COVER CROP DIVERSITY EFFECTS ON SOIL FUNCTIONS IN A CORN – POTATO ROTATION

By

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ABSTRACT

Soils provide important ecosystem services necessary for sustainable agriculture. The ability of soils to provide nutrients to plants, recycle plant residues, and store nutrients, contributes to plant productivity, organic matter (OM) formation, and the prevention of nutrient leaching and runoff. These ecosystem services are largely driven by microbially mediated soil processes. Plant diversity has been shown to positively influence microbially mediated soil functions, including nutrient provisioning and carbon cycling. Gaps remain in understanding how plant diversity affects these ecosystem services. Specifically, the role of functional diversity in promoting microbially mediated nutrient provisioning and carbon cycling in soils.

I investigated the effects of four cover crop species, two grasses and two legumes, on the ecosystem services of nutrient provisioning and carbon cycling. These cover crops were planted individually, as a mixture of one grass and one legume, and as a mixture of all four species. I found positive effects of cover crops on nutrient provisioning and carbon cycling ecosystem services. One mixture of a grass and a legume outperformed its constituent monocultures, while another mixture did not, indicating the important of both functional diversity and species level interactions. Cover crop performance was not always correlated to plant biomass, indicating the importance of diversity and species level effects. The four crop crop mixture was consistently outperformed by the two species mixtures of a grass and a legume, indicating the importance of functional diversity rather than simple species diversity. This research demonstrates the positive effects of functional diversity on microbially mediated nutrient provisioning services and carbon cycling. However the inconsistent effects between cover crop species and cover crop mixtures also indicates a need for further research on plant functional traits, plant species level interactions, and plant diversity effects on microbially mediated soil functions.

This thesis is dedicated to Catalina and Dr. Lisa Tiemann, who believed in me.

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LIST OF SYMBOLS AND ABBREVIATIONS

AR	Annual ryegrass
AWP	Austrian winter pea
BG	β-1,4-Glucosidase
C	Carbon
CBH	β–D-1,4-cellobiohydrolase
CH_4	Methane
$CHCl_3$	Chloroform
CO_2	Carbon dioxide
CR	Cereal rye
CWT	Hundredweight
EEA	Extracellular enzyme activities
EOC	Extractable organic carbon
EON	Extractable organic nitrogen
HV	Hairy vetch
KG	Kilogram
K_2SO_4	Potassium sulfate
LAP	Leucine amino peptidase
MBC	Microbial biomass carbon
MBN	Microbial biomass nitrogen
MC	Methyl coumarin
MUB	Methylumbelliferone
L-DOPA	3,4-dihydroxyl-L-phenylalanine

NAG	β-1,4-N-acetyl glucosaminidase
N	Nitrogen
N_2	Dinitrogen gas
NH_3	Ammonia
NH_4^+	Ammonium
N_2O	Nitrous oxide
NO_3^-	Nitrate
OM	Organic Matter
OXIDASE	Phenol oxidase and perioxidase
P	Phosphorous
PHOS	Acid phosphatase
SOM	Soil organic matter

INTRODUCTION

Michigan has nearly 10 million acres of farmland, comprising over 25% of its total land area (United States Department of Agriculture, 2019; US Census Bureau, 2010). In addition to producing vegetables, fruits and grains, healthy farmland soils retain nutrients for future crops, store water, control erosion, and sequester carbon from the atmosphere (Costanza et al., 1997; Millennium Ecosystem Assessment Series, 2003; Palm et al., 2006; Paustian et al., 2016; Robertson et al., 2014). Thus, farm soils in Michigan represent not just a source of food and energy, but a source of many ecosystem services for the people of Michigan.

Soil is extremely complex (Young & Crawford, 2004) and there is an incomplete understanding of how soil functions contribute to ecosystem services (Swift et al., 2004; Swinton et al., 2007). This is partially due to incomplete knowledge of how soil biota drive ecosystem services, and the difficulty in describing the variability and functioning of highly diverse soil communities (Fierer, 2017; Fierer et al., 2009; Fierer & Jackson, 2006). However we do know soils drive multiple ecosystem services, including moderating the hydrological cycle through water infiltration rates and storage, filtering water of chemicals, physically supporting plants, retaining and supplying nutrients to plants, recycling organic matter (OM), providing a habitat for soil organisms, and regulating the flux of the greenhouse gases carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) (Adhikari & Hartemink, 2016; Daily, 1997; Millennium Ecosystem Assessment Series, 2003; Palm et al., 2006).

In agricultural systems, one critical ecosystem service that soils provide is nutrient provisioning. Most ecosystems are nitrogen (N) and phosphorous (P) limited (Jilling et al., 2018; Robertson & Vitousek, 2009; Smith et al., 2015). N bioavailability drives plant productivity in most ecosystems (Jilling et al., 2018). In natural systems N and carbon (C) cycling are tightly

coupled, with plants and microbes acting as both sources and sinks of N (Gardner & Drinkwater, 2009). However, in intensively managed agroecosystems, such as in Michigan, a high proportion of N comes from fertilizer, which results in N availability becoming decoupled from C availability (Gardner & Drinkwater, 2009). When N is not coupled with C inputs, the result is N asynchrony (Daly et al., 2021). N inputs that exceed plant and microbial requirements lead to increased N mineralization, increased nitrification, and increased leaching of nitrates (Gardner & Drinkwater, 2009). Intensively managed agroecosystems that have low levels of soil OM, and therefore less microbial activity, will possess a limited ability to cycle and store excess N, exacerbating N losses from fertilizer applications (Blesh, 2019). Partially as a result, an average of 38 % of inorganic N fertilizer applied annually to farmland is lost (Gardner & Drinkwater, 2009). Furthermore, in agroecosystems with high fertilizer inputs and decoupled N and C cycling, N availability may fluctuate, leading to periods of both N excess and N limitations for plant growth (Daly et al., 2021).

Even in agroecosystems with large fertilizer inputs, organic bioavailable N remains a significant source of N for plants and microorganisms (Daly et al., 2021; Jilling et al., 2018). Soil biota facilitate plant nutrient uptake by breaking down organic residues into their constituent nutrients. Plants and microorganisms compete for bioavailable organic N in the form of monomers such as amino acids, amino sugars, and nucleic acids (Kuzyakov & Xu, 2013; Schimel & Bennett, 2004). These N containing monomers result from depolymerization of N containing molecules as well as from the desorption of N containing monomers in mineral associated organic N (Jilling et al., 2018; Schimel & Bennett, 2004).

Microorganisms mediate important soil biological processes, including degrading OM and mineralizing nutrients, through the release of extracellular enzymes into the soil environment

(Marx et al., 2001). Extracellular enzymes complex with molecular substrates and break the substrate down into smaller molecules through oxidation or hydrolyzation (Wallenstein & Weintraub, 2008). By reducing the molecular mass of compounds and breaking down OM, extracellular enzymes provide access to nutrients important for microorganism growth (Dunn et al., 2014; Wallenstein & Weintraub, 2008).

Extracellular enzymes break down polymers and release the N containing monomers such as amino acids, amino sugars, and nucleic acids (Barrios, 2007; Robertson & Grandy, 2010; Schimel & Bennett, 2004). Oxidative enzymes both break down aromatic compounds and destabilize mineral-bound compounds, thereby exposing N containing compounds to further degradation from hydrolytic enzymes (Jilling et al., 2018). By affecting the rate at which substrates are degraded and their constituents become available for plant and microbial uptake, extracellular enzymes exert an important control on microbially mediated nutrient cycling (Marx et al., 2001).

