery of fossil fuels (Chappell and LaValle, 2011). No single input more pervasively underpins every aspect of the food production chain. From mechanization that allows management of farm landscapes at a superhuman scale to the transportation of inputs and harvest, fossil fuels are being leveraged in as many ways as possible to get food to markets (Woods et al., 2010). But industrialization at the farm and food distribution scales alone would not have been enough to generate the leap in food production and population growth seen today. Farms would still face the limitations that have been set for agriculture going back over 10,000 years, including the availability of nutrients to grow plants and animals. Historically, if the growth and export of a farm exceeded the rate of biogeochemical cycling and resupply of nutrients back into the local agroecosystem, soil fertility would decline far enough to force the land to be taken out of production or converted to another stage of succession (Fox et al., 2000; Soropa et al., 2022). Now, such practices of fallowing land or more integrated internal nutrient cycling of individual farms are circumvented by the prevalence of soluble fertilizers synthesized and refined by industry (Chappell and LaValle, 2011). Many different methods or technologies support the generation of these fertilizers which amalgamate life-essential nutrients into ready packages for single growing seasons. Regardless of extraction process, all soluble fertilizer production stems from a dependency on the powerful, undervalued, deleterious energy of fossil fuels. Nitrogen for fertilizer is chemically converted from inert dinitrogen gas in the atmosphere into ammonia using heat and pressure provided by fossil fuel energy, as well as constituent hydrogen derived from

methane in natural gas (Erisman et al., 2008). Phosphorus is typically mined from phosphate-rich ore and sediments with large machinery and many physical and chemical refinement processes (Cordell and White, 2011). Both nutrient resources are geographically concentrated in certain parts of the globe (FAO, 2017; Ludemann et al., 2022) and then transported towards money.

Reliance on such a system for supplying the nutrients that support at least half of the global population (Erisman et al., 2008) combines externalities of fossil fuel use and mining (greenhouse gas emissions, land degradation, water contamination) with the risk of scarcity in a future where finite reserves run low (Cordell and White, 2011; Woods et al., 2010). Further, the relative availability and affordability of soluble forms of nitrogen and phosphorus have facilitated application of fertilizers far exceeding seasonal crop demand in many of the world's most intensified agricultural regions (MacDonald et al., 2011). Given that these two nutrients are predominant limiting factors for crop productivity metrics such as yield, growers are incentivized to apply as much fertilizer as it takes to ensure a profitable harvest (Begho et al., 2022). Through very different biogeochemical pathways, these reactive, biologically available forms of nitrogen and phosphorus are largely unused by the target crop for which they are applied and find many ways to be occluded in the soil or escape the agroecosystem entirely.

In soils, inorganic nitrogen compounds are readily emitted from the system in multiple forms. Nitrates can be carried away in soil solution via percolation or runoff. Ammoniacal fertilizers can be volatilized as ammonia while nitrate can be converted to gaseous forms as nitric oxides (NO_x), nitrous oxide (N_2O), or dinitrogen (N_2) through the microbial processes of denitrification (Butterbach-Bahl et al., 2013; Pilegaard, 2013; Sutton et al., 2013). Phosphorus is not so transient in soil, but quite the opposite. When reactive phosphates are added to soils, they quickly stabilize through a variety of physicochemical processes including adsorption to mineral or metal oxide surfaces, precipitation with cations such as calcium and aluminum, or otherwise assimilate in formation of secondary minerals (Ahmed et al., 2023; Dean, 1949). All of these processes entail a reduction in the relative bioavailability of phosphates in soils, which contributes to an accumulation of low solubility phosphorus. When this reservoir of phosphorus builds up substantially, it increases the concentration of phosphorus that can be exported from farms via soil erosion (Bennett et al., 2001). In some cases, phosphorus can be applied at such high rates that the capacity of the soil to stabilize soluble phosphorus is exceeded, increasing the likelihood of leaching phosphate as well (Hussain et al., 2021; Kleinman et al., 2000).

Fertilizer application rates that greatly exceed the immediate growth demands of a given crop will increase the amount of nutrients that are uncontrollably exported from agricultural fields (Bennett et al., 2001; Robertson, 1997). Not only is this a waste of the energy and resources that have been committed to making this fertilizer, but it also threatens human health and the welfare of the surrounding ecosystem. Nitrogen moving through soil can create unsafe drinking levels in groundwater or be transported

ultimately to natural water bodies where they cause drastic ecological phenomena, such as toxic algal blooms called 'red tides' (Erisman et al., 2013). Nitrogen exported as gas or particulate can be deposited by rainfall on nearby ecosystems, where elevated nitrogen availability can manipulate plant community composition and diversity by favoring competitive plants that require larger amounts of nitrogen (Bobbink et al., 2010). Nitric oxides behave as airborne pollutants while nitrous oxide is a potent greenhouse gas (Fowler et al., 2013). Excess phosphorus carried away in erosion, runoff, or leaching contributes to widespread eutrophication of freshwater systems (Bennett et al., 2001).

These challenges are generally recognized across many sectors involved in the process of anthropogenic nutrient cycling, including farmers, policymakers, consumers, and researchers. However, the primary narrative still centers on inorganic fertilizers being essential for feeding the human population (Erisman et al., 2008). Therefore, attempts to ameliorate the issues regularly focus on being conservative with soluble fertilizers to optimize efficiency while ignoring other agroecological processes. An example of this management philosophy is observed in the 4Rs approach that posits accurate use of fertilizers from the right source, in the right amount, at the right placement, and the right timing will optimize desired nutrient outcomes (Bruulsema et al., 2009). If determined accurately for individual farms, it is possible that this approach could reduce excessive use of soluble fertilizer while increasing plant use efficiency of imported nutrients. But these types of precision agriculture prescriptions adopt the same formulaic approach to farming that has been standardized by the general reliance on inorganic fertilizers. That is, these principles focus on supplementing small, ephemeral soil nutrient pools detected by chemical soil tests that target only easily extractable nutrients while completely ignoring total, mineralogical, and organic matter pools of nutrients that likely dictate nutrient availability across many growing seasons (Drinkwater and Snapp, 2007). Hence, this method maintains a reliance on fossil fuel derived and transported fertilizer nutrients to fulfil annual demands of plant production. The extent of this nutrient management strategy falls short of the multifunctional practices achievable through a more agroecological approach to nutrient cycling.

Enter the Ecological Alternatives

Rather than being treated as an isolated component of the farm production equation, nutrient management could be integrated into a conscientious approach that accounts for how farm operations embed within broader food system and ecosystem contexts as a whole – in short, an ecological mindset. A paper by Drinkwater and Snapp (2022) makes a case for such an approach through a set of guiding principles for Ecological Nutrient Management (ENM). The review presents both theoretical frameworks and applicable case studies. The five principles center around capturing reactive nutrients and transitioning them to more stabilized pools that remain biologically accessible but less prone to loss from the agroecosystem. Among the primary objectives of ENM is to implement effective plant diversity for

efficient nutrient cycling and storage mechanisms, thereby minimizing losses to the environment while supporting stable crop production. Of course, there are numerous ways to cycle nutrients in and out of farms without depending on synthetic fertilizers. These practices could include importing mineral nutrients, organic wastes such as livestock manure and compost, or even recaptured nutrients from human waste (Harder et al., 2021). However, the quality and quantity of such nutrient sources vary tremendously as does their availability in some regions of the world, making reliable access difficult (Norgaard et al., 2022; Nyamasoka-Magonziwa et al., 2021). On the other hand, virtually every form of agriculture works with some extent of plant variety as a basis. At a minimum, plants provide a foundation for maintaining functioning soils as an accessible mode of management, which can be supported by many other practices as appropriate.

Managing plant diversity in agroecosystems has numerous positive impacts that have been studied in various applications. The reasons for managing a diversity of plants are often multifaceted, with different plant systems suited to a wide range of goals. Some of the inherent benefits of increased plant diversity result from improved resilience to abiotic and biotic stresses such as storms, droughts, and pests (Isbell et al., 2015; Keesing et al., 2006). Plant diversity can be used to diversify farm revenue streams (and nutrition), or in a bad year could supply redundancy if most crops fail but at least one persists. Plant selection can also be based on ecologically functional criteria. For instance, utilizing leguminous plants to regulate soil nitrogen content takes advantage of the synergistic nature of legume species to increase or downregulate the amount of nitrogen fixation occurring throughout their lifecycle based on the relative supply of plant available nitrogen released from soil organic matter (Blesh, 2019). Therefore, legumes could be used to supplement soil nitrogen content with reduced chances of contributing excess nitrogen susceptible to loss when compared to attempting to match seasonal crop demand with soluble fertilizer application. Additionally, perennial plant systems can contribute greatly to restoring soil organic matter and the soil biological activity that regulates the availability of nutrients from these pools through attributes such as substantial root systems, reduced soil disturbance, and long-term carbon priming of the soil (Mosier et al., 2021). Thus, including leguminous forbs in a perennial forage mixture to graze livestock could combine improved soil health outcomes from deep-rooted, long-lived plants and some added nitrogen fixation with increased food quality of the forage plant species. It is this tendency toward multifunctionality which makes plant diversity management central to achieving the outcomes needed in agriculture.

The breadth of possibility for utilizing plant diversity in agroecosystems has been employed by land stewards throughout the history of agriculture (Altieri, 2000; Masters, 2021; Zimmerer et al., 2022) and continues to be explored today through the lens of modern research. There is vast potential for coupling traditional agrarian knowledge with present understanding of ecological science to determine,

among other things, the suitability for plant selection and management to improve soil nutrient status. One of the key opportunities is studying and relating measured traits or functions of plant species to help guide the implementation of diversified agroecosystems (Drinkwater and Snapp, 2007). Not that farmers are necessarily inclined to do so, but simply increasing plant species richness randomly might not be as effective as selecting plant species with roles and niches best tailored to the goals of the grower and the constraints of the environmental context (de la Riva et al., 2023). Hence, when assembling practices around introducing levels of plant diversity it is crucial to have access to knowledge about plant functions. Effectively, promoting functional plant diversity.

In ENM, it is beneficial to understand how plants can be incorporated in agroecosystems based on traits related to nutrient cycling. Plants are a key starting point for managing nutrients because of their role in primary productivity through carbon fixation. Primary productivity is the conduit by which energy enters soil as carbon fixed from the atmosphere. Carbon attained by plants is used as energy to do work in soils which cycles essential nutrients via two main pathways: direct/indirect mechanisms of plant growth such as root biochemistry and physical attributes (Lambers et al., 2006; Nuruzzaman et al., 2006) and serving as the energy basis for all other soil biology (Djikstra et al., 2013; Smercina et al., 2019). Plant mechanisms and soil biology often interact synergistically to transition, acquire, transport, and recycle nutrients. Throughout terrestrial ecosystems, sufficient nitrogen and phosphorus is obtained through adaptations in plant biology and relationships formed with soil organisms. Two prominent examples of plant symbiosis with soil microorganisms have evolved to address nutrient limitation in soils. Leguminous plants host specialized bacteria, such as rhizobia, capable of converting atmospheric nitrogen into ammonia, where plentiful energy and optimized (anoxic) conditions allow the bacteria to produce reactive nitrogen at increased rates relative to the more diffuse processes of host-free biological nitrogen fixation occurring outside of the symbiosis (Vitousek et al., 2013). Many plant lineages also host mycorrhizal fungi that are able to grow prolifically through soils, accessing nutrients such as phosphates which otherwise quickly become limiting around plant roots due to slow diffusion rates in soil solution (Smith and Smith, 2011). Such adaptations utilize the comparative advantage of plants as autotrophs to supply carbon-rich energy sources to heterotrophic organisms specialized in sourcing nutrients that plants need. Cooperative adaptations and coevolution are powerful attributes available to plants which have often been undermined in the industrial approach to agriculture. Ecologically based approaches such as ENM would prioritize leveraging the functional plant attributes available to agriculture which allow efficient nutrient cycling within an agroecosystem or food system.

Drinkwater and Snapp (2022) describe the general processes of plant nutrient cycling relevant to ENM. The formation and differentiation of soil organic matter is central to the role of plant diversity in nutrient cycling. Plants can uptake and conserve nutrients that are imported through management,

increasing the residence time of reactive nutrients that enter agricultural soils. Additionally, plants are active in liberating latent nutrients of soils that have existed in mineral and organic constituents from pedogenesis. Plant roots directly contribute to weathering of soils through their physical growth and the chemistry of root exudation (Wild et al., 2022). The carbon supplied by living and dead roots also serves as the foundational energy source to fuel soil biological activity which enacts further mechanical and chemical release of stored nutrients (Wild et al., 2022). All of this biological uptake of essential nutrients via plants and soil organisms is redeposited in the soil as necromass at the end of their lifecycle (Liang et al., 2019). Over time, this process of nutrient assimilation and turnover builds up reservoirs of organic matter in the soil ranging from readily labile to highly stabilized forms (Lavallee et al., 2020). The result in many ecosystems is a dynamic equilibrium whereby a relatively small concentration of reactive/decomposable organic nutrients supply seasonal growth while slow accrual and turnover of larger stable reserves buffers against the loss of nutrients (Daly et al., 2021). In agroecosystems, it is necessary to understand the relative bioavailability and synchrony of these biologically mediated soil nutrient cycles. Biogeochemical patterns of soil organic nutrient cycling are being uncovered, but the role of plant management and intervention is an active area of investigation. Working knowledge of the nutrient cycling mechanisms of different plants adapted to agriculture is not a simple task and requires thoughtful planning to be managed feasibly by a grower.

There are costs – financial, labor, time, and other opportunity costs – associated with increasing plant diversity complexity. Navigating this barrier requires careful consideration of the purposes of diversification to guide selection. Ecosystem research has demonstrated that not all services provided by biodiversity can be maximized simultaneously (Meyer et al., 2018). Even in agroecosystems which are generally more simplified, it may not be possible to supply the necessary space and resources to plant diversity to maximize all desirable outcomes in a single growing season. However, the industrialized approach to agriculture has gone far in the other direction, attempting to maximize only a few plant services – namely yield. Managing for only one ecosystem service tends to undermine multifunctionality in ecosystems (Bullock et al., 2011). By prioritizing only the most profitable plants, decreased diversity has come at the expense of all other important attributes of diverse agroecosystems (Chappell and LaValle, 2011). It is necessary to balance the costs of introducing and managing plant diversity with the value of services rendered. Part of the consideration comes down to balancing tradeoffs between regulating and provisioning services of plants. It is evident that agriculture necessitates an emphasis on provisioning services. However, disregarding the regulating services that plants offer has created, among other issues, a dependency on supplementing with importation of fertilizers (de la Riva et al., 2023). Provisioning crops such as grains and vegetables selected primarily for yield are adapted to easily accessible soil nutrients which allows them to bypass the investment of carbon into root activity and soil

biology priming (Hirte et al., 2018; Martin-Robles et al., 2018; Poyda et al., 2023). If these are the only plants used in an agroecosystem, soil nutrient cycling is suppressed, and the absence of fertilizers would result in declining yields. When plant diversity is utilized, there are many opportunities to manage complementarity in a variety of plant traits across the continuum of provisioning and regulating services. Intensive crops with high harvest index could be cycled with plants valued for mineral weathering, nutrient scavenging, nitrogen fixation, soil carbon accrual, or any number of traits that regulate nutrient availability. Often, combining traits conducive to healthy nutrient turnover goes hand in hand with other soil health functions including physical and biological characteristics (Mosier et al., 2021). The ability to stack functions makes plant diversification appealing relative to simply applying fertilizers.

Opportunities for Plant Diversity

There are numerous ways to adopt higher plant diversity which are as varied as the goals of farmers and the types of ecosystems where agriculture is practiced. There is rich historical and cultural knowledge about the merits and strategies of utilizing plant diversity in the context of food production. From traditional fire and mowing management of grasslands to kickstart succession, resulting in elevated plant diversity to provide higher number of species with human uses in Japan (Uchida and Kamura, 2020), to shifting cultivation and intercropping of complementary food crops in Malawi (Mulwafu, 2011), land stewards have maintained regionally appropriate and successful practices for ages. The future of adapting agriculture to fit human and environmental needs simultaneously will require building upon this legacy. Among the many examples of how plant diversity is managed in agroecosystems, there are two fundamental strategies. Plant diversity can be increased either temporally or spatially. That is, in the same spatial position plant diversity can be cycled over time or at the same point in time plant diversity can be employed across spatial scales. Both of these strategies are often used together, but this framework helps to categorize the opportunities for adding plant diversity to an ongoing system.

This thesis explores two distinct approaches for embedding plant diversity which pertain to the unique contexts of the systems being studied. Cover cropping involves growing plant species not intended for harvest in specific seasonal intervals between the timing of primary crops. The purposes of cover cropping are generally focused on providing regulating ecosystem services such as weed plant suppression, prevention of soil erosion, and contribution to soil health (Scavo et al., 2022). The use of cover crops is an example of temporal plant diversification. It is often practiced in parts of the world, such as temperate environments, where seasonal periods of the year not conducive to the growth requirements of primary crops can be used to fit in plants adapted to those conditions. The time from fall to spring in the upper Midwest of the United States is cold and has shorter photoperiods which makes this season unsuitable for many of the staple grain crops grown in the region, such as maize or soybean. This creates a period of time where plant diversity can be introduced via cover cropping without competing for space

needed to grow crops during the peak season. Chapter two of this thesis investigates nutrient cycling potential of cover crop management. The soil carbon and nitrogen attributes of different cover crop systems are well established in a variety of contexts (Nyabami et al., 2024; Perrone et al., 2022; Thapa et al., 2018), but there is ample room to discover the soil phosphorus cycling potential of specific cover crop plants (Hallama et al., 2019). Such information can be used to further inform management decisions reflective of the range of nutrient cycling possibilities in cover crops.