Microbes are also responsible for fixing a significant portion of soil N from the atmosphere by converting dinitrogen gas (N_2) into ammonia (NH_3) (Barrios, 2007; Peoples et al., 1995; Rees et al., 2005). Biological N fixation occurs among microorganisms that are in symbiotic relationships with plant species (*Rhizobium, Actinomycetes),* as well as in free living microorganisms (*Azotobacter, Klebsiella, Rhodospirillum*) (Barrios, 2007; Smercina et al., 2019). Symbiotic N fixation is currently a more important source of N in agriculture compared to free living N fixation (Herridge et al., 2008; Peoples et al., 1995; Smercina et al., 2019).

Microbial diversity has been shown to influence soil functions such as C and N mineralization (J. Chen et al., 2018; Delgado-Baquerizo et al., 2016; Maron et al., 2018; Strickland et al., 2009; Trivedi et al., 2019) N availability is therefore driven to a large degree by

microbially mediated processes (Scholes & Scholes, 2013), and the physiologies and ecological strategies of different microbial communities have ecosystem level effects (Fierer, 2017; Wieder et al., 2014).

As N provisioning services are lost in soils, systems become less sustainable. In order to rebuild this service and increase sustainability, farmers can work to improve soil health. There are many management practices that can improve soil health, but here I focus on cover crops and diversification, two of the USDA NRCS Soil Health Division's pillars of soil health.

Cover crops increase the overall diversity of plants in an agroecosystem. Plant diversity is often, but not always positively correlated with increased primary productivity (S. Chen et al., 2018; Hector et al., 1999; Sanford et al., 2016; Tilman, 1996; Tilman et al., 1997), increased microbial functional diversity (Tiemann et al., 2015; Wang et al., 2017), increased microbial activity (Lange et al., 2015; McDaniel, Tiemann, et al., 2014; Tiemann et al., 2015), increased processing rates and pools of C and N (McDaniel, Grandy, et al., 2014; McDaniel, Tiemann, et al., 2014; Tiemann et al., 2015), and increased soil C storage (S. Chen et al., 2018; Furey & Tilman, 2021; Lange et al., 2015).

Cover crops increase the diversity and the quality of plant residues available for decomposition (Hayden et al., 2014). The nitrogen content of cover crops affects microbial use efficiency and therefore soil organic matter (SOM) accumulation (Cotrufo et al., 2013). Microbes have a lower C to N ratio than most plant residues (Manzoni et al., 2008), meaning that N will often be a limiting nutrient in decomposition. Cover crops such as legumes with a high N content will result in quicker microbial degradation (Hobbie, 2005) and greater efficiency of decomposition, resulting in increased microbial activity, increased accumulation of microbial products, and increased microbial necromass, leading to both increased nutrient cycling and

increased SOM formation (Blesh, 2019; Cotrufo et al., 2013). Agroecosystems that utilize legume N fixation as a source of N can reduce N loss (Blesh, 2019). Conversely, crop litter with low N content, which is typical in intensively managed agroecosystems, may result in microbial degradation of existing OM rich in N (Craine et al., 2007).

Through photosynthesis, root exudation, and decomposition, plants fix C and transfer it belowground, supplying both the energy for soil biota and SOM formation (Bowsher et al., 2018; Paul, 2016). Root exudation of C molecules drives microbial activity and mineralization of N substrates, as well as release of N in mineral associated organic matter (Jilling et al., 2018, 2021; Liu et al., 2022). In agroecosystems, cover crops increase the portion of the year with living plants, leading to increased root exudates and increased microbial activity (Blesh, 2019) Plants release distinct root exudates (Bardgett et al., 1999; Griffiths et al., 1992) which lead to shifts in microbial community composition (Zwetsloot et al., 2020) and microbial respiration (Steinauer et al., 2016; Zwetsloot et al., 2018). Cover crops increase root exudate quantity and diversity, leading to increased microbial activity, increased nutrient cycling, and increased soil organic carbon (SOC) formation (Kim et al., 2020; Tiemann et. al., 2015) Both cover crop rhizodeposition and cover crop residues add low molecular weight carbon to the soil (Sokol and Bradford, 2018, Wang et al., 2021, White et al., 2020), which supports efficient microbial metabolism and mineral stabilization (Cotrufo et al., 2013; Kallenbach et al., 2015), leading to more SOM accumulation. Higher concentrations of SOM are associated with increased levels of soil and plant N, increased microbial biomass, and increased plant productivity (Oldfield et al., 2018, 2019).

Studies have effectively demonstrated relationships between plant diversity (Eisenhauer et al., 2011; Hooper & Dukes, 2004; Zak et al., 2003), crop rotational diversity (McDaniel,

Tiemann, et al., 2014; Tiemann et al., 2015), and cover crops (Curtright, 2022; Kim, 2022; Hayden, 2014) with microbially mediated nutrient cycling. While cover crops and increases in plant diversity have been linked to increases in microbial mediated nutrient cycling, as well as SOM formation, farmers require specific recommendations in order to utilize cover crops effectively.

The relationship between diversity and increased microbially mediated nutrient cycling may be driven by niche complementarity of species with different functions, root architecture phenologies or physiologies (Brooker et al., 2015; Finney et al., 2016; Grime, 1998; Lynch, 1995). In agriculture, a common cover crop mixture involves a grass and a legume, which relies on the benefits of N functional diversity (Finney et al., 2016; Hayden et al., 2014; Maher et al., 2021). Grass species such as CR or AR are able to scavenge excess nitrogen in the soil, immobilizing it in plant biomass and reducing N losses through leaching (Sainju et al., 2007; Stark & Porter, 2005; Weinert et al., 1998). When grass cover crops are killed by mowing or tillage the following year, the N may be available for the following cash crop (Sainju et al., 2007; Stark & Porter, 2005; Weinert et al., 1998). Legumes such as hairy vetch (HV) and Austrian winter pea (AWP) are able to fix large quantities of N which are available to the subsequent cash crop (Brainard et al., 2012). Therefore the mixture of a grass and a legume has been hypothesized to provide the benefits of both N retention and N fixation (Brainard et al., 2012; Hayden et al., 2014).

In addition to complementary functions, the combination of a legume and a grass may provide synergistic interactions. The presence of a cereal intercropped with a legume has been shown to increase N fixation by the legume (Izaurralde et al., 1992; Johansen & Jensen, 1996). While grasses may outcompete legumes, they can also enhance winter survival by moderating

the soil temperature (Brainard et al., 2012; Hayden et al., 2015). However it is unclear whether mixtures of two cover crops, a grass and a legume, results in increases in ecosystem services, or tradeoffs between functions (Hayden et al., 2014).

Furthermore, while legume cover crops fix N, the quantity of N fixed is dependent on soil fertility and the competitive or facilitative interactions with other plants (Blesh, 2018; Hayden et al., 2014, 2015). A combination of a grass and a legume cover crop may lead to a greater amount of N fixed by the legume, however unique plant traits of different legume species and grass species will lead to a spectrum of outcomes (Blesh, 2018; Blesh et al., 2013; Bukovsky-Reyes et al., 2019; Hayden et al., 2014). The outcome of interactions between legumes and grasses will also be influenced by existing soil fertility (Blesh & Ying, 2020). In addition, while the combination of a two cover crop mixture of a grass and a legume cover crop has been studied fairly extensively, few studies have investigated the potential benefits and tradeoffs between three or more cover crops (Finney & Kaye, 2017; Florence et al., 2019)

It is therefore unclear whether cover crop impacts on microbially-mediated nutrient cycling are driven by increases in plant diversity, by trait specific interactions between cover crop functional groups, or by a combination of the two, which also could be influenced by climate, agronomic practices, and soil characteristics.

Potato systems are characterized by intensive tillage and fertilizer use, which result in low levels of OM and poor soil aggregation (Po et al., 2009). Furthermore, sandy soils that are often used for potato production are at more risk to erosion, which can preferentially remove fine particles and OM (Stark & Porter, 2005). Therefore cover crops that are more effective at preventing erosion, such as grasses which produce high biomass, may contribute more to microbially mediated nutrient cycling in a potato system than in a different crop production

system with a heavier soil type (Hunter et al., 2019). Conversely, legumes, which have the ability to fix N, may be more important in potato systems and sandy soils that are low in nutrients (Stark & Porter, 2005). Cover crops could therefore provide outsize benefits to potato cropping systems by preventing erosion, increasing microbially mediated OM formation, decreasing N losses, and increasing N inputs through biological N fixation. However, it is important to understand which cover crop traits are most effective given the intensive tillage and sandy soils typical of potato production.