The other system investigated in chapter three focuses on cropping systems in Malawi that integrate a range of intercrop species diversity. Intercropping is a spatial crop diversity approach which involves the simultaneous growth of different plants in proximity to each other around the same time (i.e., at least some overlap in the time that different plants are growing). This is a traditional practice that could be used to maximize the variety of plants grown within a productive season or to utilize limited resources such as land space or labor (Witcombe and Tiemann, 2022). In Malawi, multiple species of plants important for household use may be grown together in the field to efficiently use space and get the most variety of production during the unimodal rain season (Mungai et al., 2016). The target for intercropping strategies in this context is usually the provisioning service of harvest and the variety of direct uses of the plants needed by farmers. Though, regulating factors could be important secondary outcomes of intercropping. For instance, the intercropping of cereal grains with pulse crops not only supplies complete protein food sources for household/community nutrition, but also provides the complementary attributes of nitrogen fixation from the legume and high carbon fixation from the grass to support soil nutrient balances (Witcombe and Tiemann 2022). The long-term contributions of higher diversity intercropping systems to building soil carbon pools has been demonstrated (Fujisaki et al., 2018; Tu et al., 2022). Influence from plant diversity on carbon cycling may have interesting implications for shaping soil microbial communities over time. Differences in soil microbiomes between intercropping practices could be informative for exploring feedbacks between plant selection and soil biology selection as it relates to functions such as nutrient cycling in future areas of research.

Both sets of practices are currently implemented for many reasons that go beyond the potential benefits of nutrient cycling. The focus of this study will be on the way that these practices influence the intersection of plant diversity, soil biology, and nutrient cycling. It is the goal that such information contributes to an ever-growing body of knowledge supporting the management of functional plant diversity to address the nutrient needs of food systems. Reducing reliance on externally sourced, fossil fuel dependent fertilizers through improved agroecosystem nutrient cycling is essential for reducing harmful agricultural externalities while also providing liberation for farmers as stewards of the land to have more sovereignty over the elements of their production.

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CHAPTER 2:
AMF Colonization Improves Leguminous Cover Crop Phosphorus Uptake in Organically Managed Soil

Abstract

Phosphorus is an essential nutrient for crop growth. Though prevalent in the soil, plants can suffer from low availability of phosphorus due to physical and chemical pathways which occlude this nutrient from plant uptake. Soil phosphorus fertility management requires more strategies for maintaining plant productivity while reducing externalities caused by over application of phosphates. Cover crops have potential to improve soil nutrient cycling, particularly in partnership with symbiotic organisms such as arbuscular mycorrhizal fungi (AMF). A greenhouse experiment was conducted using soils from contrasting agronomic management histories to compare differences in soil phosphorus distribution, cover crop species selection, and the presence or suppression of AMF colonization to determine the relative contribution of these variables to cover crop phosphorus uptake. Cover crop response to AMF colonization was highly dependent on cover crop species and the relative lability of soil phosphorus. Mycorrhizal colonization increased hairy vetch (*Vicia villosa* Roth) biomass by 121% and more than tripled total phosphorus uptake compared to plants without AMF in organically managed soil with low extractable phosphorus. In contrast, in rye (*Secale cereale* L.) total plant phosphorus uptake in conventional agronomic soil was significantly suppressed (35% less) when colonized by AMF compared to uncolonized control plants. These results demonstrate that host plant mycorrhizal dependency and phosphorus uptake potential of cover crops are highly unique traits that can be driven by soil phosphorus availability and plant host growth strategies. Further exploration building upon the methods utilized in the present study could identify optimizations in cover crop species selection for the purpose of internal phosphorus cycling given variations in soil phosphorus distributions and the efficacy of existing AMF communities.

Introduction

Phosphorus (P) is an essential nutrient for crop plants, yet is physicochemically constrained in most soils (Stutter et al., 2015). Many farmers respond to the low availability of soil P by applying soluble P fertilizers. The global P reserves that supply this input are finite, costly to extract, and have an environmental footprint from mining and processing (Cordell and White, 2015). As an intervention, cover crops may offer a lever for growers to enhance the bioavailability of P sources in farm soils (Hallama et al., 2019). Cover crops can be used to influence internal nutrient cycling of cropping systems, as is often demonstrated with nitrogen and carbon cycling (Koudahe et al., 2022; Snapp et al., 2005).

Functional plant diversity via cover cropping has the potential to change the P availability status of a soil both directly through rhizosphere activity (Cu et al., 2005) and by uptake and deposition of P in the cover crop biomass (Dube et al., 2014; Hallama et al., 2019). Plant mechanisms and interactions with

the soil microbiome have been identified as ways that cover crops have the potential to mobilize occluded P reserves in soils (Hallama et al., 2021). Broadly, we hypothesize that different cover crops can utilize low-solubility P that is otherwise unavailable for crop plants and convert this to organic pools through deposition of their residues, release of exudates, and stimulation of soil microbial activity. This could provide a stable, steadily mineralizable source of P for crops. To address this hypothesis, it is necessary to study how cover crops interact with symbiotic partners to extract P from different soil sources. Arbuscular mycorrhizal fungi (AMF) form an integral partnership with plants for the uptake of P (Smith and Smith, 2011). However, the actual role and capacity of AMF-cover crop combinations for affecting soil P distribution is not fully understood. Providing knowledge of this symbiosis applicable to growers will involve characterizing the impact of cover crops and AMF on P dynamics in a variety of soils.

Arbuscular mycorrhizal fungi (AMF) are known to access P for plants (Smith and Smith, 2011). The degree of plant benefit from this symbiosis varies with respect to plant host species (Bunn et al., 2015; Elbon and Whalen, 2015; Pandey et al., 2005), soil characteristics (Cardoso et al., 2006; Kim et al., 2017), and mycorrhizal partner (Smith and Smith, 2011). It is often considered that soils with high P bioavailability will result in plants that do not benefit from AMF P acquisition because the plants can easily access P using direct phosphate uptake mechanisms (Breuillin et al., 2010; Salvioli di Fossalunga and Novero, 2019). Meanwhile, soils with low extractable P are conducive to observable plant benefit from AMF colonization (Andrino et al., 2021; Mora et al., 2019).

In addition to nutrient availability, different mycorrhizal host plants are known to have variable responses to colonization. Plant functional group, life history, and physiology have all been shown to affect the degree to which a plant species benefits from colonization (Qin et al., 2022; Hoeksema et al., 2010). For instance, plants with more fibrous root systems may be less responsive to AMF colonization regarding nutrient acquisition due to tradeoffs between investing in root exploration versus symbiont carbon allocation (Yang et al., 2015).

Others have found that plants perform differently with AMF, such as Bunn et al. (2015) who observed that forbs have better growth response to AMF than grasses. Though these general trends have been observed in a number of settings, making consistent predictions about coupled impacts of AMF and host plant on P cycling in agricultural soils is limited by the diversity of test environments, lack of consistency in plant growth and testing methods, and the paucity of consideration for the distribution of soil P forms beyond standard extractable soil test P levels – especially organic P pools. Current understanding of these nutrient cycling dynamics is also represented by many studies utilizing AMF inocula consisting of one or very few total species which fails to represent the diversity of AMF taxa existing in soils. This is particularly true for controlled environment/potting experiments (Hoeksema et al., 2010). The present study uses the local AMF community of the agronomic sites to assess the

symbiotic nutrient cycling potential of naturally occurring AMF taxa relevant to the study context.

The Long - Term Ecological Research Main Cropping System Experiment (MCSE) at Kellogg Biological Research Station in southwest Michigan presents the opportunity to test both long-term drivers and short-term dynamics of soil P availability in cover crop-AMF interactions. Agronomic management treatments have produced divergent properties in soils found at this site. Gallaher and Snapp (2013) determined that long-term management treatments in the MCSE have resulted in contrasting levels of organic soil P, with 57% higher P associated with particulate organic matter in the organic management treatment than the conventional. This distinction in soil P pools in the MCSE is essential for testing these cover crop-AMF-soil P interactions. Simultaneously, there is the potential to link effects of management history to soil P distribution and how this influences ecologically integrated nutrient management approaches such as cover crop-AMF P mobilization.

Legacy effects of management are expected on soil P pools present in agricultural soils that have diverged in amounts and distribution of P occurring in organic or inorganic pools, but that are otherwise edaphically similar because they share the same landscape and soil series (Robertson and Hamilton, 2015). Using these soils from the same site as the plant growing medium in a controlled potting experiment allows comparisons between cover crop P dynamics driven by long-term management without confounding other differences in mineralogy and texture which could strongly influence the bioavailability of P species in soils (i.e., through sorption and metal complexation processes; for example, see Matoso et al., 2023).

A bioassay experiment was designed for comparing cover crop and AMF P acquisition from different soil P distributions. This study sought to determine the relative contribution of soil P distribution, cover crop species, and AMF symbiosis to cover crop P uptake and growth. Two cover crop species: winter rye (*Secale cereale* L.) – an annual grass with extensive rooting, and hairy vetch (*Vicia villosa* Roth) – an annual legume with sparser roots, were selected to explore this topic in addition to the two agronomic management soils. I hypothesized that i) overall biomass would be higher in conventionally fertilized field soil than the organic management treatment, ii) that cover crop P uptake would be higher in AMF colonized plants than those with suppressed AMF, iii) that cover crop response in terms of biomass and P uptake to AMF colonization would be greater in the organically managed soil, and iv) that cover crop response to AMF would be cover crop species dependent, with hairy vetch being more responsive to AMF colonization than rye.

Materials and Methods

Soil Selection

Soils used in this greenhouse experiment were sourced from agronomic field treatments T1 and T4 in the Main Cropping System Experiment component of the Long-Term Ecological Research site at

Kellogg Biological Station. Agronomic treatments of interest to this study represent annual crop systems of maize-soybean-wheat rotations. The agronomic treatments were identified to have contrasting soil P pools which provide ideal systems for testing cover crop P uptake processes from real agricultural soils. Previous research from Gallaher and Snapp (2013) demonstrated that the T4 biologically-based treatment (hereafter called "Organic") has developed greater (57% higher) quantities of P in the particulate organic matter (POM) fraction of soil organic matter when compared to the T1 conventionally managed treatment (hereafter called "Conventional").

Soils for setting up the greenhouse experiment were sampled in April of 2023. Sampling occurred between the previous crop of wheat, which had been harvested in July 2022, and the sowing of maize in May 2023. Conventional plots were tilled in October 2022 and remained bare at the time of sampling, while Organic plots had a medium red clover (*Trifolium pratense* L.) cover crop which was interseeded with the previous wheat crop in this treatment and had over-wintered. A soil auger was used to collect the top 15 cm of soil. Due to the large quantity of soil needed, soils were sampled only from a 15 m x 87 m segment of the large treatment plots designated for more destructive sampling. Enough cores were taken to fill a 5-gallon bucket per field replicate. Three field replicate plots were sampled from both Conventional and Organic treatments. The replicates were later composited to create a potting media representative of any field heterogeneity present in the blocks. The soils were passed through 6mm sieves to screen large debris and homogenize the soils. Prior to use, soils were stored in buckets with lids in a walk-in cooler at 4 C.

Key initial nutrient measurements from the soils at the time of sampling are reported in Table 1.1, illustrating the greater concentration of Bray extraction P in Conventional samples relative to Organic samples. These measurements confirmed the contrast in soil nutrient distribution based on management legacy that could be utilized in this study. Initial inorganic nitrogen concentrations were similar between both agronomic treatments.

Table 1.1 – Soil properties from the time of field sampling (Initial) and after Oven (80 C for 12 h) and Autoclave (121 C for 45 minutes) control methods to suppress AMF propagules

Agronomic Source	Treatment	Bray-P (ppm)	NO_3 – N (ppm)	NH_4 – N (ppm)
Conventional	Initial	64.0 [4.5]	19.3 [0.4]	3.2 [0.6]
	Oven	77.0 [1.9]	22.1 [1.5]	7.0 [0.9]
	Autoclave	63.8 [0.9]	19.2 [0.3]	8.4 [0.3]
Organic	Initial	10.1 [0.3]	22.9 [0.5]	2.0 [0.2]
	Oven	14.3 [0.1]	25.8 [1.5]	5.9 [0.5]
	Autoclave	20.5 [0.5]	22.2 [0.9]	11.5 [0.3]

Note. Data is provided in the format: means [standard deviations] where applicable.

Plant Selection

The two species of cover crop plants that were selected for this study are commonly used by growers in the Upper Midwest region. Additionally, the selection of cover crop types was made to facilitate comparing plant functional groups. Winter rye (*Secale cereale*) represents a standard grass cover crop, while hairy vetch (*Vicia villosa*) is a leguminous forb.

The cover crop seed was sourced from Ernst Seeds in Pennsylvania through collaborators at Cornell University. All seed was washed and surface sterilized to ensure that no external microbial spores, including that of AMF, would be introduced to the experimental control treatments. Seeds were washed in 0.5% NaOCl and 70% ethanol solutions for two minutes each, with deionized water rinses between and after the washes. Seeds were germinated on wetted filter paper in Petri dishes until the emergence of the root radicle was visible in the majority of seed; an average of three days for rye and six days for vetch. Germinated seeds were then transplanted.

Experiment 1

Soil Preparation. In order to test the impact of AMF colonization on cover crop P uptake, control conditions were created by employing a novel heating method to the soils. Many studies attempting to control soil organisms, including AMF, use high temperature and pressure approaches such as autoclaving (Al-Khaliel, 2010; Endlweber and Scheu, 2006; Hu et al., 2020; Xie et al., 2014). This approach can create artifacts, including significantly shifting nutrient pools (Endlweber and Scheu, 2006; Hu et al., 2020). To avoid drastically altering soil P pools (e.g., lysing cells, degrading organic compounds), an alternative heating method was used. Fresh soils collected from the field were spread to a depth of 1 inch in aluminum trays and placed in preheated ovens at 80 C for 12 hours. This temperature is the minimum threshold identified to effectively kill AMF propagules including hyphae and spores according to protocols available through the International Collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM, n.d.). Ideally, the use of dry heat reduces alteration to soil nutrient composition that typically occurs in autoclaved soils. Subsamples were taken before and after heating to assess changes in the soil. This oven method reduced the amount of soil P that was shifted to Bray-extractable pools in Organic soil samples relative to the standard autoclave approach (Table 1.1).

Potting Setup. Heated soil from Conventional and Organic plots was used to fill up conical treepots (6.86 cm x 20.32 cm, volume = 490 mL) as the growing medium and primary source of P to the cover crop plants. AMF control treatments received 425 g of heated soil alone, while positive AMF treatments had propagules reintroduced by adding 10% by weight of fresh soil from the Organic plots (42.5 g fresh soil + 382.5 g heated soil). All treatments also received a microbial wash at a quantity of about 2% the volume of final soil in the pots. The wash was prepared by mixing fresh field soil 1:1 by volume with deionized water and sieving the liquid down to 10 μm so that AMF spores/hyphae were excluded while prokaryotes

and small, non-target fungi could be reintroduced. This allows AMF control soils to maintain components of the soil microbiome which may be important in P cycling, such as P solubilizing bacteria, to reduce the likelihood of overestimating the role of AMF in increasing cover crop P acquisition. AMF-positive treatments also received the microbial wash to control for confounding effects such as the introduction of soluble nutrients (Fig. 1.1).

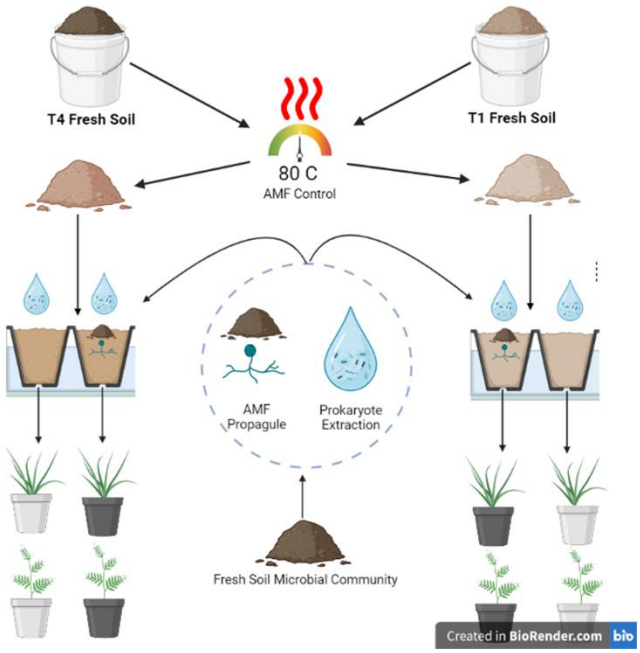
Deionized water was added to bring the soils up to 15% moisture content by weight, which was the average field conditions for these soils according to a data set compiled from annual soil sampling at KBS. Germinated cover crop seeds were planted into the pre-wetted soil a couple days later to allow time for weed seeds contained in the soils, if any, to emerge and be removed. Irrigation requirements were determined by weighing pots and adding deionized water to bring them back up to 15% moisture content as needed.

Finally, a modified Hoagland's nutrient solution was used to add plant-essential nutrients while excluding additions of P to ensure that other non-target limitations would not occur. The formulation for the nutrient solution is as follows: $Ca(NO_3)2*4H_2O$, KNO_3, $MgSO_4*7H_2O$, KCl, Fe-EDTA, H_3BO_3, $MnCl_2*4H_2O$, $CuSO_4*5H_2O$, $ZnSO_4*7H_2O$, MoO_3*H_2O. These stock nutrient solutions were diluted to a half-strength Hoagland's dosage (1.25 mL $CaNO_3$ and KNO_3, 0.5 mL $MgSO_4$, 0.5 mL KCl, 0.5 mL Fe-EDTA, and 0.5 mL micronutrient solution per liter of final volume in deionized water). Fertilizer application was determined based on a target of 30 lb N / acre rate recommended for rye which was derived from plant uptake values in literature summarized by Ozorio et al. (2022). Based on the concentration of N in the half-strength Hoagland's solution, 83.33 mL of solution per pot was applied split into doses of 10.4 mL per week over the course of plant growth.

The first potting experiment was carried out in 2023 on raised benches in a lath house to allow adequate shading and air circulation. A covered structure with a clear plastic sheet was used to exclude rainfall from the potted plants so that soils would not flood or leach. Photoperiod for the duration of the experiment was about 14-15 hours of daylight, and daytime temperatures fluctuated between 20 – 30 C. Rye and vetch plants were grown for a total of 8 weeks. At this time, the vetch had just begun to flower.

Treatments and Layout. This study is a completely randomized design (CRD) with three factors having two levels each: soil (Conventional, Organic), cover crop (rye, vetch), inoculation (control, AMF). Soils, plants, and AMF treatment were randomly assigned to pots, and were arranged randomly in trays on the growth table. Each treatment was replicated 10 times, resulting in a total of 80 experimental units.