Furthermore, potato systems are sensitive to both fertilizer deficiencies and fertilizer excesses (Powell et al., 2020; Weinert et al., 1998). While insufficient N can delay potato canopy growth and tuber productivity, excess N can delay tuber initiation and reduce tuber set, bulking and maturity (Powell et al., 2020; Weinert et al., 1998). Individual studies on the effects of cover crops have shown variable results, due to contrasting soil mineralogies, rainfall, tillage, fertilization schemes, crop rotations, planting date, length of study, and specific cover crop combinations used (Brooker, Renner, & Basso, 2020; Brooker, Renner, & Sprague, 2020; Feng et al., 2021; Gao et al., 2022; Hayden et al., 2012, 2014, 2015; Kim et al., 2020; Maher et al., 2021; Martínez-García et al., 2018; Mullen et al., 1998; Nevins et al., 2020; Nguyen et al., 2022; Thapa et al., 2021). Therefore, research is needed on how different cover crop traits affect N cycling, specific to the climate, soils, and agronomic practices of potato cultivation.

In this study, I sought to address questions about soil ecosystem services, specifically, nutrient provisioning, as relevant to farmers in Michigan who would like to incorporate cover crops into their fields, as well as the broader ecological questions regarding cover crop diversity. Specifically, I investigated the importance of cover crop diversity and cover crop functional trait diversity in relation to microbially mediated nutrient cycling, using local agronomic practices for

seed corn and potato production. To do this, I used a cover crop diversity experiment established in 2015, in a field with a seed corn-potato rotation at the Montcalm Research Center in central Michigan. At the site, four different cover crop species have been planted in various combinations. I sampled soils from cover crop treatments containing each of the four cover crop species alone, combinations of two cover crop species and a combination of all four cover crop species, as well as a no cover crop (control) treatment. The species combinations were created to pair two different functional groups, grasses and legumes, such that the two cover crop species treatments consisted of two different grass-legume pairs.

The two grass-legume pairs chosen were HV – cereal rye (CR), and AWP – annual ryegrass (AR). HV and AWP are cold tolerant legumes with the ability to fix large quantities of N (Brainard et al., 2012; Marr et al., 1998; Midwest Cover Crop Council, n.d.; U.S. Department of Agriculture National Resource Conservation Service, 2020). CR and AR are two cold tolerant grasses that are able to create large amounts of biomass and therefore scavenge large quantities of nitrogen for a subsequent crop (Marr et al., 1998; Midwest Cover Crop Council, n.d.; Sainju et al., 2007).

I hypothesize that cover crops would improve soil functioning, specifically nutrient provisioning. Enhanced nutrient, and in this case specifically N, provisioning should be accompanied by greater soil N retention in the non-growing season, increased organic forms of N, and enhanced microbial communities (e.g. increased microbial biomass, labile organic C and enzyme activities). Further, I hypothesize that there would be synergistic or facilitative interactions between grasses and legumes in paired combinations such that cover crop driven improvements in soil functioning would be greater with cover crop pairs compared to monocultures. Finally, I hypothesize that a mixture of four cover crops, two grasses and two

legumes would not increase soil functioning as compared to the paired mixtures, due to competition between the two different grasses and two different legumes. I predict the functional traits of legumes and grasses are more important than the overall number of cover crops species, or in other words, that functional diversity is more important than overall species diversity.

MATERIALS AND METHODS

Research was conducted at the Montcalm Research Center (43.3513919 N, -85.1815580 W), where there is an ongoing cover cropping field established in September 2015, with sowing of the first cover crop seeds after corn harvest. Over a 15 year average, the mean annual rainfall was 17.9 inches during the growing season (April – September, Table 1) and the maximum and minimum temperatures were 73 °F and 50 °F, respectively (Michigan Potato Research Report, 2021, Table 2).

The soils at the Montcalm Research Station are Tekenink-Elmdale loamy sands. The Tekenink series is classified as coarse-loamy, mixed, semiactive, mesic Typic Glossudalfs (National Cooperative Soil Survey, 2014a). The Elmdale series is classified as coarse-loamy, mixed, semiactive, mesic Oxyaquic Hapludalfs (National Cooperative Soil Survey, 2014b). The soil pH is 6.5 and the cation exchange capacity is 10 $cmol_c/kg$ (Soil Survey Staff et al., n.d.)

The field at the Montcalm Research Center is a seed corn (*Zea mays*, Dekalb 44-98) - potato (*Solanum tuberosum L.*, 'Superior') rotation, with either crop grown in alternating years. Corn was grown in 2015, 2017, 2019, and 2021 (Table 3). Potatoes were grown in 2016, 2018, and 2020 (Table 4). The field was chisel plowed to 12" in early April and vertically tilled by disking to 2" approximately two days later (Table 3 and Table 4). Disking was done east to west, so as not to move plant residues into other treatment plots, which run north to south. Corn or potato seed were planted in the beginning of May (Table 3 and Table 4). Corn seed was planted at a rate of 34,000 seeds per acre by a four row corn planter, and potato seed was planted at 12" inch spacing within rows and 34" spacing between rows with a two row potato planter. All fertilizer applications were incorporated on the same day. Corn years received 100 lbs/acre of ammonium sulfate fertilizer (21-0-0-24S) and 275 lbs/acre of dry granular fertilizer in the form

of urea (46-0-0), with NDURE 2.0 N stabilizer applied at a rate of 1 quart/ton, all applied at planting (Table 3 and Table 4). Acuron herbicide (S-metolachlor 23.40%, Atrazine 10.93%, Mesotrione 2.60%, Bicyclopyrone 0.65%) was applied after corn planting at a rate of 2.5 qt/acre (Table 3). Potato years received 40 gallons/acre of liquid starter at planting, which was a combination of 50% ammonium nitrate liquid fertilizer (28% N) and 50% ammonium phosphate fertilizer (10% N, 34% P, Table 4). Potatoes were hilled in late June/early July (Table 4). At hilling potatoes received 100 lbs/acre of dry granular urea fertilizer (46-0-0, Table 4). As a late side dressing potatoes received an additional 100 lbs/acre of dry granular urea fertilizer (46-0-0, Table 4). During 2020, potatoes received 12.1" of irrigation in addition to 15.9" of rain during the growing season. Potato years received fungicides between June and August in the following quantities: two applications of Echo 720 at 16 oz/acre, 5 applications of Echo 720 at 24 oz/acre, two applications of Mancozeb at 2lbs/acre, one application of Bravo at 20oz/acre, and one application of Pencozeb at 2lbs/acre (Table 4). Potato years received insecticides between June and July in the following amounts: Blackhawk at 3.3oz/acre, Coragen at 6oz an acre, Besiege at 9oz/acre, and Mustang Maxx at 3oz/acre (Table 4). The corn growing season lasts from April/May to September. The potato growing season lasts from April/May to October. Corn was harvested using a combine and potatoes were harvested using a one row digger. Soil samples collected between April-October are designated as 'growing season' and samples collected between November - March as 'non-growing season'.

Cover crop treatments are organized in the field in a randomized-block design. There are eight treatments: the individual cover crop species alone, which includes 1) annual rye (*Lolium multiflorum*), 2) CR (*Secale cereale*), 3) HV (*Vicia villosa*), 4) AWP (*Pisum sativum*); grass-legume pairs of cover crop species, 5) AR + HV, 6) CR + AWP; 7) all four cover crop species

together; and 8) a no cover crop control (Table 5). Each treatment is represented across five replicate blocks in experimental plots (n=5) that are 6.1 x 6.1 m, which encompasses seven planted rows of potatoes.