Figure 1.1 - Visualization of the Bioassay Setup



Infographic depicting the workflow to establish the bioassay. Fresh Soil (top) is collected from the field (T1: Conventional; T4: Organic); subsample of fresh soil (bottom center) is retained for the Prokaryote Extraction and reintroducing AMF Propagules; soil is heat treated for AMF Control; all treatments receive Prokaryote Extraction; positive treatments (black pots) receive addition of fresh soil containing AMF Propagules, while controls (white pots) do not; Cover crops rye (*Secale cereale*) and hairy vetch (*Vicia villosa*) planted. Image created with BioRender.com.

Experiment 2

The second experimental trial, conducted in 2024, repeated the dry heat method (80 C oven, 12 h) in addition to an autoclaving method (121 C, 45 minutes). The second experiment included the same factors as the first trial (soil history, cover crop species, AMF treatment) with the added factor of AMF suppression method (oven or autoclave). This experiment was a 2x2x2x2 factorial CRD of 16 treatments, all with 7 replicates totaling 112 experimental units. The experiment was executed as described previously except that the plants were grown in a greenhouse receiving 16 hours of light per day for 8 weeks from January to March rather than 14-15 hours of daylight per day in Experiment 1.

Plant Measurements

Plant samples were collected at the end of each experiment after 8 weeks of growth. Plant roots and shoots were separated, with dry biomass taken for each. Above-soil rye and vetch tissues were dried at 70 C for 12 hours, ground, and passed through a 1-mm sieve. Ground shoot and leaf tissues were submitted to a commercial laboratory for P, N, and C analysis. Before drying the root biomass, a subsample of fine, fresh roots was taken from each plant for measuring AMF colonization. In Experiment 2, roots were also ground and analyzed for total P, C, and N. Due to the relatively low biomass produced

during both years of experiments, tissue samples from treatment replicates were composited for nutrient analyses resulting in 24 samples per AMF suppression method per year.

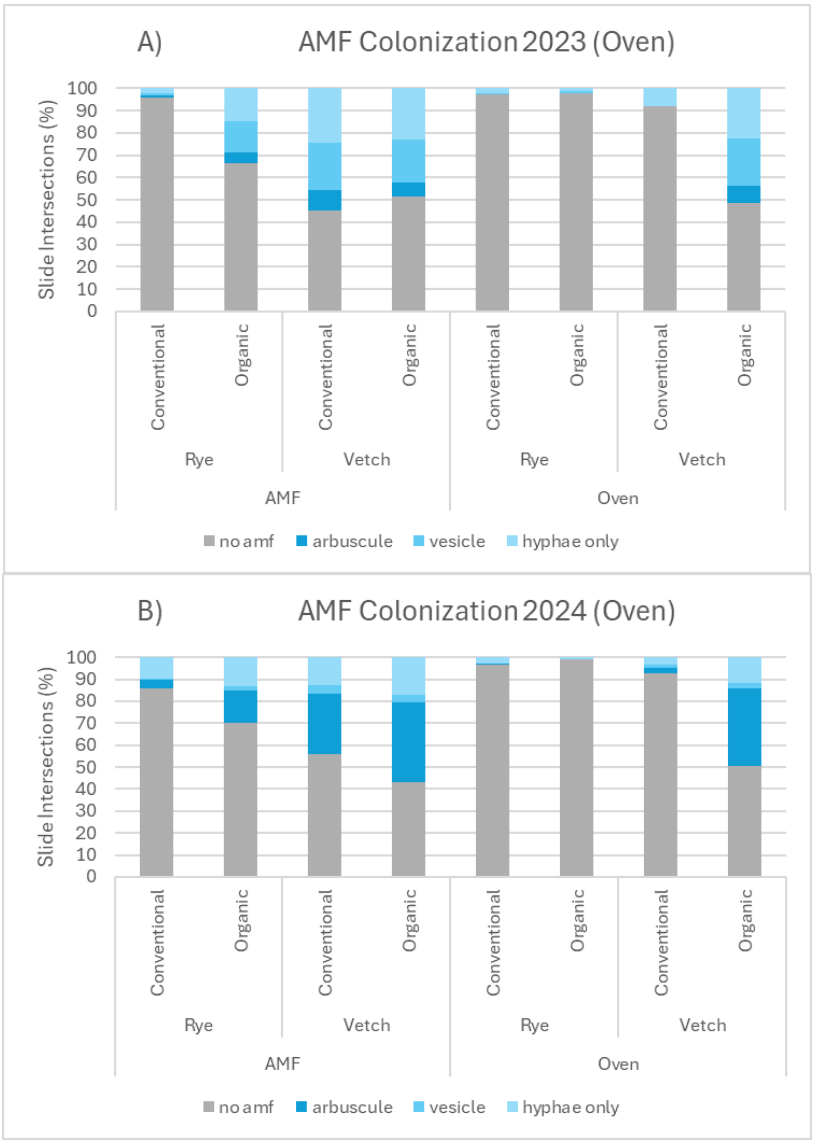
Determination of AMF colonization was done following a staining procedure by INVAM based on the approach in Giovannetti and Mosse (1980) and quantification method based on McGonigle et al. (1990). Briefly, fine roots of rye and vetch plants were cleared in heated 10% KOH for 10 minutes, acidified in 2% HCl for 20 minutes, then stained with heated 0.05% methyl blue solution (glycerin, methyl blue, HCl) for 5 minutes. Stained roots were stored in deionized water at 4C until microscopy was conducted. Stained root slides were prepared by randomly selecting roots which had been cut to 3-cm segments with a total of 5 root segments per slide. This allowed root length (approximately 15 cm per sample) to be consistent in comparisons of treatments. AMF colonization was measured using the magnified intersections method from McGonigle et al. (1990) which uses a magnification of up to 40x, facilitating proper identification and quantification of arbuscules. Given that arbuscules are the active site of exchange between fungal and plant partners relevant to P uptake dynamics (Luginbuehl and Oldroyd, 2017), this allowed a determination of more active AMF colonization structures salient to the P dynamics questioned here.

Statistical Analyses

Statistical analyses of plant and soil measurements were conducted using R Software (v 4.1.1) and R Studio (v 2023.12.0.369) (Posit Team, 2023; R Core Team, 2021). Multiple linear regression models were made to assess the main and interaction effects of study factors (soil source, AMF colonization, cover crop species) on plant biomass and phosphorus content response variables using the "lm" function. Each year (2023 or 2024) and each control method (Oven or Autoclave) were analyzed separately. Assumptions of normality and equal variance were confirmed by visualizing residual plots and conducting Levene's test, respectively. Weighted least squares regression models were used to weight variances separately for factors that did not pass the homogeneity of variance test using the generalized least squares model fitting function "gls" from the *nlme* package (Pinheiro et al., 2021). Analysis of variance (ANOVA) was performed on full, three-factor models to determine significance of main and interaction effects of treatment on cover crop responses. Any significant interaction terms were explored using a slicing approach with the "testInteractions" function from the *phia* package to determine significant differences of one factor across levels of corresponding interacting factor(s) (De Rosario-Martinez, 2015). Further, the relationships of interaction terms were visualized by plotting the interaction means. Mean comparisons of factor levels were conducted using the *emmeans* package, with letters representing significant mean separation of all pairwise comparisons determined by the "cld" function (*multcomp*) employing Bonferroni p-adjustment to account for multiple comparisons (Hothorn et al., 2008; Lenth, 2023).

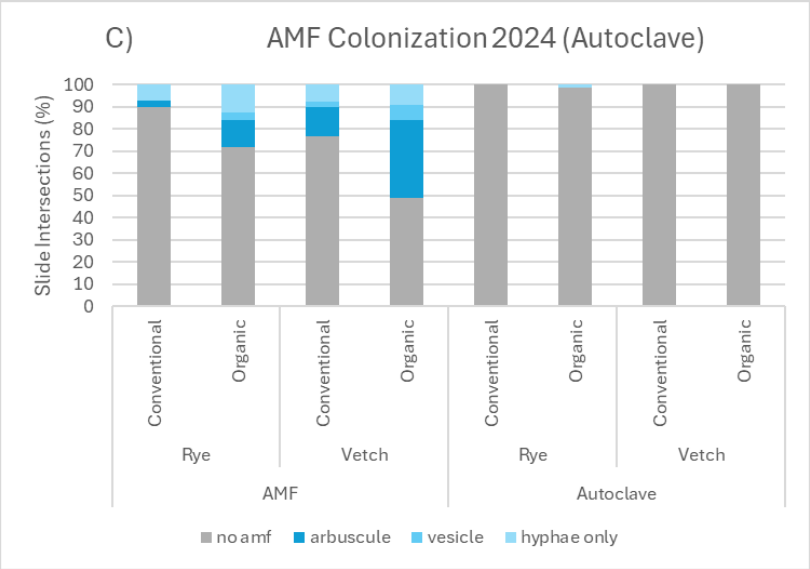
Results

Figure 1.2 – AMF Colonization Quantification in Cover Crop Roots



Stacked bar plot showing the percentage of slide intersections where AMF structures were observed at 40x magnification under the microscope for each treatment in A) the first trial (Oven 2023) and the second trial B) Oven 2024 and C) Autoclave 2024. Legend color refers to the dominant AMF structure (no amf, arbuscule, vesicle, or hyphae only) quantified at each slide intersection; Conventional and Organic soil history, AMF reintroduced or Oven/Autoclave control, Rye or Vetch cover crop species.

Figure 1.2 (cont'd)

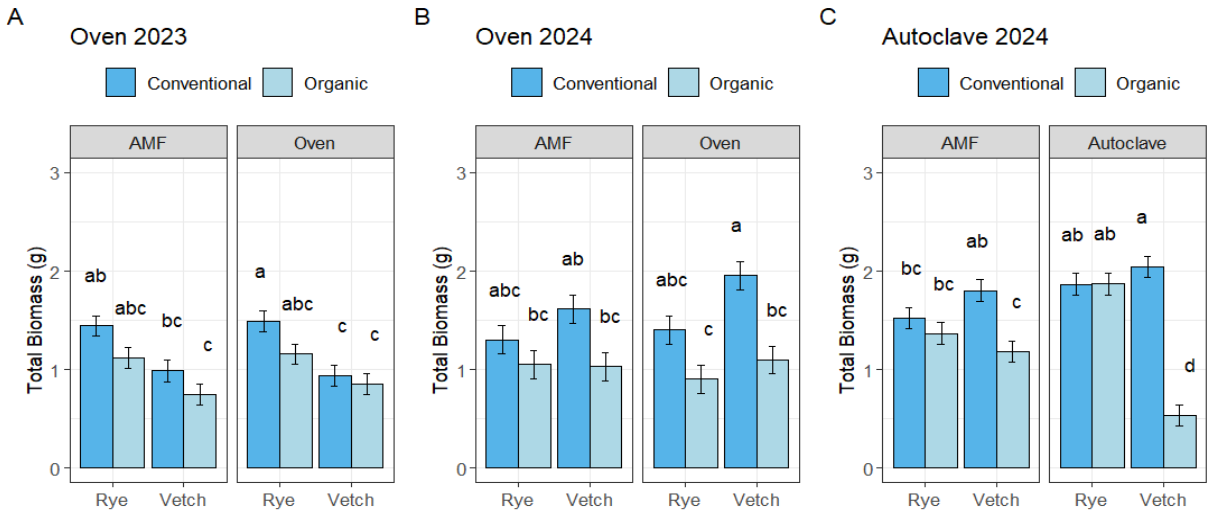


Measurement of AMF colonization in the plant roots demonstrated a drastic difference between the effectiveness of AMF control methods used. In 2023 when the oven control method was employed to suppress AMF propagules in the soils, suppression was effective for all rye plants regardless of soil management history. However, suppression appeared to be intermediate in vetch grown in the conventional soil and completely ineffective when vetch was grown in the oven-treated soil from organic management history. These findings were replicated in 2024 when colonization was again determined to be nearly as high in vetch grown in oven-treated control soil as in the AMF positive treatment when propagules were reintroduced. This phenomenon was pronounced in the organically managed soil and minimal in the conventional soil (Fig. 1.2A and 1.2B).

The autoclaved heat treatment introduced in the 2024 study was determined to be much more effective at suppressing AMF colonization. Identification of AMF colonization structures (arbuscules, vesicles, or intraradical hyphae) was nearly zero in all autoclave control samples (Fig. 1.2C).

Across all control methods and in both years, vetch had higher rates of AMF colonization on average compared to rye.

Figure 1.3 – Total Cover Crop Biomass

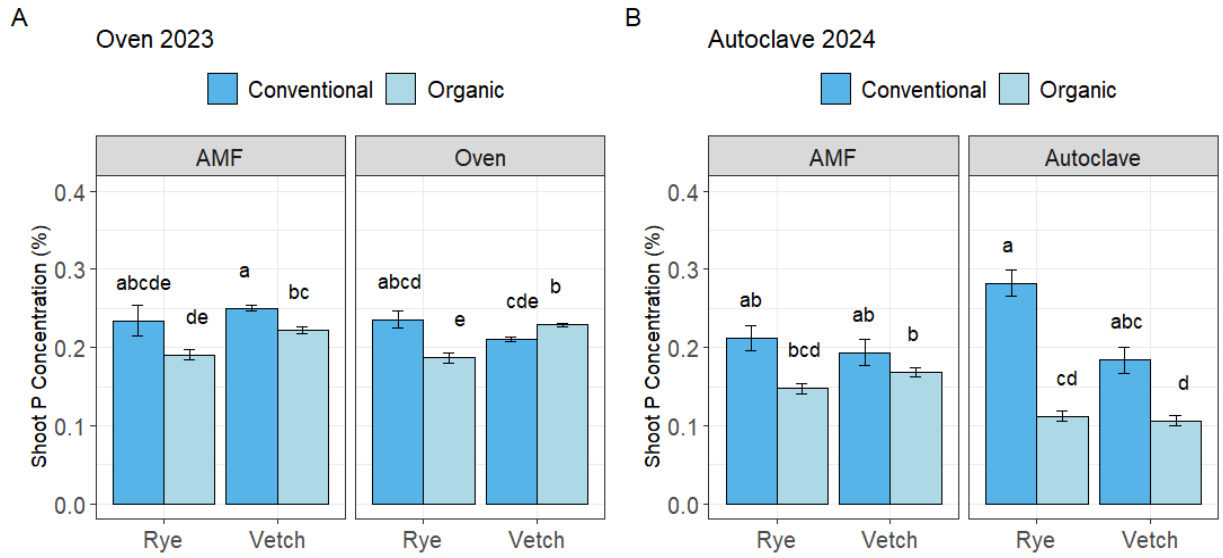


Bar graph of total biomass across all treatments in the A) oven treated 2023, B) oven treated 2024, and C) autoclaved 2024 soils; Conventional and Organic soil histories, AMF reintroduced or Oven/Autoclave control, Rye or Vetch cover crop species. Error bars represent the standard error (n = 7). Bars in the same plot (A, B, or C) with different letters are significantly different at $\alpha = 0.05$.

Winter rye and hairy vetch growth varied in response to soil management history relative to the AMF control method used and between the two experiment years. In the first year when the oven control treatment was used, rye had the highest average biomass (1.30 g for rye compared to 0.88 g for vetch). In 2024, vetch growth improved, as can be seen in the relatively higher biomass in both oven-treated and autoclave-treated soils from that year (Fig. 1.3). Cover crop biomass was not affected by AMF colonization in either 2023 or 2024 when the oven-treated soil was used as the AMF control. Significant pairwise differences between treatments were only measured in combinations of soil management history and cover crop species. For instance, plants grown in the conventional soil had higher biomass overall ($p = 0.001$ in 2023 and $p < 0.001$ in 2024).

When the autoclave control method was used, however, hairy vetch biomass was approximately 121% higher in the AMF positive treatment than samples where AMF was suppressed in the organically managed soil. This biomass response to AMF treatment was not observed in vetch grown in the conventional soil nor in any of the rye samples.

Figure 1.4 – Cover Crop Shoot Phosphorus Concentration



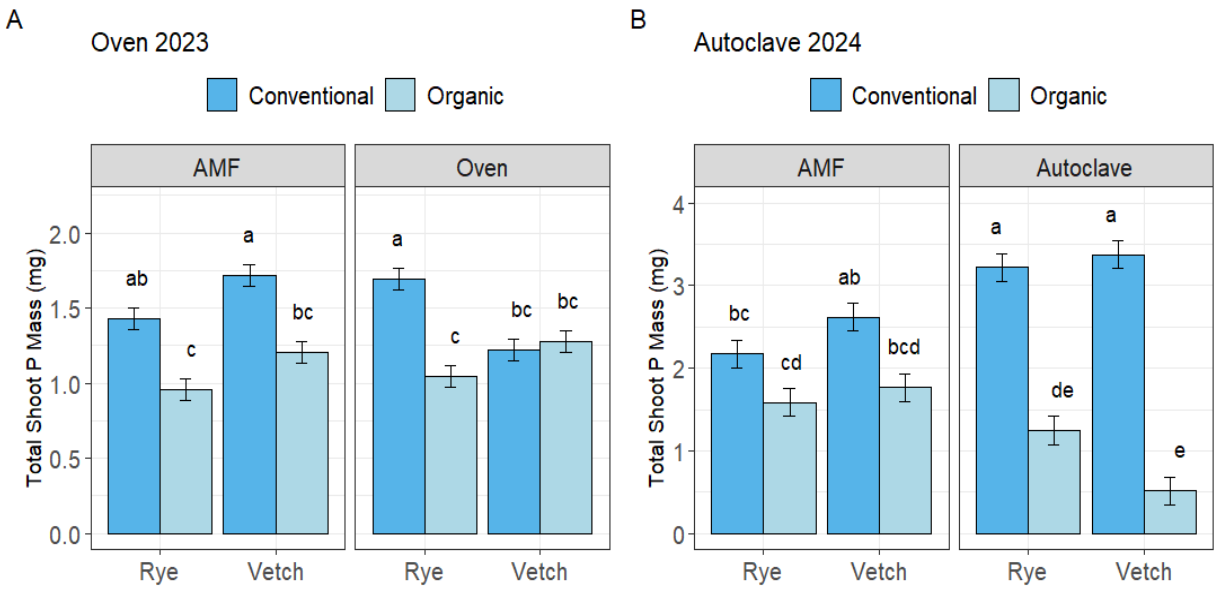
Bar graph of shoot tissue phosphorus concentration across all treatments in the A) oven treated 2023, and B) autoclaved 2024 soils; Conventional and Organic soil histories, AMF reintroduced or Oven/Autoclave control, Rye or Vetch cover crop species. Error bars represent the standard error of A) individual treatments and B) soils due to dissimilarity in variance. Bars in the same plot (A or B) with different letters are significantly different at $\alpha = 0.05$.

Cover crop P cycling potential was significantly affected by interactions between soil management history, cover crop species, and AMF treatment as illustrated by plant tissue P content measurements. Aboveground tissue (shoot) P concentration was typically highest in plants grown in the conventional soil. This was also true for total shoot P mass which translates to plant P uptake (Fig. 1.5). Some significant pairwise differences occurred between treatments in the 2023 experiment, but the range in shoot P concentration was much narrower (0.18 – 0.25%) than that measured in the autoclave-treated study from 2024 (0.10 – 0.21%) (Fig. 1.4).

AMF treatment had a unique effect on vetch in the organic soil treatment. In the autoclave samples, significantly higher P concentration was measured when vetch was grown in organic soil where AMF was reintroduced than the autoclave control. In combination with the higher biomass observed in this treatment (Fig. 1.3C), this resulted in significantly higher total P uptake in the shoot tissue for vetch plants with AMF colonization in the organic soil than those without colonization. Conversely, this AMF effect on total shoot P was not seen in the oven trial from 2023 (Fig. 1.5A).

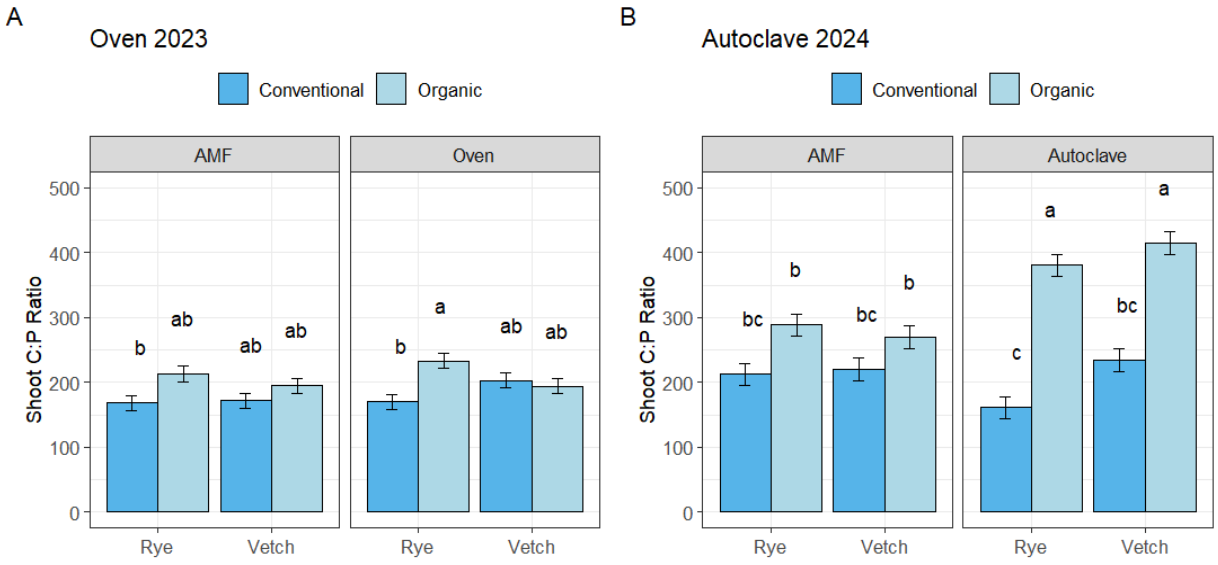
Rye P dynamics were not driven by AMF colonization except for one instance in the autoclave trial. Rye shoot P content was suppressed by AMF treatment in the conventional soil when compared to the autoclave control. This corresponds to the slightly lower biomass and shoot P concentration from this treatment (Fig. 1.3C and Fig. 1.4B).

Figure 1.5 – Total Cover Crop Shoot Phosphorus Mass



Bar graph of shoot tissue phosphorus mass across all treatments in the A) oven treated 2023, and B) autoclaved 2024 soils; Conventional and Organic soil histories, AMF reintroduced or Oven/Autoclave control, Rye or Vetch cover crop species. Error bars represent the standard error (n = 7). Bars in the same plot (A or B) with different letters are significantly different at $\alpha = 0.05$.

Figure 1.6 – Cover Crop Shoot Carbon to Phosphorus Ratio (C:P)



Bar graph of shoot tissue carbon to phosphorus ratios across all treatments in the A) oven treated 2023, and B) autoclaved 2024 soils; Conventional and Organic soil histories, AMF reintroduced or Oven/Autoclave control, Rye or Vetch cover crop species. Error bars represent the standard error (n = 7). Bars in the same plot (A or B) with different letters are significantly different at $\alpha = 0.05$.

Finally, the ratio of carbon to phosphorus (C:P) for plant shoots followed similar trends to the

other plant P metrics. Shoot C:P was driven primarily by soil history, with plants grown in conventional soils having lower C:P than those in organic soil. AMF colonization significantly reduced C:P in both vetch and rye grown in organically managed soil compared to the autoclave control samples (Fig. 1.6B). Tissue P quality did not differ between most treatments in the 2023 trial, on the other hand.

Discussion

Cover crop phosphorus cycling potential was demonstrated to have distinct responses to colonization by AMF as driven by differences in host plant traits and the context of soil P distribution determined by long term agronomic management. These cover crop responses illustrate the contextual nature of P cycling potential, providing evidence which supported three out of the four hypotheses tested by this study. The first hypothesis, that plant growth would be higher overall in the conventionally managed soil compared to organically managed soil, was supported. However, there was not support for the hypothesis that cover crop growth would be higher overall among plants colonized by AMF than those without AMF. This may relate to observed differences in plant response to combinations of soil P availability and host response to AMF colonization that supported the third and fourth hypotheses.

Cover Crop Outcomes

In this comparison of cover crop responsiveness to soil phosphorus stratification and AMF colonization, there was a clear distinction between the cover crop species tested. As predicted by the fourth hypothesis in this study, hairy vetch (*Vicia villosa*) was identified to be much more responsive to AMF symbiosis than winter rye (*Secale cereale*). In both experiments, hairy vetch was more highly colonized by arbuscule-forming Glomeromycota fungi than rye, as quantified by the proportion of root segments containing AMF structures (Fig. 1.2A-1.2C). High colonization of vetch corresponded to differences in plant growth and plant P dynamics. In the organically managed soil using the autoclave control method, vetch growth was reduced compared to that grown in autoclave soil that received AMF inoculum, as measured by the decline in vetch biomass. This was likely due to soil P limitation, given that Bray extractable P in the organic soil was only about 33% of that measured in the conventional soil from the autoclave treatment (Table 1.1). Evidence for this is shown by the significant increase in vetch tissue P concentration (Fig. 1.4) and total P uptake (Fig. 1.5) in the AMF positive treatment when the cover crop was grown in the organic soil. This was not the case in conventional soil samples, where no relationship between vetch growth or P acquisition was detected between vetch plants with and without AMF. High biomass and plant P indicators suggest that soil P availability was sufficient for vetch in the conventional soil regardless of AMF colonization. Hence, there is support for the third hypothesis in this study, that cover crop response to AMF colonization would be most prominent in the organically managed soil.

In contrast, AMF colonization did not increase rye P acquisition or growth despite differences in soil P availability between the two soils. If anything, colonization was slightly suppressive to rye, as AMF

reduced the total amount of rye P uptake in the conventional soil. Rye growth and plant P measurements were otherwise independent from AMF colonization, with the exception of rye shoot C:P in the organic soil from the autoclave trial. In the P-limited soil treatment, rye had lower tissue C:P in the AMF positive samples without differing in total P uptake, which could suggest that carbon allocated from rye host plants to AMF symbionts may have been greater than P acquisition. Thus, the second hypothesis predicting that P uptake would be higher across soils and plant hosts when cover crops are colonized by AMF was not supported.

The low colonization and lack of positive response to AMF from the rye in the present study contradicts previous findings that AMF improves rye growth and P uptake outcomes. For example, when comparing rye, triticale (*Triticale octoploide*), and wheat (*Triticum aestivum*), Pandey et al. (2005) found that rye had high AMF colonization and the highest increase in P uptake (64%) resulting from colonization compared to rye plants without inoculation. The field soil that resulted in this rye response to AMF colonization was reported to only have extractable soil P levels of 7.8 ppm (Pandey et al., 2005), which is considerably lower than either the conventional or managed soils used in the present study. Additionally, Pandey et al. utilized an isolated strain of *Glomus macrocarpum* AMF as the inoculation which may be a more beneficial symbiont to rye compared to the consortium of AMF used in the present study. Therefore, it is possible that differences in available soil P and the AMF communities used in these studies contributed to divergent findings.

The comparison of rye and hairy vetch in this study demonstrates how plant host response to AMF colonization is highly context dependent. There is potential for predicting AMF contributions to cover crop P uptake based on knowledge of plant host traits and soil P status. In the context of cover crop use in agroecosystem P cycling, this information is critical. Total plant biomass, total plant P uptake, and the nutrient quality of cover crop tissues all bear important implications for the amount, timing, and pathways for soil P pool transformations.

Soil Legacy Influences AMF Function

For the sake of preserving soil nutrient distributions, this study attempted a lower intensity of dry heating as an alternative to the standard autoclaving approach to suppressing naturally occurring field AMF. The autoclave control method was effective at suppressing AMF colonization in all control treatments undergoing this approach (Fig. 1.2C). Though the alternative heating method of 80 C was introduced to reduce artifacts of intense heating on soil nutrient availability, autoclaving was demonstrated to maintain sufficient contrast in soil P distribution as measured by plant response. The first hypothesis that soil sourced from the conventionally managed agronomic treatment would support the most cover crop growth was confirmed by the results of this study for both autoclaved and oven-treated soils. Across all AMF control method trials, cover crop biomass was consistently highest in the

conventional soil (Fig. 1.3). Hence, the autoclave treatment was most efficacious for comparing the relative impact of AMF colonization on cover crop P dynamics as it produced adequate AMF controls while maintaining intended soil nutrient contrasts.

Surprisingly, the introduction of this less intense, dry-heating control method presented an unexpected outcome in the comparison of AMF communities. Persistent colonization of hairy vetch in soil from long-term organic management after 80 C heat exposure was found to be reproducible in this study (Fig. 1.2A and 1.2B). One implication of this observation is the potential for AMF taxa present in the organic management fields to propagate easily after acute heat and water stress. These taxa were seemingly not present in similar quantities to colonize hairy vetch grown in heat treated soils from conventionally managed fields. Alternatively, soil structure differences, such as aggregation, may protect AMF propagules in the organically managed soil. Given that soil samples came from replicated field blocks it is likely that differences in AMF community survival could have been driven by contrasting long term management rather than by spatial differences.

Studies have demonstrated that individual AMF species perform differently regarding colonization, plant host responsiveness, and the benefits conferred by the fungal organisms to plants (Begum et al., 2019; Jansa et al., 2005). Taxonomic differences among the Glomeromycota may correspond to functional differences in AMF communities formed under varying selection conditions. For instance, it has been speculated that certain agricultural management regimes, such as tillage and heavy fertilizer use, select for fast growing AMF taxa that emphasize sporulation (Bowles et al., 2016). These organisms may be geared to rapid colonization and diversion of host plant photosynthates but have reduced capacity for nutrient transport, potentially contributing to the reported phenomenon of parasitic AMF-host interactions (Verbruggen and Kiers, 2010).

In corn-soy-wheat cropping systems of the KBS LTER, the potential functional differences of AMF communities to respond to heat stress may have diverged based on distinct management between the organic and conventional treatments. Other differences in functional performance of these AMF communities have been demonstrated previously. In 2017, Gottshall et al. compared AMF communities from the same LTER agronomic treatments and found that the organic field had a community of AMF that significantly increased wheat biomass, whereas AMF sourced from the conventional fields had no effect on host biomass. Functional differences in these two communities may have been attributed to distinctions in the prevalence of certain AMF taxa, with a member of the genus *Diversispora* identified as an indicator species for the organic system while a taxon belonging to the *Acaulospora* was an indicator species for the conventional system (Gottshall et al., 2017). Future research in these agronomic treatments could expand upon functional differences occurring between AMF communities formed by management legacy.

Methodological Considerations

Further, this experiment represents a simple framework to study the P cycling potential of cover crop species through association with AMF. Phosphorus pool distributions formed under field conditions driven by agricultural management could be better utilized to study context-dependent P cycling processes. Field scale studies addressing the comparative impact of AMF colonization are still limited by nontrivial hurdles (Brito et al., 2009). AMF control methods at the field scale can be challenging and expensive, while very few studies have been effective at allowing a direct comparison AMF colonization and suppression (Ryan and Graham, 2018). Though the methods used in this experiment are not without artifacts, this approach was able to discern cover crop growth and P uptake differences based on the agronomic treatments of origin. Using real field soils in a potting experiment grants the comparison of complex, realistic soil nutrient compositions formed under conditions of interest while being much more feasible to execute than many field-scale alternatives for studying AMF interactions.

Studying the effects of AMF colonization from field soils requires effective control methods which can remove or sufficiently suppress AMF propagules from the soil. This is a non-trivial step in designing AMF experiments which can present a few challenges. One limitation can be the non-target effects of biocontrol methods used to suppress AMF organisms which can eliminate many other soil organisms that might otherwise actively contribute to phosphorus cycling mechanisms during the life cycle of cover crop growth. This can be remedied by making a water extraction of the soil to reintroduce prokaryotes and non-target fungi that can be screened smaller than the spores and hyphae of AMF species (Emery and Rudgers, 2012; Glassman and Casper, 2012; Gottshall et al., 2017; Johnson et al., 2008).

A second necessary consideration for studies looking at nutrient dynamics from field soil contexts is the effect of AMF control methods on soil nutrient pools. Autoclaving is a standard approach in soil studies that seek to broadly suppress soil organisms (Gan et al., 2021; Williams-Linera and Ewel, 1984). By extension, this has become common for suppressing naturally occurring AMF in field soils (Al-Khaliel, 2010; Xie et al., 2014). The impact of severe heating and pressure which make this approach so effective for biological agents also has the drawback of drastically shifting soil conditions. For instance, Hu et al. (2020) found that autoclaving significantly increased soil extractable P, and that a duration of autoclaving beyond 2 hours approximately doubled the amount of P that could be extracted compared to the untreated control. These nutrient shifts pose the risk of diminishing the salience of planned treatment contrasts.

Few studies have looked at alternatives to suppressing AMF in field soil for microcosm based experiments. One example by Endlweber and Scheu (2006) looked at soil nutrient mobilization outcomes from heating soils at 60, 80, 100, and 120 C for 4 hours, autoclaving at 120 C for 2 hours, and fumigating with chloroform for 24 hours. They found that low temperature dry heating (60 C) was effective at

significantly suppressing AMF (<1% colonization) while minimizing shifts in mobile nutrient pools. This study only validated methods from one soil source – thus one AMF community – in combination with one host plant (*Plantago lanceolata*). However, it is possible that the relative susceptibility of AMF propagules to lower heat approaches might be different based on community, soil, and target host plant characteristics. A more recent study by Hu et al. (2020) looked at AMF colonization suppression following different durations of autoclaving and found that half an hour was sufficient for suppressing *Rhizophagus irregularis*. Though this approach minimizes the amount of autoclaving necessary to suppress AMF, it is possible that the occurrence of soil nutrient pool shifts compromise treatment comparisons. Control methods for AMF research warrants further investigation, but a simple comparison of heating intensity made in the present study determined that autoclaving could be appropriate for addressing research goals related to multipartite interactions of cover crop phosphorus cycling.

Conclusion

Cover crop growth and phosphorus acquisition were shown to be variably dependent on soil phosphorus distribution and AMF colonization based on the cover crop species. Cover cropping has potential to influence the internal P cycling of agroecosystems, thus we must bolster our understanding of the mechanisms which drive cover crop P acquisition. The present study used only a few levels of each factor as a proof-of-concept. Further exploration could use the basic structure of this experimental design to compare more types of cover crop species, soil nutrient conditions, and AMF communities.

Given the distinct responses of the two cover crop species presented here, it is evident that plant species traits play a big role in determining P cycling potential of cover cropping as an agricultural practice. How much of this difference is attributable to individual plant species as opposed to broader physiological categorizations such as grasses/forbs or leguminous/non leguminous remains to be seen.

Agricultural soils vary widely, as well, with myriad differences in pedology and management histories which can influence soil P conditions. Similar potting experiments can address more intricate questions about soil P stratification from a variety of mineral and organic sources. For instance, looking across soils with low extractable P but varying levels of P associated with soil organic matter fractions could identify the capacity with which cover crops and AMF are able to access organic sources of P. Preparing more assays with logical gradients of edaphic and land management factors could contribute extensively to the present understanding of how cover crop species utilize soil P resources, and under what circumstances this is facilitated by AMF symbiosis.

This experimental design could also incorporate more levels in the AMF treatment factor to account for the comparison of different AMF communities. Future comparisons of AMF community consortia sourced from contrasting agronomic management regimes could inform much more about i) the selection pressure of different agricultural practices on AMF diversity and ii) the functional variation of

AMF communities in soil P acquisition. The home-and-away (or cross inoculation) approach could be used to compare how symbiosis outcomes of AMF communities might interact with edaphic properties of the site where a community was formed relative to a site with different characteristics based on potential adaptations (Gan et al., 2021). Using a consistent control method, such as autoclaving, and introducing local versus external AMF consortia would build upon current understanding of the functional redundancy, plasticity, or specificity of AMF communities recruited by different agroecosystem settings.

There is vast, untapped potential to better utilize the phosphorus present in many agricultural soils of the world. Plants, together with functioning soil biology, can be a lever to improve nutrient cycling. Building upon this knowledge will provide more robust options for farmers to utilize functional plant diversity through cover cropping to address their soil fertility goals.