Cover crops were interseeded by hand into corn as close to the V6 growth stage as possible in June during maize growing years, to minimize competition effects between corn and cover crops while allowing time for cover crop growth (Brooker, Renner, & Basso, 2020; Brooker, Renner, & Sprague, 2020). Cover crops were hand seeded in the fall, following the potato harvest, in potato growing years. Cover crop seeding rates are: AR (15lbs/acre), CR (90lbs/acre), HV (20lbs/acre), AWP (70lbs/acre), AR + HV (11.25 lbs/acre and 15lbs/acre respectively), CR + AWP (45lbs/acre and 52.5 lbs/acre respectively), and AR + CR + HV + AWP (7.5lbs/acre, 22.5lbs/acre,10lbs/acre, 35lbs/acre respectively, Table 5). Soil sampling began in 2016 and continued through 2021 (Table 6). Soil samples were collected two to three times a year, both within the growing season and outside of the growing season on the following dates: October 12, 2016; January 16, 2017; September 1, 2017; November 13, 2017; June 25, 2018; July 13, 2018; August 17, 2018; April 8, 2019; July 25, 2019; October 18, 2019; February 4, 2020; and October 7, 2020 (Table 6).

Soils were sampled at a depth of 10 cm, using a 1.9 cm diameter soil probe, with three cores taken from each plot homogenized to create a composite sample. Soils were kept on ice in the field and brought back to the lab for processing. All soils were sieved through a 2 mm mesh. Approximately 10 g of fresh soil were immediately weighed as soils were processed and dried to 65 ℃ in a drying oven, in order to determine gravimetric soil moisture content. Soils used for microbial biomass, dissolved organic C and N and inorganic N determination were stored at 4 ℃

for no more than two weeks. Soils used to determine extracellular enzyme activities (EEA) were stored at -20 ℃ until assays could be completed.

Inorganic N and Extractable Organic C and N Determination

Inorganic N and extractable organic C (EOC) and N (EON) were extracted from soils by adding 40 mL 0.5 M potassium sulfate (K_2SO_4) to 8 g of fresh soil and placing on an orbital shaker for 24 hours. After being mixed on the orbital shaker, soils were filtered through 2.5 µm pore size filter paper (Whatman #5), in order to retain all the microbial cell constituents and all EOC and EON that is readily available for uptake by microbes or breakdown by extracellular enzyme activity (Tiemann & Billings, 2011)

Concentrations of inorganic N, as nitrate (NO_3^-) and NH_4^+, in extracts were determined colorimetrically. Nitrate reductase (EC 1.1.7.1-3; NaR) was used to catalyze the conversion of NO_3^- to nitrite in the presence of NADH as reductant. Sulfanilamide and N-(1-napthyl) ethylenediamine dihydrochloride were then added to the resulting nitrite (a combination of original nitrite and nitrite produced by the reduction), creating a pink color (NECi (Method N07-0003, Revision 9.0, March 2014)). Assays were conducted in clear 96-well plates and the final absorbance measured at a wavelength of 540 nm using a spectrophotometer (Synergy HT plate reader, Biotek, Winooski, VT,USA). Concentrations of NH_4^+ were also determined in clear 96-well plates by adding salicylate and ammonia cyanurate reagent packets (Hach Company, Loveland, Colorado, USA) according to the methods outlined in Sinsabaugh et al., 2000. The final absorbance was determined using the Synergy HT at a wavelength of 610 nm.

Microbial Biomass C and N Determination

Microbial biomass C (MBC) and microbial biomass N (MBN) were determined using the chloroform fumigation-extraction method (Jenkinson et al., 2004; Vance et al., 2002). Two

milliliters of ethanol-free chloroform ($CHCl_3$) were added to 8 g of soil and incubated at room temperature for 24 h in a sealed 50 mL test tube. Following the incubation, the test tubes were vented in a fume hood for 2 hours. Chloroform fumigated soils were extracted using 0.5 MK_2SO_4 as described above.

Soil extracts, both fumigated and unfumigated were analyzed for extractable organic nitrogen (EON) and extractable organic carbon (EOC) using a Vario Select TOC/TN analyzer (Elementar Americas, Ronkonkoma, NY). Microbial biomass C or N was calculated as the difference between the EOC or EON extracted from fumigated and nonfumigated samples. Fumigation with $CHCl_3$ lyses an estimated 45% of microorganisms (Joergensen & Mueller, 1996; Vance et al., 2002). We therefore divided the results by an efficiency factor of 0.45, to take into account the partial lysing of microbial cells.

Total Soil Carbon and Nitrogen

One set of soil samples (July of 2019) were air dried after sieving and ground to a fine powder for determination of total soil C and N using a Costech ECS 4010 elemental analyzer. (Costech Analytical Technologies Inc, Valencia, CA, USA).

Extracellular Enzyme Activity

I measured the rate of activity of seven enzymes, *β-1,4-Glucosidase*, (BG), β–*D-1,4-cellobiohydrolase,* (CBH), β-*1,4-N-acetyl glucosaminidase* (NAG), *leucine amino peptidase* (LAP), *acid phosphatase* (PHOS), phenol oxidase and perioxidase. These enzymes represent labile C acquisition enzymes (BG and CBH), recalcitrant C acquisition enzymes (phenol oxidase and perioxidase), N acquisition enzymes (LAP and NAG), and a P acquisition enzyme (Tiemann & Billings, 2011c). These enzymes can be separated into two groups: hydrolases and oxidases. Of the labile C degrading enzymes, CBH catalyzes the hydrolysis of cellulose, resulting in

cellobiose (McDaniel, Grandy, et al., 2014) and BG catalyzes the hydrolysis of cellobiose, a disaccharide, resulting in glucose (Dunn et al., 2014). Recalcitrant C acquisition enzymes measured include phenol oxidase and perioxidase, which oxidize aromatic and polyphenol compounds(Dunn et al., 2014). The high molecular weight and polymorphic structures of substrates broken down by oxidative enzymes require more enzymatic steps and have a higher activation energy than, for example, cellulose (Trasar-Cepeda et al., 2007). Phenol oxidase and perioxidase activity were measured jointly and are referred to in the results as oxidase enzyme activity. Nitrogen acquisition enzyme NAG is a chitinase which cleaves N-acetyl glucosamine from chitin and peptidoglycan oligomers (McDaniel, Grandy, et al., 2014; Tiemann & Billings, 2011). Chitin is a polysaccharide abundant in nature and is a component of fungal cell walls and insect exoskeletons (Flach et al., 1992; Madigan et al., 2019; Russell, 2014). Therefore, chitin represents an important source of N in the soil. LAP hydrolyzes peptide bonds, cleaving N-terminal amino acids from proteins (Sipler & Bronk, 2015). Finally, I measured one phosphate acquisition enzyme, PHOS, which releases phosphate groups from organic P through hydrolysis (Dunn et al., 2014; McDaniel, Grandy, et al., 2014).

Extracellular enzyme assays followed methods described by Tiemann and Billings (2011) and German et al. (2011). Briefly, using a hand-held immersion blender we homogenized 1 g of soil in 125 mL of ultrapure water for 30 seconds. We then pipetted 200 ul of each soil slurry into 96-well microplates. Substrates, fluorescently labeled with either methylumbelliferone (MUB) or methyl coumarin (MC), corresponding to each enzyme were added to the microplates. I used serial dilutions of 50 mM MUB and MC to create a standard curve and assays included substrates alone as well as soils plus MUB or MC only as controls. To assess oxidase enzyme activities, I used 3,4-dihydroxyl-L-phenylalanine (L-DOPA) as a colorimetric reagent, and a

previously established extinction coefficient (Weintraub et al., 2007). Once substrates were added, soils were incubated at 24℃ for ~18 hours. Immediately before fluorescence measurement, I pipetted 10 ul 0.5 M NaOH into each well in order to maximize MUB and MC fluorescence (Tiemann & Billings, 2011).

I measured fluorescence and absorbance on a Synergy HT-1 plate reader (Biotek, Winooski, VT, USA) set at 370 nm excitation and 455 nm emission for MUB and 350 nm excitation and 430 nm emission for MC. For the oxidative enzyme activities, I measured color change associated with the breakdown of L-DOPA using an absorbance of 460 nm.

Cover Crop Biomass

In October of 2016 cover crop above and belowground biomass was measured, however no data was available on aboveground biomass for the control.

Potato Harvest

In September of 2020, two rows of potatoes were harvested from each plot, weighed for yield in hundredweight (cwt), and examined for incidence of diseases using standard grading metrics for scab, hollow heart, brown center, internal black spot, and viral diseases (Driscoll et al., 2009; Ninh et al., 2014).

Statistics

Before running statistical analyses, the residuals of response variables were visually evaluated using histograms and density plots. Data that did not pass tests of normality were transformed using a log or exponent transformation. For enzyme activities, I converted to relative activity levels by dividing each individual rate by the highest rate measured across the entire data set. Enzyme activities, inorganic nitrogen, ammonium, nitrate, EOC, EON, MBC, and MBN were analyzed using a repeated measures ANOVA (SAS OnDemand for Academics) using

the *Proc Glimmix* function, with treatment as a fixed effect, repetition as a random intercept, and date assigned as a random effect with plot ID as the subject. Different covariance structures were tested for each model to account for interactions over sampling dates and between seasons. The covariance structures tested for each model included: unstructured covariance, heterogeneous compound symmetry, heterogeneous first order autoregressive, and spatial power. The best fitting model was chosen by finding the lowest Akaike information criterion (AIC) value among all covariance structures. Least-squares means tables were generated for all pairwise comparisons with Tukey-Kramer adjusted *P* values to minimize error (Tiemann & Billings, 2011d). In addition, Dunnett's test for multiple comparisons was conducted with all cover crop treatments compared directly to the control.

RESULTS

Soil Ammonium

There were no significant differences in soil a NH_4^+ values between cover crop treatments (P<0.203, Table 7) or significant interactions between cover crop treatments and sampling dates (P<0.2596, Table 7). However, there was a significant difference in soil NH_4^+ values between sampling dates (P<0.0001, Table 7). For example, on the post corn sampling date of 9/1/2017, soil NH_4^+ values were significantly higher than the post corn sampling dates of 11/13/2017 (P<0.0001), 10/18/2019 (P<0.0001), and 2/4/2020 (P≤0.0022), as well as the post potato sampling dates of 10/12/2016 (P≤0.0008), 11/4/2016 (P≤0.0006), 1/1/6/2017 (P≤0.0005), 8/17/2018 (P≤0.0092), 4/8/2019 (0.0022), and 10/7/2020 (P<0.0001., Figure 1).

Soil Nitrate

There were significant differences in soil NO_3^- between cover crop treatments (P < 0.0121, Table 8), sampling dates (P < 0.0001, Table 8) and in cover crop treatments by sampling date (P < 0.0005, Table 8). Overall, the soil NO_3^- in the AWP and CR mixture was 7.5% higher than the control. When comparing treatments by sampling date, the CR cover crop had significantly (10/12/16) and marginally (1/16/17) lower NO_3^- values than the control on two years following potato harvests (Figure 2; potato harvests in 2016, 2018, and 2020; Table 4). The HV cover crop, AR and HV mixture, and the four cover crop mixture all had significantly lower NO_3^- values than the control on 10/12/2016, following a potato harvest (Figure 2; potato harvests in 2016, 2018, and 2020; Table 4). The CR and AWP mixture had marginally higher NO_3^- values than the control on 9/1/017 and 11/13/2017, following a corn harvest (Figure 2; corn harvest in 2017 and 2019; Table 3).

Extractable Organic Carbon

There were no significant differences in EOC values between cover crop treatments ($P<0.5279$, Table 9) or significant interactions between cover crop treatments and sampling dates ($P<0.9561$, Table 9). However, there was a significant difference in EOC values between sampling dates ($P<0.0001$, Table 9). For example, the EOC values on the post-potato sampling date of 11/9/2016 was significantly higher than the corn sampling dates of 9/1/2017 ($P<0.0001$), the corn sampling date of 10/18/2019 ($P<0.0001$), and the potato sampling date of ($P<0.0001$, Figure 3).

Extractable Organic Nitrogen

There were no significant differences in EON values between cover crop treatments, however there were significant interactions between cover crop treatments and sampling dates ($P<0.0260$, Table 10). There was also a significant difference in EON values between sampling dates ($P<0.0001$, Table 10).

The EON varied significantly between treatments on one post-potato harvest sampling date, 11/6/2016 (Figure 4). On this sampling date, all four monocultures (AR, CR, HV, AWP) as well as the AR and hair vetch mixture had significantly higher EON compared to the control ($P<0.0044$, $P<0.0280$, $P<0.0048$, $P<0.0781$, and $P<0.0025$, respectively; Figure 4). Overall, the AR and HV mixture had the highest EON relative to the control (91% higher than control).

Microbial Biomass Carbon

Cover crop treatments had a significant effect on MBC ($P<0.0427$, Table 11). The AWP monoculture had moderately less MBC than the control (6% lower, Figure 5). The interaction between cover crop treatment and sampling date was not significant (Table 11), but sampling date alone did affect MBC. On one post-corn sampling date (9/1/2017), MBC was significantly

lower compared to the post-potato sampling dates of 11/9/2016 (P<0.0001), 2/4/2020 (P<0.0001), and 10/7/2020 (P<0.0001). The post-corn sampling date (9/1/2017) MBC was also significantly lower compared to the other post-corn sampling date, 10/18/2019, (P<0.0002). On another post-corn sampling date (10/18/2019), MBC was significantly lower compared to the post-potato sampling dates of 11/9/2016 (P<0.0065), 2/4/2020 (P<0.0163), and 10/7/2020 (P<0.0063).

Microbial Biomass Nitrogen

Cover crop treatments did not have a significant effect on MBN (Table 12). The interaction between cover crop treatment and sampling date was not significant, but sampling date alone did affect MBN (Table 12). On the post-potato sampling date of 11/9/2016, MBN was significant higher compared to the post-corn sampling dates of 9/1/2017 (P<0.0001), 10/18/2109 (P<0.0001), and 2/4/2020 (P<0.0001), as well as the post-potato sampling date of 10/7/2020 (P<0.0001, Figure 6). On the post corn sampling date of 9/1/2017, MBN was significantly lower compared to the post-corn sampling dates of 10/18/2019 (P<0.0001) and 2/4/2020 (P<0.0001), as well as the post-potato sampling dates of 11/9/2016 (P<0.0001) and 10/7/2020 (P<0.0001, Figure 6).

Labile Carbon Extracellular Enzyme Activity

Cover crop treatments had moderate, but not significant effects on BG activity (P<0.2423, Table 13). The annual rye and HV mixture had moderately higher BG enzyme activity (24% higher) compared to the control (P<0.0792, Figure 7). The interaction between cover crop treatment and sampling date was not significant (P<0.8013, Table 13), but sampling date alone did affect BG enzyme activity (P<0.0001, Table 13). For example, BG enzyme activity on the corn sampling date of 5/22/2017 was significantly lower compared to corn

sampling dates of 9/1/2017, 11/3/2017, 7/25/2019, 10/18/2019, 2/4/2020 (P<0.0029, P<0.0291, P<0.0001, P<0.0001, P<0.0001 respectively) as well as potato sampling dates of 6/25/2018, 7/13/2018, 8/17/2018 and 4/8/2019 (P<0.0002, P<0.0001, P<0.0001, P<0.0043). In addition, BG enzyme activity on the post-potato sampling date of 8/17/2018 was significantly higher than the potato sampling dates of 6/25/2018, 7/13/2018, 4/8/2019, and 10/7/2020 (P<0.0001, P<0.0374, P<0.0001, and P<0.0001), as well as the corn sampling dates of 5/22/2017, 9/1/2017, 11/3/2017, and 10/18/2019 (P<0.0001, P<0.0430, P<0.0001, and P<0.0049).