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APPENDIX: SUPPLEMENTARY TABLES

Table 1.2 - Total Biomass Dry Weight (g) Main Effects for Oven Treatments

Oven 2023	Mean (CI)	P-Value
Soil History		0.0014
Conventional	1.21 (1.09 – 1.33)	
Organic	0.97 (0.85 – 1.08)	
Cover Crop		<0.001
Rye	1.30 (1.18 – 1.42)	
Vetch	0.88 (0.76 – 1.00)	
AMF		0.68
Positive	1.07 (0.955 – 1.19)	
Control	1.11 (0.992 – 1.23)	

Oven 2024	Mean (CI)	P-Value
Soil History		<0.001
Conventional	1.57 (1.405 - 1.73)	
Organic	1.02 (0.856 - 1.19)	
Cover Crop		0.013
Rye	1.17 (1.00 - 1.33)	
Vetch	1.43 (1.26 - 1.59)	
AMF		0.38
Positive	1.25 (1.09 - 1.42)	
Control	1.34 (1.18 - 1.50)	

Summary F-test results for main effects of Soil History, Cover Crop, and AMF factors on Total Biomass (root and shoot mass) in the two trial years of the Oven control method; CI: Confidence Interval. P-values were considered significant below 0.05.

Table 1.3 - Autoclave 2024 Total Biomass Dry Weight (g) ANOVA Results

Factor	DF	Sum Sq	Mean Sq	F-Value	P-Value
Soil History	1	4.54	4.54	54.12	<0.001
Cover Crop	1	1.00	1.00	11.90	0.0012
AMF	1	0.17	0.17	2.01	0.16
Soil History x Cover Crop	1	3.42	3.42	40.85	<0.001
Soil History x AMF	1	0.46	0.46	5.50	0.023
Cover Crop x AMF	1	1.37	1.37	16.34	<0.001
Soil History x Cover Crop x AMF	1	0.95	0.95	11.37	0.0015
Residuals	48	4.02	0.08		

ANOVA results for main and interaction treatment effects on Total Biomass from the Autoclave control method. P-values were considered significant below 0.05.

Table 1.4 – Shoot Phosphorus Concentration ANOVA Results

Oven 2023

Factor	DF	F-Value	P-Value
Soil History	1	9.11	0.008
Cover Crop	1	56.62	0.004
AMF	1	11.22	<0.001
Soil History x Cover Crop	1	21.51	<0.001
Soil History x AMF	1	48.00	<0.001
Cover Crop x AMF	1	0.05	0.83
Soil History x Cover Crop x AMF	1	4.33	0.054
Residuals	16		

Autoclave 2024

Factor	DF	F-Value	P-Value
Soil History	1	91.19	<0.001
Cover Crop	1	0.02	0.88
AMF	1	41.28	<0.001
Soil History x Cover Crop	1	14.06	0.002
Soil History x AMF	1	20.13	<0.001
Cover Crop x AMF	1	7.83	0.013
Soil History x Cover Crop x AMF	1	2.31	0.15
Residuals	16		

ANOVA summary for main and interaction treatment effects on Shoot Phosphorus Concentration from the Oven (2023) and Autoclave (2024) control methods. P-values were considered significant below 0.05.

CHAPTER 3:
Smallholder Farm Crop Management Promotes Soil Microbiome Diversity in Central Malawi

Abstract

Biodiversity is a key asset in ecosystems, including managed ecosystems such as agriculture. Soil microorganisms are a crucial aspect of agroecosystem biodiversity which can influence soil fertility and plant health, among many other processes. It was once believed that soil microbes are ubiquitous and redundant in soils. These days, we understand much more about how soil microbial composition, or soil microbiomes, can differ between locations. Prevailing climatic and edaphic conditions impose abiotic forces which can select for unique soil microbiomes, as can land use history. Perturbations and inputs typical of cropping systems have the potential to influence soil microbial communities over long term implementation. In this study, soils sampled from 300 smallholder farm fields across Central Malawi were sequenced (16S and ITS rDNA) to measure microbiome diversity and composition differences related to both environmental and farm management parameters. Location clusters based on regions known as Extension Planning Areas (EPAs) explained the most variation in soil prokaryote and fungal diversity, followed by mean annual temperature and mean annual precipitation showing strong geographical trends. In particular, Golomoti and Linthipe EPA regions were shown to have the most dissimilarity in microbial community composition, with Linthipe tending to have higher alpha diversity than Golomoti. These findings followed known macroecological gradients where Golomoti is generally more marginal agricultural land in slightly hotter, drier, sandier conditions, while Linthipe is a more mesic region conducive to agricultural production. Interestingly, farm practices related to plant inputs to field systems had significant impacts on fungal alpha diversity. Sites with high crop residue retention had greater fungal richness, evenness, Shannon Diversity index, and Inverse Simpson index levels than those with low residue incorporation. High crop diversity sites also had higher fungal evenness, Shannon, and Inverse Simpson values than low crop diversity fields. Additionally, sites with high crop diversity had many more unique, prevalent taxa at the Family and Genus level for both fungi and prokaryotes than what was observed for low crop diversity samples. These findings show that, not only are soil microbiomes shaped by environmental context at an intermediate scale, but that microbial diversity can also be intentionally managed by application of different practices across farms. In particular, crop diversification and crop residue retention show promise for promoting soil microbial diversity on smallholder farms in Central Malawi. Further investigation could uncover if such shifts in microbial diversity have functional implications for soil and plant health in these settings.

Introduction

Soil biology is an active component which forms the basis of terrestrial life. Organisms in the soil modify physical and chemical aspects of soils creating a dynamic system of biogeochemical flows. Soil

biological indicators are necessary tools to understand the capacity of a soil to support other organisms, such as plants. The importance of soil ecological function for agriculture is well supported (Bender and van der Heijden, 2014; Drinkwater and Snapp, 2022; Kleijn et al., 2019). Many of the processes governed by soil biology rely upon the interactions of myriad organisms ranging vastly in size and spanning the tree of life (de Vries et al., 2013; Morrien et al., 2017). The smallest of these soil biota are the microorganisms. Microbial communities are foundational to numerous cascading interactions in soils. Microorganisms are recognized to have profound direct and indirect effects on soil properties, especially at the plant-soil interface (Jacoby et al., 2017; Wagg et al., 2014). These interactions are fundamental to the productivity and stability of agricultural soils.

Microbe Diversity Matters

Soils consist of many types of microorganisms with substantial breadth in diversity. Bacteria and fungi are among the most abundant and diverse groups of organisms in soils (Bastida et al., 2021). They also have highly adaptive, specialized, and powerful roles in driving soil functions. Fungi are most often noted for their ability to decompose a wide range of substrates, especially the more recalcitrant organic compounds that accumulate in soils (Janusz et al., 2017). They are also impactful in mobilizing nutrients such as phosphorus from other sources including primary minerals and metal complexations (Brazhnikova et al., 2022; Osorio and Habte, 2013). Bacteria are critical in the fixation of nitrogen from atmospheric gas to inorganic forms usable by plants (Levy-Booth et al., 2014). Specific groups of bacteria are also specialized in converting nitrogen to other forms through nitrification and denitrification, thus completing the nitrogen cycle (Levy-Booth et al., 2014). Both bacteria and fungi are important groups to track because they are useful indicators for the functional capacity of soils to meet agricultural and environmental needs. Characterizing shifts in these communities indicates how different selective pressures shape their resilience and function.

Different Drivers of Microbial Communities

Microbial communities are known to differentiate based on biogeographical conditions. For instance, soil characteristics such as pH and soil carbon, and climatic conditions such as precipitation and temperature have been identified as major forces in determining microbial community differences across broad scales (Bastida et al., 2021; Cowan et al., 2022; Delgado-Baquerizo et al., 2018). These properties determine the differences observed in soil microbiomes between fields, sites, and regions in studies (O'Brien et al., 2016). Outside of these macroecological selection pressures, disturbances can also push soil communities in new directions.

Agricultural management directly alters soil systems through manipulation of vegetation, soil disturbance, and the import/export of nutrients. Many studies have considered the ramifications of these actions for soil biology (de Graaff et al., 2019; Garcia-Orenes et al., 2013; Mbuthia et al., 2015; Stefan et

al., 2021). Some trends have been observed, but the impacts of agriculture on soil microbiomes is often context dependent (de Graaff et al., 2019). Consequently, interactions between soil and geographical conditions with varying agricultural practices could shape microbial communities substantially.

Biodiversity in Undersampled Regions

Given that microbial actors are critical for the productivity of agricultural soil, it is necessary to determine how these communities are distributed across farms. There are many teams researching environmental and anthropogenic drivers of soil biological community selection in the Americas (Fierer and Jackson, 2006; Xue et al., 2013), Europe (de Vries et al., 2013; Wagg et al., 2014), and select parts of Asia (Chen et al., 2014; Tian et al., 2018; Zhang et al., 2017). Fierer and Jackson (2006) measured biogeographical drivers of soil bacterial diversity across different ecosystems of North and South America, identifying pH to be the most significant factor in distinguishing these communities. Other factors describing soil water availability, temperature, and soil texture were not nearly as relevant as explanatory variables. Contrary to these findings, Tian et al. (2018) looked at a more regional transect of biomes in China from tropical to temperate forests and observed that mean annual temperature and mean annual precipitation were highly significantly correlated with differences in soil bacterial diversity, along with soil pH and organic matter, indicating both environmental and edaphic determinants of microbial diversity. Additionally, Zhou et al. (2016) demonstrated more emphasis on the role of temperature gradients in shaping soil bacterial and fungal communities in North America with higher correlations than was observed by Fierer and Jackson. It is evident that more work is needed to understand how and in what contexts different macro scale environmental variables affect soil microbiomes. This is especially true for tropical areas, as temperate climates have been disproportionately studied to date (Dickey et al., 2021).

Other studies have shown how human intervention in managed landscapes, such as agricultural fields, can also impact soil microbial diversity. For example, Xue et al. (2013) measured greater diversity in soil microbial functional genes corresponding to N, C, and P cycling in a lower-input cropping system relative to a conventionally managed corn-soy-wheat rotation in a Michigan (US) long-term field experiment. Another study by Chen et al. (2014) showed that different inputs of plant material (living clover vs corn stalk mulch) in the form of ground cover selected for different soil bacterial community compositions in an apple orchard of the Loess Plateau region in China. Types of land use and management differ greatly around the world, influenced by market demands, resources of producers, and cultural context, among many other aspects. Hence, much remains to be seen regarding overlap of specific land management practices and the environmental framework they are nested in for impacting soil biology.

The above studies demonstrate the work that has been done to characterize soil microbiomes in specific regions of the world. The African continent, however, remains either highly under sampled, under

reported, or both, regarding bacterial and fungal biodiversity (Cameron et al., 2018; Guerra et al., 2020). Broad swaths of Africa have very low distribution of sample sites reporting on soil biodiversity, particularly in Central and North Africa, while other portions of the continent that have been sampled pale in comparison to the number of sample sites identified in regions of Europe and North America (62% of all sample sites globally were from temperate landscapes) according to an overview by Guerra et al. (2020). This is a critical lack of data, given how much land mass Africa represents, along with the uniqueness of geographical, edaphic, and climate characteristics that are largely unaccounted for in previous macroecological studies of soils. Cowan et al. (2022) set out to address this gap by surveying soil microbial diversity in 9 countries of Sub-Saharan Africa. They measured biogeographical factors influencing fungal, bacterial, and archaeal diversity in a comprehensive sampling effort. This work has contributed to representing the context of Sub-Saharan Africa in macroecological analysis of soil microbiomes. To build upon this expanding body of knowledge, we identify two key opportunities: i) coupling environmental drivers with agricultural management regimes to look at context-specific land use effects on soil microbiomes, and ii) targeting Malawi, which was not previously featured.

Malawi Agroecosystems

In Malawi, agriculture is dominated by smallholder production (Snapp et al., 1998). This production is characterized by rainfed cropping systems which have become centered around maize (Waddington, 1994). Still, practices vary considerably from farmer to farmer (Mhango et al., 2013; Mungai et al., 2016). Management of crops and their interplanting, rates of fertilizer application, and the use, quality, and treatment of organic soil amendments can have distinct impacts on soil processes. A recent study by Tu et al. (2022) has identified both environmental and farm management drivers of differences in soil carbon pools among Malawi farms. Some of the important drivers determined by this study were temperature, pH, slope, clay content, residue management, and crop diversity. It is known that soil carbon and soil microbial community dynamics are undeniably, but often inextricably connected (Jiang et al., 2022; Lange et al., 2015). As such, it is valuable to see how these components of soil carbon determinants might overlap with response from the soil microbiomes of Malawi farms.

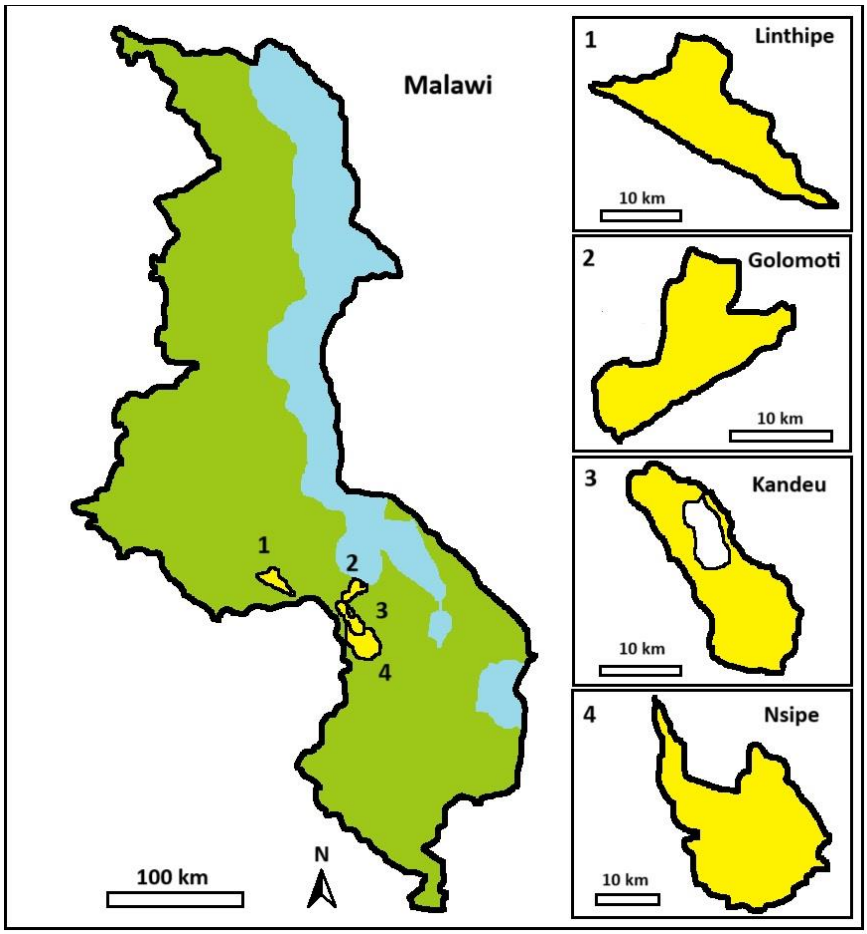
The present study seeks to expand upon the work done with the Africa Research in Sustainable Intensification for the Next Generation (Africa RISING) project in Central Malawi. Africa RISING research has provided a wealth of information from in-depth farm household surveys combined with environmental sampling of farm fields collected over 10 years. This presents a unique opportunity to couple a robust dataset with molecular sequencing of farm soils to observe patterns in community development. Thus, in this study we not only present data on soil biodiversity from an underrepresented region in global databases, but also relate characteristics of these communities to key environmental and agricultural drivers. Specifically, we are interested in the role of crop diversity management in shaping

soil microbial communities under divergent environmental contexts. Several studies have demonstrated increased soil fungal and bacterial diversity with greater crop rotational or intercrop diversity (Wang et al., 2020; Williams et al., 2023; Yang et al., 2023) but Stefan et al. (2021) observed that the influence of crop diversity as a soil microbiome driver was determined by how limiting the environment was between cropping systems in Switzerland and Spain. Further analysis will contribute to the understanding of how much leverage a practice like intensified crop diversity could have on microbial communities along environmental gradients. In this study, we focus on agroecosystems across Central Malawi.

The objective of the study is to determine, at the regional scale, how environmental and agricultural management drivers relate to soil bacterial and fungal communities among smallholder farms of Central Malawi. We hypothesize that 1) microbial communities will differ between EPA regions based on predominant environmental conditions, 2) microbial communities differences will be significantly related to farmer practices, specifically 3) sites with high crop diversity will be more microbially diverse and have distinct microbial composition compared to those practicing low crop diversity.

Materials and Methods

Figure 2.1 – Malawi Sample Site Map



Map of Malawi indicating the regions where smallholder farm data was collected.

Site Description/Selection

Smallholder farms located in Central Malawi were selected from across four regions referred to as Extension Planning Areas (EPAs). Nsipe, Kandeu, and Golomoti are latitudinally arranged from south to north, with the northern part of Golomoti coinciding with Lake Malawi (Fig. 1). Linthipe is latitudinally similar to Golomoti but located further west inland away from the lake. Previous work with these sites has identified the four EPAs as being high (Linthipe), medium (Kandeu and Nsipe), and low (Golomoti) agricultural potential landscapes (Mungai et al., 2016). These EPAs were chosen to represent a gradient of climatic and landscape conditions experienced by growers across the region. Mean annual precipitation (MAP), mean annual temperature (MAT), and normalized difference vegetation index (NDVI) were determined for each site to measure these regional-scale differences between locations. Remote sensing data was obtained from National Aeronautics and Space Administration (NASA) Moderate Resolution Imaging Spectroradiometer (MODIS) and Climate Hazards InfraRed Precipitation with Station (CHIRPS) databases as described by Tu et al. (2022). Additionally, the slope of the field was measured for each plot and is used here as an explanatory variable. Slope was assessed visually into the following categories: nearly level, gentle, moderately steep, and steep. Further background on these sites is provided by Mungai et al. (2016) and Tu et al. (2022).

Descriptive environmental characteristics are presented in Table 2.1 with the extension area (village cluster) scale as averaged across the plot level for this study conducted in Central Malawi. The marginal site is the Golomoti area with less than 800 mm annually, often poorly distributed rainfall, high evapotranspiration (mean annual temperature more than 27 C) and sandy soil. Linthipe is the mesic site with generally consistent rainfall patterns, mean annual precipitation over 900 mm, and mean annual temperature less than 24 C, with relatively fertile, well drained, loamy sand soils. Nsipe and Kandeu are intermediate sites.