There were significant differences in CBH enzyme activity between treatments (P<0.0291, Table 14). Using Dunnett's test, the annual rye -HV mixture had significantly higher CBH enzyme activity (38% higher) compared to the control (P<0.0133, Figure 8). Both the AWP and the AWP-CR mixture had moderately higher CBH enzyme activity (23% and 25% higher respectively) than the control (Figure 8). The interaction between cover crop treatment and sampling date was not significant (P<0.9215, Table 14), but sampling date alone did affect CBH enzyme activity (P<0.0001, Table 14). For example, CBH enzyme activity on the corn sampling date of 7/25/2019 was significantly higher than all other sampling dates (P<0.0001). In addition, the CBH enzyme activity on the post-potato sampling date of 10/7/20 was significantly lower than the corn sampling dates of 9/1/2017 (P<0.0018), 11/3/2017 (P<0.0001), 7/25/2019 (P<0.0001), 10/18/2019 (P<0.0001), and 2/4/2020 (P<0.0001), as well as the potato sampling dates of 6/2/5/2018 (P<0.0087), 7/13/2018 (P<0.0265), 8/17/2018 (P<0.0001), and 4/8/2019 (0.0005).

Nitrogen Enzyme Activity

There were no significant differences in LAP enzyme activity between cover crop treatments (P<0.656, Table 15). The interaction between cover crop treatment and sampling date

was not significant (P<0.525, Table 15), but sampling date alone did affect LAP enzyme activity (P<0.0001, Table 15). For example, the LAP enzyme activity on the post corn sampling date of 7/25/2019 was significantly higher than all of other sampling dates (P<0.0001, Figure 9). In addition, the LAP enzyme activity on the post-corn sampling date of 9/1/2017 was significantly lower than the potato sampling dates of 7/13/2018 (P<0.0001), 8/17/2018 (P<0.0001), 4/8/2019 (P<0.0001), and 107/2020 (P<0.0203, Figure 9), as well as the corn sampling dates of 5/22/2017 (P<0.0001), 11/3/2017 (P<0.0003), 7/2/5/2019 (P<0.0001), and 2/4/2020 (P<0.0128, Figure 9).

The annual rye and HV mixture had moderately higher NAG enzyme activity (5.7% higher) compared to the control (P<0.0603, Figure 10). The interaction between cover crop treatment and sampling date was not significant (P<0.3820, Table 16), but sampling date alone did affect NAG enzyme activity (P<0.0001, Table 16). For example, the post-corn sampling date of 10/18/2019 was significantly higher than the potato sampling dates 6/25/2018 (P<0.0001), 7/13/2018 (P<0.0001), 8/18/2018 (P<0.0001), 4/8/2019 (P<0.0005), and 10/7/2020 (P<0.0001, Figure 10), as well as the corn sampling dates of 5/22/2017 (P<0.0001), 9/1/2017 (P<0.0070), and 2/4/2020 (P<0.0001, Figure 10).

Extracellular Enzyme Activity (Oxidase)

There was a marginally significant interaction between treatment and date for oxidase enzyme activities (P<0.0698, Table 17). The AR monoculture, HV monoculture, and AR - HV mixture had significantly greater oxidase enzyme activity than the control on 10/18/2019 (P<0.0002, P<0.0075, P<0.0001, respectively, Figure 11). The CR monoculture and the AWP monoculture had moderately higher oxidase activity than the control on 10/18/2019 (P<0.0469, P<0.0793, respectively, Figure 11). Sampling date alone significantly affected oxidase enzyme

activity (P<0.0001, Table 17). On the sampling date of 11/3/2017, oxidase enzyme activity was significantly higher than all other sampling dates (P<0.0001).

Extracellular Enzyme Activity (PHOS)

The AWP cover crop had significantly higher PHOS enzyme activity (16.9% higher, Figure 12) than the control (P<0.0402, Table 18). There was a significant interaction between date and treatment for PHOS enzyme activity (P<0.0001, Table 18). The annual rye and HV cover crop mixture had a significantly higher PHOS enzyme activity than the control (P<0.0039) on 11/3/2017. The AR monoculture, CR monoculture, HV monoculture, AWP monoculture, annual rye + HV mixture, and CR + AWP mixture all had significantly higher PHOS enzyme activity than the control on 8/17/2018 (P<0.0001, P<0.0006, P<0.0078, P<0.0001, P<0.0001, P<0.0001, P<0.0001, respectively).

Cover Crop Aboveground Biomass

There were significant differences between cover crop aboveground biomass in fall 2016 (Table 19). AR monoculture had the highest aboveground biomass at 454.36 kg/ha (Table 20), which was significantly more aboveground biomass than the CR (41.1% higher), the AWP monoculture (52.1% higher), the CR -AWP mixture (124.7%), the AR – HV mixture (148.0%), and the four cover crop mixture (348.7%, Figure 13). No data was available on the control above-ground biomass.

Cover Crop Belowground Biomass

There were significant differences between cover crop below ground biomass between treatments (Table 21). All cover crop treatments had greater belowground biomass than the control (Figure 14). The AR had the highest belowground biomass 12.0083 g/kg soil, which was 1,606.7% higher than the control (Table 20, Figure 14). The AR – HV mixture had the second

highest belowground biomass at 6.31 g/kg soil, which was 741.3% higher than the control (Table 20, Figure 14. The CR had the third highest belowground biomass at 4.89 g/kg soil, which was 552% higher than the control (Table 20, Figure 14). The control plot had the lowest belowground biomass at 0.75 g/kg soil (Table 20, Figure 14).

Potato Harvest

Cover crop treatments had a significant effect on potato harvest weight (Table 22). The control had the highest potato yield (226.6 cwt/a, Table 20, Figure 15), which was significantly higher than the AR monoculture (16% higher), the CR monoculture (12.5% higher), the annual rye-HV mixture (12.9% higher), the CR-AWP mixture (12.5% higher), and the four cover crop mixture (12.9%). There were no significant differences between treatments for scab rating (Table 23, Figure 16), hollow heart (Table 24, Figure 17), brown center (Table 25, Figure 18), or viral diseases (Table 26, Figure 19).

DISCUSSION

Cover crops have the potential to improve soil health and thus enhance or sustain important soil ecosystem services such as nutrient provisioning. I hypothesized that cover crops would improve soil functioning, specifically nutrient provisioning, and would be accompanied by greater soil N retention in the non-growing season, increased organic forms of N, and enhanced microbial communities (e.g. increased microbial biomass, labile organic C, labile C enzyme acquisition activity and labile N acquisition activity). Further, I hypothesized there would be synergistic or facilitative interactions between grasses and legumes in paired combinations such that cover crop driven improvements in soil functioning would be greater with cover crop pairs compared to monocultures. Finally, I hypothesized that a mixture of four cover crops, two grasses and two legumes, would not increase soil functioning as compared to the paired mixtures due to deleterious effects of competition between the two different grasses and two different legumes.

Hypothesis 1: Cover crop effects on nutrient provisioning

I hypothesized that cover crops would improve soil functioning, particularly nutrient provisioning, and would be accompanied by greater soil N retention in the non-growing season, increased organic forms of N, and greater microbial activity related to nutrient cycling as indicated by increased microbial biomass, increased N enzyme acquiring enzyme activities, and increased labile organic C and labile C acquiring enzyme activities (Austin et al., 2017; Blesh, 2018; Curtright & Tiemann, 2021; Hayden et al., 2015; Kim et al., 2020; Kong & Six, 2012; Nguyen et al., 2022; Tribouillois et al., 2016).

In support of Hypothesis 1, all cover crop treatments, except for the four cover crop mixture, significantly increased PHOS enzyme activity compared to the control on the 8/17/2018

sampling date. In addition, over all treatment dates, the AWP monoculture had significantly greater PHOS enzyme activity compared to the control, even though it may have had the lowest belowground biomass of all cover crop treatments. (Figure 12, Figure 14). Living cover crops may stimulate the microbial community through root exudation, leading to increased enzyme activity (Hallama et al., 2019). Increased PHOS enzyme activity may also indicate an increased availability of phosphorous from cover crop residue breakdown, driven by the ability of cover crops to directly access phosphorous through their unique root architecture (Hallama et al., 2019). In a study of three legumes in the Southeast United States, Liang et al. (2014) found that AWP had comparable or greater effects on enzyme activity compared to HV and crimson clover, despite producing up to 40% less biomass than HV and crimson clover (Liang et al., 2014). These results indicate that factors such as residue biochemistry and root exudate quality may have stronger effects than total cover crop biomass on microbial activity.

In support of Hypothesis 1, three cover crop treatments had significantly higher labile carbon acquisition enzyme activity compared to the control (AR + HV mixture, CR + AWP mixture, AWP monoculture, Figure 7 and Figure 8).

Increased labile carbon acquiring enzyme activity may be indicative of increased available inorganic nitrogen (Bowles et al., 2014; Jian et al., 2016; Sinsabaugh & Moorhead, 1994) or increased labile carbon substrates locally (Phillips et al., 2011; L. K. Tiemann & Billings, 2011). As cover crops introduce both additional labile carbon sources and nitrogen sources, both are likely (Blesh, 2018; McDaniel, Grandy, et al., 2014; McDaniel, Tiemann, et al., 2014; L. Tiemann et al., 2015). Labile carbon substrates require less energy to break down and therefore are more efficient energy sources for microbes than high molecular weight and polymorphic structures such as lignin (Allison & Vitousek, 2005; Silva et al., 2021; Sinsabaugh,

2010; Sinsabaugh et al., 2013). Access to more labile carbon sources has been linked to greater microbial metabolic efficiency, greater microbial activity, and greater microbial biomass (Kallenbach et al., 2019; Sinsabaugh et al., 2013; Tiemann et al., 2015; Tiemann & Billings, 2011), leading to increased N cycling (Cheng & Kuzyakov, 2015; Kuzyakov, 2002; Phillips et al., 2011) and greater SOM accumulation through microbial growth, turnover, and necromass accumulation (Cotrufo et al., 2013; Grandy & Neff, 2008; Kallenbach et al., 2015; Liang et al., 2017; Miltner et al., 2012; Tiemann et al., 2015).

The AR + HV mixture had significantly higher nitrogen acquisition enzyme activity compared to the control (20.9% greater NAG, Figure 10). These results partially support hypothesis 1: some cover crop treatments increased microbial nutrient cycling compared to the control, however this was treatment dependent.

The cover crop treatments of AR, CR, HV, and AR + HV all had moderately significantly higher soil oxidative enzyme activity on the 10/18/2019 sampling date (Figure 11). Oxidase enzyme activity is associated with breaking down high molecular weight and polymorphic structures such as lignin, which require more enzymatic steps and have a higher activation energy (Silva et al., 2021; Sinsabaugh, 2010). It was hypothesized that the increase in labile carbon sources from cover crop root exudates and residue chemistry would lead to increases in labile carbon acquiring enzyme activity (BG and CBH), and a decrease in oxidase enzymes breaking down chemically recalcitrant, and more energy intensive carbon sources (McDaniel, Tiemann, et al., 2014; Mooshammer et al., 2022; Sinsabaugh, 2010; Tiemann et al., 2015; Zhang et al., 2021). Access to more labile carbon sources has been linked to greater microbial metabolic efficiency and greater SOM accumulation (Cotrufo et al., 2013; Kallenbach et al., 2015; Liang et al., 2017; Miltner et al., 2012; Tiemann et al., 2015). However oxidative enzyme activity does

not consistently decrease when comparing mixtures to monocultures (Curtright & Tiemann, 2021; Zhang et al., 2021) and there are cases when labile carbon in the soil environment increases oxidative enzyme activity (Phillips et al., 2011). The increase in oxidative enzyme activity may also have been driven by changes in microbial biomass (Moorhead et al., 2013; Mooshammer et al., 2022; Nannipieri et al., 1983). Enzyme activity is a result of microbial stoichiometry, soil nutrient availability, and microbial community activity (Allison & Vitousek, 2005; Sinsabaugh et al., 2008, 2014; Sinsabaugh & Moorhead, 1994; Waring et al., 2014)**.** This suggests that enzyme production is commonly, though not always related to microbial biomass (Mooshammer et al., 2022; Nannipieri et al., 1983). MBC on 10/18/2019 was 8.3% lower than the AR treatment, 10% lower than the CR treatment, 7.8% lower than the HV treatment, and 4.6% lower than the AR + HV mixture (Figure 20). Therefore the lower oxidative activity in the control may have been due to overall lower enzyme activities in the control caused by lower overall microbial activity, represented by MBC.

Across all sampling dates, the AWP had moderately lower MBC (4.3 %, Figure 5)) than the control while the HV monocultures had significantly lower microbial biomass carbon than the control (6.0% lower, Figure 5). MBC is generally associated with increased microbial activity and increased nutrient cycling (Cheng & Kuzyakov, 2015; Hartman & Richardson, 2013; Jian et al., 2016; Moorhead et al., 2013; Phillips et al., 2011; Sinsabaugh et al., 2008, 2016; Waring et al., 2014) although this is not always the case (Tiemann & Billings, 2011). It was expected that treatments with cover crops would result in higher MBC and MBN, due to higher quality plant residues, a diversity of plant residues, and increased proportion of the year with living plants in the ground (Eisenhauer et al., 2010; McDaniel, Tiemann, et al., 2014; Zak et al., 2003). In addition, there was no significant effect of cover crops on MBN (Table 12). Legumes

have higher nitrogen content than non-legumes, leading to rapid decomposition and increased microbial activity (Thapa et al., 2021). High N content and favorable substrate chemistry of cover crops drive increases in microbial carbon use efficiency and therefore increased microbial demand for N acquisition (Kallenbach et al., 2015, 2016). Therefore, legume cover crops were expected to drive increases in MBN. The lack of legume effects on MBN, whether in mixtures or as monocultures, contradicts the first hypothesis because it suggests there was N limitation in the system. The lack of interaction between cover crop treatment and MBN may also be due to the large amount of fertilizers applied during the growing season, which has been shown to both decouple microbially-mediated nitrogen cycling (Blesh, 2019; Daly et al., 2021; Gardner & Drinkwater, 2009; Recous et al., 2019; Tiemann et al., 2015) and obscure cover crop effects (Barel et al., 2018).

In partial support of Hypothesis 1, all cover crop treatments, with the exception of the CR – AWP mixture and the four cover crop mixture, had significantly higher EON than the control on the 11/9/2016 sampling date (Figure 4). Legumes and grasses may contribute to increase EON in different ways (Blesh, 2018; Finney & Kaye, 2017; Lin et al., 2011). Legumes may contribute to EON by fixing N and increasing N availability in the soil (Blesh, 2019; Hayden et al., 2014; Herridge et al., 2008; Rees et al., 2005), while grasses may temporarily immobilize N and prevent N losses, until the N becomes available to microorganisms as root exudates or plant residues (Hayden et al., 2014; O'Connell et al., 2015). These results partially support Hypothesis 1, as different treatments composed of monocultures of grasses, monocultures of legumes, and mixtures all resulted in increased EON compared to the control. However, these results also indicate significant differences between cover crop mixtures, as the CR – AWP mixture and the four cover crop mixture had no significant effect on EON levels on 11/9/2016 (Figure 4).