Soil Sampling

Soil sampling was conducted during the dry season prior to planting for each year analyzed. Samples were taken randomly throughout fields at depths of 0-20 cm using 5 cm diameter augers, homogenized per field, air-dried, and sieved to 2 mm. Soils for farming households were sampled to identify a suite of biogeochemical pools and processes. For the scope of this study, soil characteristics were limited to a subset of properties expected to be highly influential for determining soil microbial community aspects across the agroecosystems studied. Percent clay content and pH are the main soil properties used as factors in this study. Soil pH was measured using a 1:2 ratio of soil and deionized water with a standard bench-top pH probe. Soil texture was determined using the micropipette method described by Burt et al. (1993).

Table 2.1 – Environmental and edaphic characteristics of Extension Planning Areas (EPAs) averaged across field sites over three years (2016-2018) from remote sensing and soil sampling as explanatory variables for soil microbial community differences in this study

	Golomoti (n = 72)	**Kandeu** (n = 77)	**Linthipe** (n = 81)	**Nsipe** (n = 67)
Latitude/Longitude	14.39S/34.58N	14.63S/34.61N	14.22S/34.11N	14.87S/34.74N
Mean Annual Precipitation (mm)	781 (754-867)	940 (912-989)	962 (925-1048)	981 (937-1072)
Mean Annual Temperature (C)	27.2 (26.6-27.7)	25 (24.8-25.5)	23.9 (23.2-24.3)	24.6 (24-25.4)
NDVI	0.534 (0.46-0.59)	0.544 (0.47-0.66)	0.537 (0.46-0.59)	0.574 (0.51-0.66)
Slope	0.57	0.80	0.83	0.87
Clay content (%)	12.6 (4.0-33.4)	15.6 (3.2-30.4)	16.5 (5.0-35.6)	14.2 (4.9-37.6)
Soil pH	6.53 (4.99-8.03)	6.13 (4.92-8.00)	6.11 (5.23-7.29)	6.3 (4.79-7.29)

Note. Where applicable, values are reported in the format: mean (minimum - maximum).

Survey Information

In addition to soil sample and remote sensing data, the Africa RISING dataset consists of survey results from participating farmer households revisited over a period of 10 years. A subset of survey data from the original Africa RISING panel of respondents was used representing 300 field sites across the four EPAs mentioned above. Several categories of farmer practices were measured in these surveys, while a selection of these practices was focused on in the present study based on their importance for determining soil carbon dynamics in Malawi as reported by Tu et al. (2022). Compost use, crop diversity, nitrogen application rates, and crop residue management were used as factors to identify farm management impacts on soil bacterial and fungal communities.

All management variables were averaged across the three most recent years of survey data, from 2016 to 2018. This was done to provide more representative values for management variables than one site year alone – given that management was reported differently across years on each field – and to provide an estimate of the cumulative effect of agricultural practices on soil microbiomes.

Table 2.2 presents a summary of the distribution of this management data from the sites used in this study. While the distribution of management practices is similar between the EPAs, note the slightly lower average fertilizer rate, crop residue retention, and intercrop diversity reported from Golomoti farms. Overall, there is a wide range of nitrogen fertilizer rates (0 to 300 kg/ha) being implemented on these farms across Central Malawi. Increases in the proportion of sites reporting compost/manure application

and crop residue incorporation have occurred in more recent years' responses relative to earlier surveys from the same project (see Mungai et al. 2016). For instance, in Linthipe removal/burning of residues dropped from 60.3% of sites in the year 2013 to the 28.4% shown in the present study.

Compost application, referring to the use of composted plant materials as well as manure, was reported on a presence/absence basis (High/Low application). Residue management consists of three alternative practices: incorporated (residues retained in the field), burned (residues burned on the field surface), or removed (residues exported from field). Due to a majority of sites adopting residue incorporation practices, crop residue removal and burning were grouped in order to have adequate sample size for statistical comparisons (High: residue retention or Low: residue burned/removed). Nitrogen application rates (kg/ha) were calculated based on mineral fertilizer types and amounts reported by each farmer. Nitrogen fertilizer rates were grouped into categories of Very Low (< 30 kg/ha), Low (30 – 50 kg/ ha), Medium (50 – 100 kg/ha), and High (> 100 kg/ha).

Maize is the dominant staple crop across this farming region. It represents the primary crop used at all sites either grown solely or intercropped with other plants as reported by the farmers for each growing season. Intercropping is a traditional means to introduce crop diversity in Malawi, as opposed to rotational diversity which is common in temperate climates, due to the unimodal rain system which dictates the growing season from November to April. Hence, the crop diversity metric describes the number of species intercropped together in a given field per growing season. Crop diversity was split into categories of Low (< 1.74 intercrops), Medium (1.74 - 2.6 intercrops), and High (> 2.6 intercrops) based on the distribution of the data over three site-years.

Table 2.2 – Farm practice survey responses averaged over three years (2016-2018) as explanatory variables of soil microbial community differences

	Golomoti	**Kandeu**	**Linthipe**	**Nsipe**
N Fertilization (kg/ha) - average [range] *Standard deviation*	48 [0-310] *55*	71 [0-243] *53*	70 [0-237] *51*	59 [0-250] *52*
% Fields Composted[a]	30.8	23.6	52.0	28.3
Crop Residue[b]				
% incorporated	62.5	88.3	71.6	67.2
% burned or removed	37.5	11.7	28.4	32.8
Crop Diversity[c] - average [SD]	2.0 [0.57]	2.4 [0.56]	2.7 [0.55]	2.2 [0.60]

[a] Compost refers to the application composted plant matter and/or manure. [b] Crop residue as percent of fields responding as either incorporated for all three years or with some years of residue burn/removal. [c]

Crop diversity is the number of species intercropped per growing season, ranging from 1 (monoculture) to 5 species per field per year and averaged over 3 years of responses.

Sequencing

Soils used for bacterial and fungal sequencing were sampled in the year 2020 following the procedure described above for other soil analyses. Since sampling occurred during the end of the dry season (October) in Malawi, it was assumed that microbial communities were relatively stable and materials would remain consistent in air-dried samples as they were shipped to the US for subsampling and DNA extraction. Soils were submitted to a commercial laboratory (Biome Makers, Sacramento USA) for sequencing. The workflow for extracting DNA, sequencing, and processing raw sequence reads was carried out by the company. DNA was extracted with the DNeasy PowerLyzer PowerSoil Kit from Qiagen. The 16S rRNA and ITS marker regions were targeted by company-specific primers for characterizing bacterial and fungal communities, respectively. Libraries were prepared following the two-step PCR Illumina protocol using custom primers amplifying the 16S rRNA V4 region and the ITS1 region described by Acedo et al. (2018). Sequencing was conducted in an Illumina MiSeq instrument using pair-end sequencing (2×300bp). Primers were removed from paired end reads using Cutadapt (Martin, 2011). Then the trimmed reads were merged with a minimum overlapping of 100 nucleotides. Next, the sequences were quality filtered by Expected Error with a maximum value of 1.0 (Edgar and Flyvbjerg, 2015). After quality pre-processing, reads having single nucleotide differences were iteratively clustered together to form ASVs (Amplicon Sequencing Variants) using Swarm (Mahe et al., 2021). *De novo* chimeras and remaining singletons were subsequently removed (Edgar et al., 2011). Finally, taxonomy was assigned from ASVs using a global alignment with 97% identity, against a curated reference database from SILVA 138.1 for 16S sequences, and UNITE 8.3 for ITS sequences (Glöckner et al., 2017; Nilsson et al., 2019).

Analyses and Statistical Methods

The following analyses were conducted using R Software (v 4.1.1) and R Studio (v 2023.12.0.369) (Posit Team, 2023; R Core Team, 2021). Significance was determined at an alpha of 0.05 for all statistical tests. Distribution of data was visualized to confirm normality and boxplots of residuals were visualized to assess homogeneity of variance.

Data Filtering and Transformations. First, ASVs represented by only one sequence were removed from the data set for both ITS and 16S data. Community diversity measurements were conducted using a rarefied subsampling of the ASV data. Rarefaction helped to make comparisons across soil samples from different locations by accounting for differences in sampling effort based on sequence counts for the observations. This is particularly important for certain alpha and beta diversity metrics which are sensitive to differences in sampling effort which makes analysis more prone to falsely detecting differences in

samples and treatments groups (Schloss, 2023). Determination of the sequencing depth threshold to perform rarefaction at was made by considering the distribution of sequence counts across the samples, visualizing rarefaction curves to observe where the rate of species richness response to sequencing depth was near saturation, and adjusting rarefaction depth to retain samples. Rarefaction was performed using the "rarefy_even_depth" function in *phyloseq* (McMurdie and Holmes, 2013) at 10,000 and 14,000 sequence counts for ITS and 16S ASVs, respectively, with results from random subsampling analyzed and reported.

Alpha Diversity. Alpha diversity refers to the analysis of diversity within a given sample, which can be used to quantify and compare sample diversity as it relates to independent variables of interest. Observed richness (Simpson, 1949; Whittaker, 1972), Shannon diversity index (Hill, 1973), and Inverse Simpson diversity index (Simpson, 1949) were analyzed concurrently to compare alpha diversity of samples across the study factor groups. Different methods convey distinct information about taxonomic distribution, the number of taxa, dominance, and shared taxa (Whittaker, 1972). Using multiple alpha diversity quantification methods allows for assessment of the different representations of richness and evenness expressed by the multiple methods while determining overall trends in diversity that might be consistent amongst all three methods of measuring alpha diversity. Observed richness, evenness, Shannon index, and Inverse Simpson index were calculated using the "estimateR" and "diversity" functions from the *vegan* package (Oksanen et al., 2022).

Statistical analysis was performed for each alpha diversity metric by starting with a main effects model of all 11 explanatory variables of interest in the study. Analysis of variance (ANOVA) was performed to identify the relative significance of each explanatory variable for sample differences in alpha diversity. Pairwise comparisons of levels within a categorical variable were conducted for those determined significant by the full main effects model using Šidák correction for multiple comparisons. Given that crop diversity was a key factor of interest in this study, pairwise comparisons were conducted for all alpha diversity metrics even in instances where crop diversity was not determined significant in the main effects ANOVA; however, the slightly more conservative Bonferroni correction was employed in these cases. Significant differences between factor level means for alpha diversity were visualized by creating boxplots with the *ggplot2* package (Wickham, 2016).

Pearson Correlation tests were conducted for continuous explanatory variables. This identified both the significance of covariation relationship as well as direction (positive or negative). Linearity of the relationships of explanatory variables and alpha diversity was confirmed visually by plotting correlations using "ggscatter" (Kassambara, 2020).

Beta Diversity. Beta diversity addresses differences in community composition between samples, which allows comparisons of sample community similarity as it relates to explanatory factors. Rarefied ASV

data was used to calculate a distance matrix based on the Bray-Curtis method using the “distance” function in *phyloseq* (Bray and Curtis, 1957; McMurdie and Holmes, 2013). Permutational multivariate ANOVA (PERMANOVA) was conducted on the main effects model of all 11 explanatory variables to determine relative significance of these factors using the “adonis2” function from *vegan* (Oksanen et al., 2022). Following the initial PERMANOVA, pairwise comparisons were made for any significant categorical variables as well as crop diversity using the “pairwise_adonis” function (Martinez Arbizu, 2017). Principal coordinate analysis (PCoA) ordination plots were then generated based on Bray-Curtis dissimilarity of sample data to visualize clustering of sample communities based on significant study factors.

The within-factor group dispersion versus between-factor group dispersion was assessed to confirm that significance in community dissimilarity was driven, at least in part, by factor influence and not simply by high variability in the samples of the same group by using the “betadisper” function from *vegan*. Permutational testing of the significance of group dispersions from centroids was used to assess the reliability of PERMANOVA output.

Due to the overall significance of EPA as an explanatory variable for sample beta diversity, samples were subset based on EPA to analyze beta diversity within each EPA to assess how the relative explanatory effect of environmental and management variables differed by region. Bray-Curtis distances, PERMANOVA, and PCoA plotting were all performed as above with the subset data for each individual EPA.

Model Selection Comparison. For alpha and beta diversity statistical analyses, appropriate model selection approaches were used to compare the full main effects models from the previous sections to parsimonious models using stepwise selection processes for identifying the most explanatory environmental and management variables. For alpha diversity, the “stepAIC” function from the *MASS* package was used to perform forward and backward selection of model terms based on the full main effects model as the benchmark (Venables and Ripley, 2002). Selected models were ANOVA tested to confirm significance of the model terms and then these parsimonious models were used to test both main and interactive effects. This was done to determine if there were any key interactions between select explanatory variables which had not been explored in the initial alpha diversity analysis.

Similarly, for beta diversity redundancy analysis (RDA) was performed both on the full model and by using forward selection with the “ordiR2step” function to select a simplified model and perform ANOVA on main effects (Oksanen et al., 2022).

Unique, Shared, and Prevalent Taxa. To determine the distribution of taxonomic groups at different levels among EPAs and crop diversity groups, results were grouped at the Family and Genus level for each taxonomic category where abundance was greater than zero. The “unique” function determined how

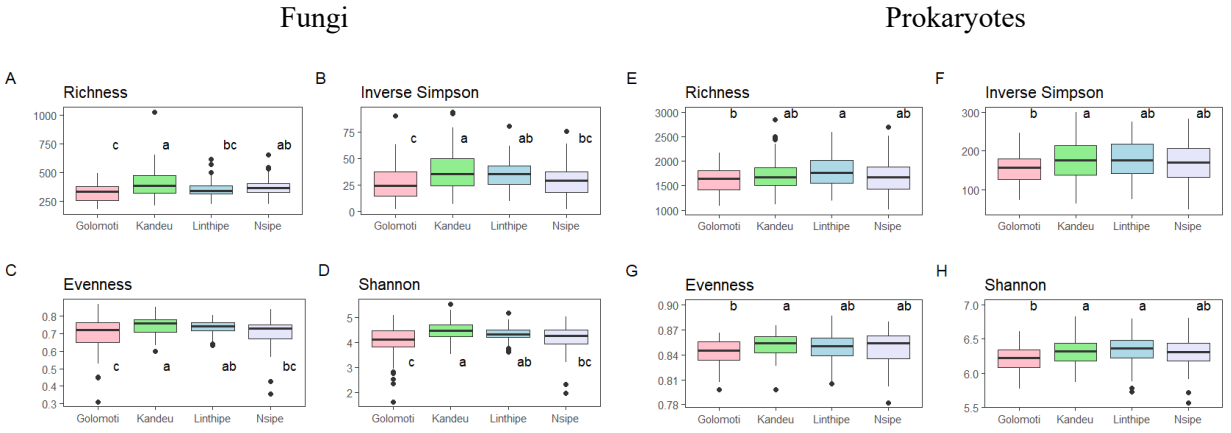
many shared and unique families and genera occurred between EPAs and between crop diversity levels. These results were plotted as Venn diagrams with "ggvenn" function (Yan, 2023).

The prevalence of top occurring taxa was explored for EPAs and levels of crop diversity. Taxa with a relative abundance greater than 0.1% that were present in at least 90% of samples for each group were identified using the "core_members" function in the *microbiome* package (Lahti and Shetty, 2019). Venn diagrams were plotted to show the occurrence of unique and shared taxa for the groups, and unique taxa for each EPA and crop diversity level were summarized at the species level.

Results

Alpha Diversity Responses

Figure 2.2 - Alpha Diversity by EPA



Boxplots of Fungal (ITS; plots A-D) and Prokaryote (16S; plots E-H) alpha diversity; differing lower case letters in graphs depict significant mean separation.

Alpha diversity of soil microbial communities in Malawi agroecosystems was found to be consistently influenced by environmental parameters, and to a much lesser extent impacted by certain agricultural practices. When included as a factor, grouping samples by EPA had the most explanatory power of all variables tested for Observed Richness, Evenness, Shannon's Diversity Index, and the Inverse Simpson's Index in both fungal and prokaryotic measurements. Models tested excluding EPA as a factor revealed that all of the environmental factors in different combinations explained the most variation in alpha diversity. Fungal and prokaryotic alpha diversity results were explained by many of the same factors with a few key distinctions. The climate variables MAT and MAP were among the most consistently influential factors, being inversely related to each other regarding impact on alpha diversity (Table 2.3). NDVI was significantly positively related to both prokaryotic and fungal richness, but not the other alpha diversity metrics. The edaphic variables, soil pH and clay content, were significantly related to prokaryotic alpha diversity, but not fungi. Clay content was especially negatively correlated with

prokaryote Evenness, Shannon Diversity, and Inverse Simpson, as well as Richness. Field slope was significantly related to fungal richness as well as prokaryote richness and Inverse Simpson Index.

Table 2.3 – Pearson correlation coefficients for continuous environmental variables and alpha diversity metrics for Fungi (ITS) and Prokaryotes (16S)

Fungi	MAT	MAP	NDVI	pH	Clay
Richness	**-0.132***	**0.163****	**0.194****	0.028	-0.062
Evenness	**-0.193****	**0.170****	-0.011	-0.080	0.005
Shannon	**-0.214*****	**0.202*****	0.053	-0.062	-0.015
Inv. Simpson	**-0.135***	**0.141***	0.034	-0.070	-0.028
Prokaryotes					
Richness	**-0.193****	**0.154***	**0.145***	0.103	**-0.186****
Evenness	**-0.194****	**0.147***	0.017	-0.088	**-0.293*****
Shannon	**-0.259*****	**0.198****	0.109	0.021	**-0.315*****
Inv. Simpson	**-0.220*****	**0.168****	0.009	-0.032	**-0.243*****

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Main effects of farm management variables were not significant in explaining alpha diversity variation, with the exception of crop residue for fungal Evenness, Shannon Diversity, and Inverse Simpson (Table 2.4). Sites with high crop residue retention had greater fungal alpha diversity than low residue fields (Fig. 2.3). Given that crop diversity was of primary research interest in this study, pairwise comparisons of crop diversity group means were conducted despite lacking significant main effect in the overall model. It was determined that crop diversity levels did differ with regards to fungal Evenness, Shannon Diversity, and Inverse Simpson (Fig. 2.3). However, this was not the case for prokaryote communities.

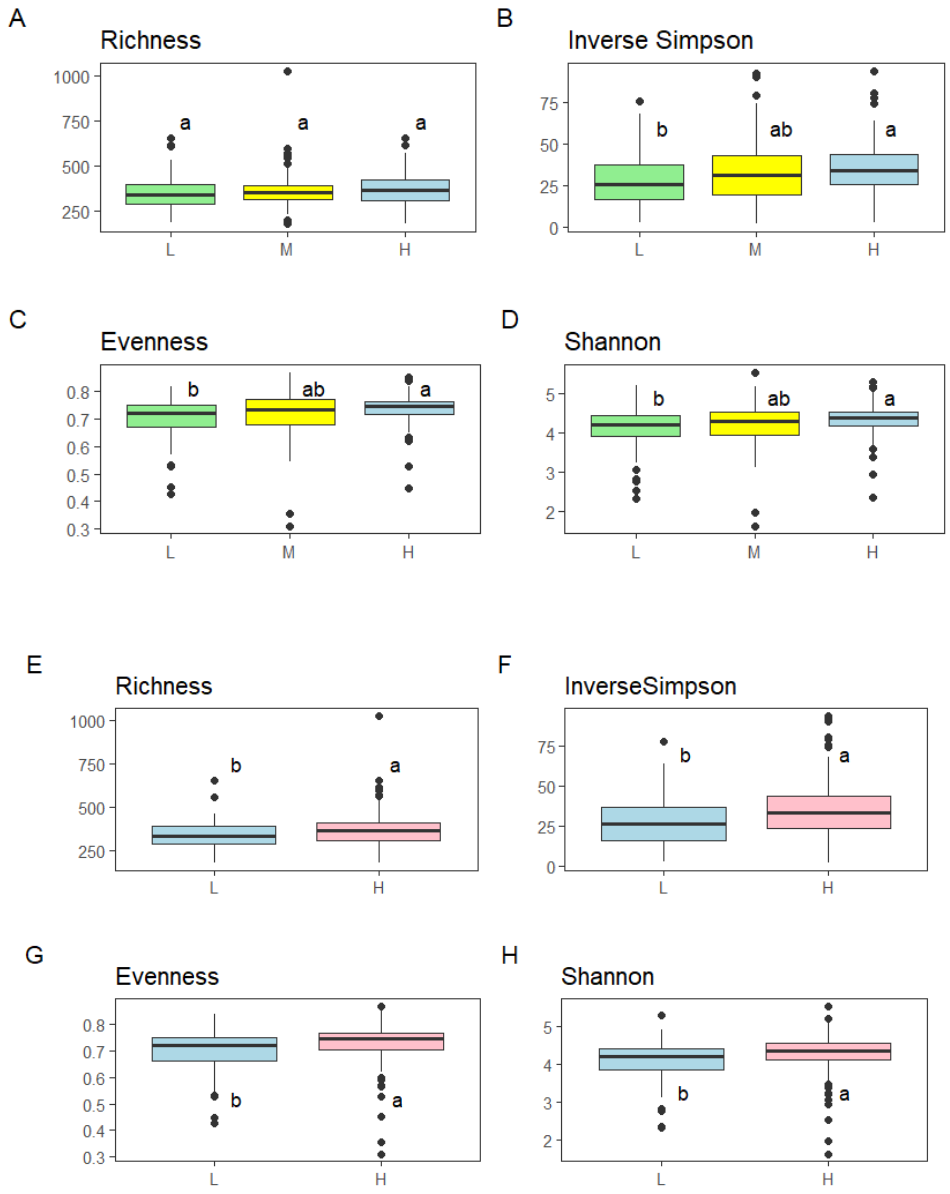
Table 2.4 – Summary of management factor results from ANOVAs run for alpha diversity of Fungi (ITS) and Prokaryotes (16S)

Fungi							
Richness:	df	F-value	p-value	**Shannon:**	df	F-value	p-value
Crop Diversity	2	0.35	0.703	Crop Diversity	2	1.38	0.254
Fertilizer	3	1.74	0.159	Fertilizer	3	0.99	0.398
Residue	1	3.74	0.054	Residue	1	8.06	0.005**
Compost	1	0.12	0.727	Compost	1	0.80	0.373
Evenness:	df	F-value	p-value	**Inv. Simpson:**	df	F-value	p-value
Crop Diversity	2	1.64	0.197	Crop Diversity	2	1.34	0.265
Fertilizer	3	0.39	0.762	Fertilizer	3	0.43	0.729
Residue	1	6.98	0.009**	Residue	1	8.07	0.005**
Compost	1	0.70	0.403	Compost	1	0.34	0.558
Prokaryotes							
Richness:	df	F-value	p-value	**Shannon:**	df	F-value	p-value
Crop Diversity	2	0.13	0.878	Crop Diversity	2	0.27	0.761
Fertilizer	3	0.18	0.910	Fertilizer	3	1.80	0.147
Residue	1	0.03	0.857	Residue	1	0.02	0.878
Compost	1	1.59	0.208	Compost	1	0.91	0.341
Evenness:	df	F-value	p-value	**Inv. Simpson:**	df	F-value	p-value
Crop Diversity	2	0.40	0.670	Crop Diversity	2	1.04	0.356
Fertilizer	3	2.56	0.055	Fertilizer	3	0.63	0.596
Residue	1	0.20	0.651	Residue	1	0.01	0.906
Compost	1	0.03	0.875	Compost	1	0.45	0.505

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Pairwise comparisons revealed significant differences between groupings of EPA, crop diversity, and slope for alpha diversity. Golomoti was consistently lowest in all alpha diversity metrics for both fungal and prokaryotic communities (Fig. 2.2). Kandeu had among the highest alpha diversity with Linthipe and Nsipe being intermediate for fungal communities. Prokaryote alpha diversity was highest among Linthipe and Kandeu with Nsipe being intermediate. Sites with steep slopes were determined to have greater fungal richness than flat and relatively little slope. Prokaryote richness was also highest in steep slope sites relative to flat locations, while the medium slope level was significantly different than flat sites for Inverse Simpson (higher variance in high slope category prevented significant mean separation). Crop diversity levels did not differ for fungal richness, but high crop diversity samples had higher mean Evenness, Shannon Diversity, and Inverse Simpson results than sites with low crop diversity.

Figure 2.3 - Fungal Alpha Diversity by Crop Diversity and Residue level



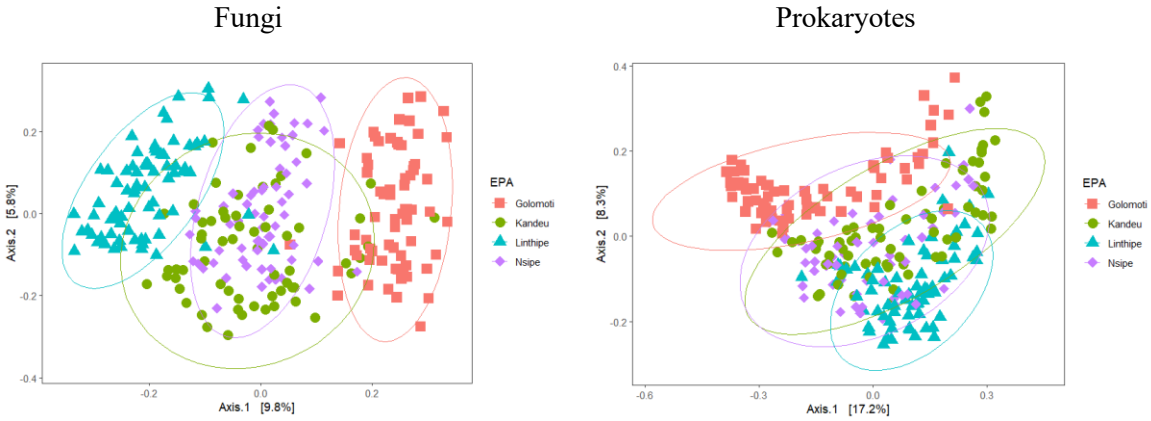
Boxplots of fungal (ITS) alpha diversity metrics for levels of Crop Diversity (A-D) and Residue retention (E-H); differing lower case letters in graphs depict significant mean separation; x-axis labels - L: Low, M: Medium, H: High.

Stepwise forward and backward selected models for fungal richness and Inverse Simpson retained clay as one of the key factors in parsimonious models though the main effect of clay was not apparent in the initial full model ANOVA. Though the main effect of clay on alpha diversity metrics was not clearly significant, parsimonious models that included main effects and interaction terms showed that clay content interacted with EPA, MAT, slope, and residue management. This suggests that clay content may not be a main driver of alpha diversity in this context but that its effect is conditionally nested within

other more prominent drivers. The same process of model selection and testing of interaction terms identified significant interactions of fertilizer application level with EPA, MAT, and clay content for prokaryote community evenness. In particular, it appears that Kandeu has a different relationship to clay content in the presence of higher fertilizer rates than it does at other fertilizer levels as opposed to the other EPAs. While the overall trend for prokaryote community evenness is downward with increasing clay content, evenness slightly increases with clay content solely in high fertilizer application sites of Kandeu.

Beta Diversity Responses

Figure 2.4 – Bray-Curtis PCoA Plots



Principal Coordinate Analysis (PCoA) ordination plots indicating Fungal (ITS) and Prokaryote (16S) rDNA community dissimilarity based on Bray-Curtis distance. Shapes, colors, and ellipses identify clustering of samples based on origin of EPA location.

Beta diversity results displayed similar trends as alpha diversity regarding the relative importance of environmental factors in explaining community differences. Main effects models showed that EPA was the most influential study factor explaining beta diversity for fungal and prokaryote results when included in PERMANOVA tests. All environmental variables were statistically significant in explaining sample dissimilarity for fungal and prokaryote communities, however the overall amount of variation explained by these factors was quite low compared to the residual variance. For fungal beta diversity, when EPA was included as a predictor, the only significant management variable was fertilizer application level. However, when EPA was excluded from the model, crop diversity and residue were determined significant (Table 2.5). This could indicate that there is a degree of shared explanation of variance for beta diversity between crop diversity and residue management with EPA for fungal communities. Crop diversity and fertilizer had significant effects in prokaryote communities in models with and without EPA as a factor.

Table 2.5 – Permutational ANOVA results on Fungi (ITS) and Prokaryote (16S) Bray-Curtis dissimilarity for environmental and farm management study factors

Fungi: permutations = 999					
	df	SS	R^2	Psuedo-F	p-value
MAT	1	6.54	0.0755	22.92	0.001***
MAP	1	1.19	0.0137	4.17	0.001***
NDVI	1	0.95	0.0109	3.31	0.001***
Slope	3	1.07	0.0123	1.25	0.036*
pH	1	0.43	0.0049	1.49	0.020*
Clay	1	0.95	0.0109	3.32	0.001***
Crop Diversity	2	0.85	0.0098	1.49	0.007**
Fertilizer	3	1.16	0.0134	1.35	0.008**
Residue	1	0.41	0.0047	1.42	0.044*
Compost	1	0.35	0.0041	1.23	0.118
Residual	255	72.81	0.8397		
Prokaryotes: permutations = 999					
	df	SS	R^2	Psuedo-F	p-value
MAT	1	4.26	0.0813	25.99	0.001***
MAP	1	0.62	0.0118	3.79	0.001***
NDVI	1	1.62	0.0309	9.87	0.001***
Slope	3	0.73	0.0140	1.49	0.017*
pH	1	0.79	0.0150	4.79	0.001***
Clay	1	1.42	0.0272	8.69	0.001***
Crop Diversity	2	0.64	0.0122	1.95	0.004**
Fertilizer	3	0.75	0.0142	1.51	0.013*
Residue	1	0.24	0.0046	1.48	0.066
Compost	1	0.16	0.0031	0.99	0.398
Residual	251	41.16	0.7856		

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Pairwise comparisons revealed that clusters of samples grouped by EPA region were all significantly different from each other. Although, Linthipe and Golomoti were the most dissimilar as was verified by visualizing the ordination (Fig. 2.4). Testing homoscedasticity with PERMDISP revealed that Linthipe had potentially different dispersion than the other three EPAs, and so within group dispersion differences could not be completely ruled out for comparisons with Linthipe. However, Golomoti clusters for fungal and prokaryote communities had little overlap with Linthipe, which supports the conclusion that significant differences in group centroids for these two regions was driven by EPA status.

When comparing the effects of fertilizer levels on fungal beta diversity, it was determined that sites with very low fertilizer application rates were significantly different than those with low, medium, or high rates. However, the very low group had significantly different dispersion than the medium and high groups, hence it is possible that significant results from the pairwise comparison were reflecting differences in within group sample dispersion. When tested for prokaryote communities, very low fertilizer was different than the other fertilizer levels but also had significantly different dispersion.

Otherwise, low and high fertilizer groups were significantly different while maintaining similar dispersion, as were medium and high fertilizer groups.

All three crop diversity levels were significantly different from each other for fungal beta diversity, but the high crop diversity group had significantly different dispersion than the other two groups. Given that medium and low crop diversity sample clusters had significantly different centroids while having similar dispersion they are likely to have different beta diversity. Though it is possible that within group variation might explain part of the overall difference, low and high crop diversity clusters ordinated apart from each other, indicating that there could be a crop diversity effect on fungal community dissimilarity. Crop diversity groups were also all different for prokaryotes, while dispersion differed between medium and high groups. In this case, differences between low and high crop diversity samples are maintained.

Beta diversity within each EPA was determined by distinct emphasis on study factors. Fungal community beta diversity was explained by multiple factors in Linthipe, Kandeu, and Nsipe, while in Golomoti only MAT was significant. MAT, NDVI, and clay were significant in Linthipe, Nsipe, and Kandeu, MAP was significant in Linthipe and Nsipe, slope was significant in Linthipe, and pH was significant in Kandeu. Crop diversity was significant in Linthipe, but further exploration showed that only medium crop diversity was significantly different than the other two levels, while low crop diversity had fewer samples than the other two groups in this EPA. Hence, this crop diversity effect within Linthipe was considered inconclusive. Fertilizer was significant in Nsipe. Very low and high fertilizer groups had different dispersion, but significant centroid differences between very low and low as well as low and high were maintained.

In Golomoti, prokaryote beta diversity was significantly explained by MAT, MAP, and slope. In Nsipe and Kandeu, most of the environmental variables were significant excluding pH in Nsipe and slope in both Kandeu and Nsipe. All of the environmental factors were significant in Linthipe, as was crop diversity. Only medium and high crop diversity were significantly different from one another, once again possibly due to small sample size for low crop diversity in this region. Crop diversity was also significant in Nsipe, but in this case driven by differences between low and medium groups.

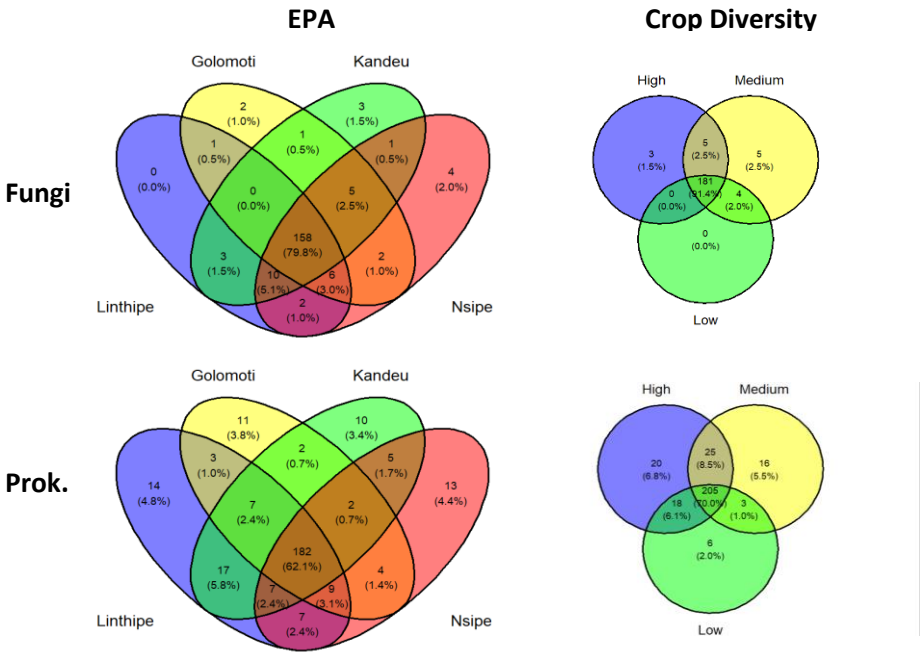
Forward selected RDA models revealed similar findings as the full main effects models for drivers of beta diversity. EPA, MAT, MAP, NDVI, pH, clay, and fertilizer were selected to explain the most variation in fungal beta diversity. The prokaryote model included EPA, MAT, MAP, NDVI, pH, clay, slope, and crop diversity.

Unique, Shared, and Prevalent Taxa

Unique and shared taxa were explored at the family and genus taxonomic levels to assess the distribution of taxa by EPA and crop diversity groupings. Overall, groups were found to share a large

percentage of fungal and prokaryote families and genera. All four EPAs shared nearly 80% of the detected families and 68% of the genera for fungi, and around 60% overlap for prokaryote families and genera. Crop diversity groupings had even more overlap between taxonomic groups (see Fig. 2.5). Despite similarities, there were ultimately more families and genera unique to high and medium crop diversity sites than those unique to low crop diversity sites for fungal and prokaryote communities.

Figure 2.5 – Unique and Shared Families by Group



Venn diagrams showing the unique and shared Families present in samples grouped by EPA or Crop Diversity level based on Fungal (ITS) and Prokaryote (16S) rDNA.

Core taxa representative of each group were noted for each EPA and crop diversity level. Kandeu and Nsipe were determined to have fewer core taxa than Golomoti or Linthipe. High crop diversity samples had the greatest quantity of prevalent taxa as well as more core taxa than low or medium crop diversity groups. There were more shared taxa with high prevalence for prokaryotes than fungi overall. Core taxa unique to each group are listed in Table 2.6 and Table 2.7.

Table 2.6 – Prevalent taxa (relative abundance > 0.1% in at least 90% of samples) unique to each EPA for Prokaryotes (16S) and Fungi (ITS) rDNA

	Golomoti	Kandeu	Linthipe	Nsipe
Prokaryote ID	*Bacillus funiculus*	*Acidovorax* sp.	*Candidatus* Nitrososphaera sp.	*Gaiella* sp.
	Candidatus Nitrosocosmicus sp.	*Gaiella* sp.	*Candidatus* Udaeobacter sp.	*Microvirga vignae*
	Geodermatophilus sp.	-	*Conexibacter* sp.	-
	Geodermatophilus tzadiensis	-	*Gaiella* sp.	-
	Microvirga sp.	-	*Ramlibacter* sp.	-
	Pseudonocardia hispaniensis	-	*Ramlibacter* sp.	-
	-	-	*Rhizobacter* sp.	-
	-	-	*Rhodoplanes* sp.	-
	-	-	*Sphingomonas* sp.	-
Fungal ID	*Aspergillus fumigatiaffinis*	-	*Aspergillus niger*	*Plectosphaerella sinensis*
	Didymella exigua	-	*Fusarium equiseti*	-
	Epicoccum thailandicum	-	*Humicola olivacea*	-
	Penicillium pimiteouiense	-	*Mortierella* sp.	-
	Subramaniula asteroides	-	*Penicillium levitum*	-
	-	-	*Penicillium pimiteouiense*	-

Table 2.7 – Core taxa (relative abundance > 0.1% in at least 90% of samples) unique to each Crop Diversity level for Prokaryotes (16S) and Fungi (ITS) rDNA

	Low Crop Div.	Medium Crop Div.	High Crop Div.
16S Species ID	*Solirubrobacter* sp.	-	*Gaiella* sp.
	-	-	*Gaiella* sp.
	-	-	*Nocardioides* sp.
	-	-	*Siccirubricoccus deserti*
ITS Species ID	*Didymella exigua*	-	*Aspergillus niger*
	-	-	*Fusarium oxysporum*
	-	-	*Penicillium pinophilum*
	-	-	*Setophoma terrestris*

Note: Medium Crop diversity had no prevalent taxa that were not shared with Low and High crop diversity samples.

Discussion

This study explored the relationships of key environmental conditions and agricultural management decisions with differences in soil microbial communities among smallholder crop fields of Central Malawi. Results corroborated the influence and trends of climate, landscape, and edaphic properties on soil microbiome diversity and composition seen in other macroecological studies, with support for the first hypothesis that microbial communities differ at a broad scale related to these factors. Although, at this regional scale there was less support for the second hypothesis that farm management choices would correspond with differences in soil microbiomes. There were a few exceptions, including the significant relationships of crop management practices with soil microbial diversity as was anticipated by the third hypothesis. These findings suggest that certain farmer practices could manipulate soil microbial community dynamics over time, even across a range of prevailing environmental parameters.

Environment as Dominant Driver

Large-scale differences in environmental conditions were the most impactful for bacterial and fungal diversity. Both alpha and beta diversity of prokaryotes and fungi were significantly different between the EPAs. The location of each EPA exists within a gradience of environmental conditions, wherein soil microbial communities likely diverge based on biogeographic setting. This is supported by the fact that microbial community diversity was consistently best explained by MAT, MAP, NDVI, slope, clay content, and pH. In particular, the climate variables tended to be most influential for alpha and beta diversity, aside from EPA itself. These temperature and rainfall factors tend to vary in predictable ways across the region, possibly dictating some of the location specificity in microbial diversity metrics. Specifically, Golomoti and Linthipe sites had the most contrast in beta diversity which seemed to correspond with differences in precipitation and temperature regimes in these two locations (see Table 2.1 and Fig. 2.4). For fungal and prokaryote alpha diversity metrics, Golomoti was also consistently lowest out of all the EPAs. Golomoti is a hotter, drier agricultural context, which seems to select for distinct soil fungal and bacterial communities compared to the more mesic conditions of Linthipe. Kandeu and Nsipe were intermediate between Golomoti and Linthipe with regards to Bray-Cutis dissimilarity. Though, Kandeu was highest among alpha diversity metrics for fungal communities.

Landscape and edaphic properties are also influential, as illustrated by the significance of slope, soil clay content, and pH in determining sample beta diversity (Table 2.5). Slope and clay content further distinguish the regions, with crop fields in Golomoti being slightly more level and with less clay on average than the other locations. However, there is clearly more variation within EPAs than between them for slope, pH, and clay content (Table 2.1). Hence, it is possible that these factors explain some degree of variation in soil microbial diversity across the region as a whole in addition to determining microbial community patterns within the geographic context of each EPA individually.

The importance of environmental drivers in soil microbiome composition is demonstrated at many scales around the world (Delgado-Baquerizo et al., 2018; Fierer and Jackson, 2006; Tedersoo et al., 2014). Cowan et al. (2022) sampled 9 countries in Sub-Saharan Africa (SSA) and found that environmental factors were important determinants of soil microbial community differences, especially pH, precipitation, and temperature. We corroborated these continental-scale trends in our more regional study of Central Malawi, which is a country not previously represented in the larger study.

Cowan et al. (2022) found that pH was the most influential factor for selecting different soil microbial taxa among the SSA countries studied. Fierer and Jackson (2006) found similar importance in soil pH for shaping bacterial communities in North and South America, noting that diversity was highest in neutral soils and declined with greater acidity. In our study pH impacted community composition, but not alpha diversity, except perhaps for prokaryote richness. The full main effects model and stepwise selected model for prokaryote richness identified pH as a significant factor, though the correlation test was not significant. Nonetheless, pH was not significantly associated with fungal alpha diversity in this region. Though pH was a significant factor for fungal beta diversity according to the PERMANOVA results, R^2 was lowest for pH compared to the other significant environmental factors. These results suggest that soil pH was less influential for fungal community metrics than for prokaryotes. This was similar to findings from Cowan et al. (2022), where bacterial and archaeal diversity was highly explained by soil pH, but only climate and distance parameters were influential in soil fungal community divergence. In a global comparison of biogeographical selectors, Tedersoo et al. (2014) determined that climate factors were more broadly impactful for fungal community composition, while soil pH was influential for community selection among specific guilds such as ectomycorrhizal fungi and certain saprotrophs.

It is evident that the environmental context is integral in shaping the soil microbiome of agricultural fields. These characteristics determine water availability, rate of respiration and decomposition, and so much more. For instance, soil texture itself infers much about water dynamics, nutrient exchange, and the potential to accrue organic matter (Dharumarajan et al., 2019; Feller and Beare, 1997; Nichols, 1984). It is noteworthy that in this study prokaryote alpha diversity was highly influenced by clay content, while this was not the case for fungal communities. Correlation analyses identified that prokaryote alpha diversity was negatively related to clay content while there was no main effect of clay on fungal alpha diversity. Prokaryotes and fungi have very different growth habits, structures, motility, and life history strategies in soils. It is possible that the filamentous nature of many fungal organisms makes them much less constrained by the distribution of soil particle size classes and reactivity when compared to many prokaryotes which are dependent on water films and water-filled pore spaces for mobilization. Clay content was a significant factor in sample community dissimilarity in

prokaryotes and fungi, however, suggesting that it still has a role to play in community composition for both groups of microorganisms.

The lack of explanatory power for the two edaphic variables in this study in describing soil fungal relative to prokaryote diversity is confirmed in other reports. Egidi et al. (2019) describe how edaphic features explain much less in terms of dominant fungal community composition than climate and vegetation status.

Along with other environmental variables, NDVI was determined to be significant for explaining community differences. This is notable because, as a proxy for plant coverage and primary productivity, NDVI itself is often driven by specific feedbacks from environmental characteristics. Combinations of climate and soil properties determine differences in NDVI across ecosystems (de la Peña-Domene, 2022). In this study, NDVI was significantly positively associated with prokaryote and fungal richness, but not the other alpha diversity metrics. It could be that, as an index for primary productivity, NDVI relates to higher species richness due to availability of plant resources entering soils but also encourages more dominance of certain microbial taxa which contradicts evenness and weighted diversity metrics such as Shannon's and Inverse Simpson's. NDVI was also a significant factor in beta diversity. This further supports that NDVI may contribute to both richness and selection of soil microbial taxa, perhaps those that dominate in more copiotrophic conditions. Other reports document relationships between NDVI and bacterial diversity (Carvalho et al., 2016; Sun et al., 2023). This is a useful insight, as future studies to determine soil microbiome processes can incorporate more geospatial data to analyze soil communities at scale. Understanding the relationship between NDVI and soil biology could be an important step in developing large-scale microbial ecological research in agricultural contexts.

Contextual Impact of Farm Management

As anticipated, it was challenging to pick up a signal of overall management effects on soil microbiomes at the broadest scale across Central Malawi. Though the range of practices considered may be expected to influence soil dynamics, these factors did not explain soil microbial composition as prominently as environmental factors did. This could be attributed to sampling from agricultural soils among real farms where management is more variable than in controlled experiments, and where the long-term effects of consistent human cultivation may be normalized across the sample sites. Despite these conditions, we were surprised to identify soil microbial outcomes dictated by farmer practices in this study. Crop residue management, nitrogen fertilizer application, and intercrop species diversity each had specific instances of significance for microbial diversity metrics.

Sites with high residue retention were higher in all alpha diversity metrics than low residue sites for fungi. However, residue was not significant for prokaryote alpha diversity. This could be related to the high levels of lignin typical for aboveground plant structures. Given that fungi have a propensity to break

down recalcitrant, lignified biomass (Janusz et al., 2017), it is possible that this feedstock from residue retention is best utilized by soil fungi and increases their overall richness and evenness. Indeed, stepwise model selection chose EPA and residue retention as the two key factors for explaining fungal evenness and Shannon's diversity values. Residue was not nearly as influential for fungal beta diversity ($p = 0.044$), with the lowest R^2 of all significant factors. Furthermore, residue was not present in the forward selection RDA model, indicating its reduced explanation of fungal community dissimilarity. Nevertheless, residue management could be impactful for increasing the richness and spread fungal taxa in Malawi agroecosystems.

Nitrogen application did not explain soil microbial alpha diversity, with the sole exception of an interaction between fertilizer level with EPA and clay content for prokaryote evenness. Exploration of this interaction showed that while all other combinations of these factors resulted in either negative or neutral relationships with clay content, prokaryote evenness seemed to increase with clay content in the presence of high nitrogen application only in Kandeu. This is not a conclusive result about the context dependency of nitrogen application rates affecting soil microbial community evenness, but could warrant further investigation. A different study looking at maize cropping system in Germany found that soil microbial responses, such as richness, to N application rates on farms could be short-lived from season to season (Fernandez-Gnecco et al., 2022), and therefore less likely to result in major shifts captured at a coarser temporal or spatial scale (Babin et al., 2019). Beta diversity, on the other hand, was determined to be significantly affected by nitrogen application levels in our study. Sites with very low nitrogen application rates (< 30 N kg/ha) were significantly different than all other fertilizer levels based on fungal community composition. Although, the variation of samples within the very low fertilizer group was significantly different than the medium and high groups, which means that part of the significance of this result could be due these differences in group variation. Similarly for prokaryote beta diversity, very low fertilizer sites were significantly different from all others, but the same challenge in group variation arises. In this case, it is difficult to conclude that Bray-Curtis dissimilarity results were solely related to differences in very low fertilizer community composition. However, prokaryote beta diversity also differed based on the high fertilizer (> 100 kg/ha) grouping of samples, where high fertilizer was significantly different than medium and low fertilizer groups. These groups all had similar variation, therefore the conclusion that high fertilizer groups had different community composition is maintained. Hence, high nitrogen application had distinct prokaryote communities, and very low nitrogen application potentially produces distinct prokaryote and fungal communities.

Geisseler and Scow (2014) synthesized data from other systems and found that microbial response to long-term N applications could be explained more in terms of feedbacks, such as impact of N fertilizers on crop productivity or soil pH. Similarly, de Graaff et al. (2019) found pronounced effect of N

fertilization on soil microbes when N rates are moderate and coupled with increases in organic matter. Further, both meta-analyses stress the differences in the form of N applied (ammoniacal, nitrate, etc.) in terms of specific impact on microbial groups, which are not captured in the factor of total N applied in this study. Thus, direct impacts of N application rates on soil microbiomes may be limited to shifting some select microbial groups particularly sensitive to nitrogen enrichment and not as prominent in macro-scale diversity attributes. Given that microbial diversity responses to N rates overall are often coupled with the status of organic matter availability it could be useful to look at the independent and interactive effects of N fertilizer application with sites of varying levels of SOM to see if coupled effects of carbon and nitrogen would explain more about these microbial communities.

Of the practices analyzed in this study, compost application was the least influential. Due to difficulty in capturing accurate accounts of seasonal application rates, compost was reported as presence/absence of application in the fields. Therefore, without knowing how much compost was being applied among the different farms that used it, it is difficult to conclude the overall impact of this amendment on soil microbiomes.

Role of Crop Diversity

Crop diversity was a primary management factor of interest in this study. Many studies have observed significant trends for plant diversity, asserting its importance for shifting and shaping soil microbial communities (Eisenhauer et al., 2010; Lange et al., 2015; Schmidt et al., 2018; Stefan et al., 2021). This is supported by a well-documented body of knowledge on the intimate relationships between plants and their associated microbes (Bennett and Klironomos, 2019; Bever et al., 2012; Cordovez et al., 2019; van der Putten et al., 2013). These plant diversity determinants of soil microbial community composition have numerous explanations, including many direct and indirect effects on the soil biology mediated by plants (Bais et al., 2006; De Long et al., 2019; Philippot et al., 2013). In the context of agroecosystem soil microbiomes of Central Malawi, crop diversity seemed to have a specific role in affecting microbial communities. Sites with high intercrop diversity tended to have higher fungal alpha diversity than low crop diversity sites. This relationship was not observed for alpha diversity among prokaryotes, however. Crop diversity did appear to explain part of fungal and prokaryote community dissimilarity. High, medium, and low crop diversity groups were significantly different from each other, suggesting shifts in community composition. In fact, high and medium crop diversity sites tended to be represented by many unique taxonomic groups not occurring in low crop diversity sites. Though all three groups shared around 91% of fungal families and 70% of prokaryote families, there were 13 fungal families 61 prokaryote families observed in high and/or medium crop diversity sites that were not detected in low crop diversity sites. Conversely, there were no fungal families and only 6 prokaryote

families unique to low crop diversity sites, indicating the potential for higher phylogenetic diversity among soil microbiomes at the sites with greater numbers of intercrops.

Prevalent taxa also depict unique differences between crop diversity levels. The unique fungal taxon prevalent in at least 90% of samples for the low crop diversity group belongs to the *Didymella* genus, many of which are plant pathogens (Chen et al., 2017). The taxa prevalent in high crop diversity sites consist of genera including *Aspergillus, Fusarium,* and *Penicillium,* all of which contain some known pathogens and food pests, but are more broadly ubiquitous saprotrophs involved in decomposition of organic matter. Many individuals within these genera are also known to affect the solubility of soil nutrients, potentially making them important actors in soil nutrient exchange (Gorain et al., 2022; Maity et al., 2014). Bacteria prevalent in the high crop diversity group included the genera *Gaiella, Siccirubricoccus,* and *Nocardioides*. Members of the *Nocardioides* are known to be capable of degrading complex substrates including aromatic compounds and hydrocarbons (Ma et al., 2023). A *Siccirubricoccus* taxon has also been associated with crude oil degradation (Li et al., 2021). This could suggest that plant species diversity provides a suite of diverse exudates and tissue substrates which support the metabolic capacities of *Nocardioides* and *Siccirubricoccus*, but this would need to be further substantiated. Crop diversity might maintain soil microorganisms with important, distinct environmental functions.

Other agroecosystem studies have identified the potential for crop diversity to affect soil microbiome diversity and composition. In an agroecosystem in California, cover crop mixes were determined to impact soil microbial abundance and shifted the community towards organisms with wider metabolic capacities through increased and diversified C inputs which provide a range of substrates to soil microbes (Schmidt et al., 2018). In an intercropping study looking at 40 combinations of diverse crop mixes in Spain and Switzerland, short-term impacts of crop richness as well as plant functional group presence had apparent impacts on soil bacterial and fungal compositions (Stefan et al., 2021). Our study provides additional evidence for the importance of plant diversity for manipulating soil microbial communities. These findings offer a starting point to consider the impacts of crop diversity on soil microbial functions. In a grassland context, Lange et al. (2015) linked plant species richness impacts on soil organic carbon (SOC) increases with positive trends in microbial diversity and activity. In particular, their model suggested that plant diversity was not linked directly to correspondent increases in soil carbon, but was the result of elevated microbial activity responding to higher rates of root exudates in high plant species compositions. Following the many reports of plant diversity influencing soil microbial dynamics through carbon contributions, it is logical to deduce that such feedbacks between crop diversity and select bacterial and fungal taxa could occur among the Malawi farm soils. This is especially interesting given that previous research on these farm fields from Tu et al. (2022) determined crop

diversity management had a positive relationship with soil carbon (SOC and permanganate oxidizable carbon). Clearly, there would seem to be important feedback between crop diversity, soil carbon, and soil microbial communities across these agroecosystems, yet the mechanisms and causal direction of these processes remain to be verified. Hence, to understand the relationship of crop diversity in Malawi to biogeochemical processes, it may be necessary to take a closer look at the interactions of these crops with the soil microbiome. This could inform what kind of shifts in soil processes, life history strategies, and plant-microbe interactions are occurring under different cropping regimes in Central Malawi.

Conclusion

Soil microbial diversity across four EPAs of Central Malawi was most strongly shaped by environmental parameters that distinguished farm fields from one another. The EPAs themselves were quite distinct in microbial alpha and beta diversity, as well as taxonomic distribution. This geographic gradient is of interest, as to our knowledge no other study has looked at macroecological patterns in soil microbial diversity at this intermediate regional scale (clusters at 5 to 10 km^2). Interestingly, contrary to previous studies soil pH was not shown to be as influential in determining soil microbial diversity, perhaps due to this difference in scale comparison, while soil texture seemed to play an important role specifically in prokaryote diversity. Though constrained at the regional scale, there were also notable influences of farm practices on soil microbiomes. Practices of nitrogen fertilizer rate, crop residue management, and intercrop diversity all had select influences on soil microbiomes. Following work done by Tu et al. (2022), which highlights the contribution of some of these practices including crop diversity and crop residue retention to the accumulation of soil carbon, further research on the nature of farm management impact on plant:soil:microbe interactions could elucidate strategies for maintaining optimal soil health in agroecosystems of Central Malawi. Such investigations could also provide more in-depth assessment of fungal and prokaryote diversity that characterizes this region, along with more detail about their functional and ecological roles, and contributions to plant and soil health.

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