The CR cover crop, HV cover crop, AR and HV mixture, and the four cover crop mixture all had significantly lower soil NO_3^- values than the control on 10/12/2016, following a potato (Figure 2; potato harvests in 2016, 2018, and 2020; Table 4).

Lower soil NO_3^- values found on a post-harvest sampling date support Hypothesis 1, which predicted that cover crops, especially grass species, would scavenge excess N and retain it through the winter. However the CR and AWP mixture had marginally higher soil NO_3^- values than the control on 9/1/017 and 11/13/2017, following a corn harvest (Figure 2; corn harvest in 2017 and 2019; Table 3). The significantly greater soil NO_3^- levels of the CR - AWP mixture may indicate a lack of N immobilization by the CR or an abundance of N addition by the leguminous AWP (Blesh, 2018; Schipanski & Drinkwater, 2012).

All cover crop treatments had significantly greater belowground biomass compared to the control (Table 20, Figure 13, and Figure 14). The AR monoculture had the highest belowground biomass (12.76 g/kg soil), which was 1597.49% greater than the control, and more than twice as much biomass as the second highest cover crop treatment (AR + HV mixture, 6.3 g/kg soil, Table 20, Figure 14). The increased quantity and duration of the year with living roots was expected to increase rhizosphere-microbe interactions, including plant root exudates which provide labile C substrates for microbial growth (Bowsher et al., 2018; Kong & Six, 2012; Liu et al., 2022; Wang et al., 2021). Higher root biomass may have partially driven the significant differences in labile C acquisition enzyme activity reported between the control and treatments of AR + HV mixture, CR + AWP mixture, AWP monoculture.

In support of Hypothesis 1, I found significant evidence of cover crop effects on nutrient provisioning, including increased EEA associated with labile C, N, and P acquisition, increased EON, and reduced soil NO_3^-. However, the effects of cover crop treatments compared to the

control were not uniform. While some metrics of nutrient provisioning were significantly different from the control across all cover crop treatments, many metrics were affected by only some of the cover crop treatments. Specifically, the annual rye and HV mixture had significantly higher BG enzyme activity (24 % higher) compared to the control ($P<0.0792$, Table 13, Figure 7), while no other monoculture or mixture had significant differences compared to the control. The annual rye-HV mixture also had moderately higher NAG enzyme activity (5.7 % higher) compared to the control ($P<0.0603$).

The significant positive interactions between cover crop treatments and labile C acquiring enzyme activity (BG and CBH), as well as the significant positive interaction between cover crop treatments and P acquiring enzyme activity, both indicate that the addition of a cover crop may significantly increase microbially mediated nutrient cycling, in support of the first hypothesis (Figure 7 and Figure 8). Significant positive interactions on specific sampling dates between some cover crop treatments and levels of EON also provide partial support for the first hypothesis.

However, the lack of uniformity across cover crop treatments indicates species and functional characteristics were important determinants of cover crop effectiveness. In addition, the efficacy of cover crop treatments was not entirely correlated with above or belowground biomass, suggesting that plant functional traits, such as N acquisition, root exudate quantity, root exudate diversity, or complementarity between plant functional groups, played a role in determining cover crop effects on microbially mediated nutrient cycling.

Hypothesis 2: Synergistic or facilitative interactions between grasses and legumes

I hypothesized there would be synergistic or facilitative interactions between grasses and legumes in paired combinations such that cover crop driven improvements in soil functioning

would be greater with cover crop pairs compared to monocultures (Blesh, 2018; Bukovsky-Reyes et al., 2019; Hayden et al., 2014; Maher et al., 2021; Schipanski & Drinkwater, 2011; White et al., 2017).

In support of the second hypothesis, the cover crop mixture of AR – HV had the most consistent effect on enzyme activities, with BG, CBH, and NAG enzyme activities significantly higher than the control (24% higher, 38.6% higher, and 5.7% higher, respectively, Figure 7, Figure 8, and Figure 10). While the AR monoculture may generally have greater belowground biomass compared to the AR – HV mixture (Table 20, Figure 14), the AR monoculture had no significant effects on BG, CBH, or NAG, (Table 13, Table 14, Table 16, Figure 7, Figure 8, and Figure 10). This may indicate that cover crop residue biochemistry, root exudate biochemistry, and N content in cover crop residue is more important than total crop residue in facilitating microbial activity (Finney et al., 2016). There may also be a facilitative interaction in the AR - HV mixture beyond simply belowground biomass (Blesh, 2018; Hooper & Dukes, 2004; Tilman et al., 2006; White et al., 2017). Combinations of two plant mixtures have been shown to produce synergistic rhizosphere microbial communities that are more than the sum of their parts (Taschen et al., 2017). For example, Pivato et. al (2017) found higher abundances of N cycling microbial communities in two plant species grown together compared to their constituent monocultures. This may explain why the AR – HV mixture had significantly higher labile C and N enzyme activities compared to the control while neither the HV monoculture nor the AR monoculture had significantly different enzyme activities in these categories. In addition, the AR – HV mixture had significantly higher PHOS enzyme activity than the control on both 11/3/2017 and 8/17/2018, while the constituent monocultures had significantly higher PHOS enzyme

activity only on 8/17/2018. These results indicate that a facilitative interaction may be taking place that increases microbial activity, supporting Hypothesis 2.

Furthermore, the AR – HV mixture had the highest EON levels on 11/9/2016, more than twice the EON of the HV monoculture and a third more EON than the AR monoculture (Figure 4). A combination of a grass and a legume cover crop was hypothesized to have complementarity effects: the higher N content of legumes is expected to increase microbial activity through a more energy efficient microbial decomposition process (Cotrufo et al., 2013; Schmidt et al., 2011), while the faster growth and larger root system of grasses is expected to help immobilize nitrogen during the growing season, as well as drive microbial activity through increased root exudation, increased root exudate diversity, and increased root exudate residence time in the soil after main crop harvest (Hayden et al., 2014; Liu et al., 2022; Wang et al., 2021). The presence of a cereal intercropped with a legume has also been shown to increase N fixation by the legume (Izaurralde et al., 1992; Johansen & Jensen, 1996). The high EON levels in the AR – HV mixture on 11/9/2016 support the second hypothesis, suggesting a facilitative interaction may be taking place that increases soil nitrogen, possibly through increased legume nitrogen fixation, favorable biochemistry of the grass-legume mixture, or the quality, quality, and diversity of their root exudates (Blesh, 2018; Blesh & Ying, 2020; Izaurralde et al., 1992; Johansen & Jensen, 1996; Kallenbach et al., 2015; Liu et al., 2022; Schipanski & Drinkwater, 2012; Steinauer et al., 2016; Thapa et al., 2021).

While the AR – HV mixture significantly affected enzyme activity across more categories than all four monocultures, the CR – AWP mixture did not affect enzyme activity any more significantly than the AWP monoculture (Table 13, Table 14, Table 15, Table 16, Table 17, Table 18, Figure 7, Figure 8, Figure 9, Figure 10, Figure 11, and Figure 12). This inconsistent

effect of legume grass mixtures may be due to the due to the competitive and facilitative interactions between cover crops unique to each cover crop mixture (Blesh, 2018; Blesh & Ying, 2020; Schipanski & Drinkwater, 2011, 2012). In 2016, the AR – HV mixture had 249% higher belowground biomass than the CR – AWP mixture, which if this trend held from year-to-year, may have contributed to the differences in enzyme effects (Table 20). While the biochemistry of cover crop residues matters as much as total biomass (Finney et al., 2016), higher cover crop residue biomass with similar N content may facilitate microbial activity through more energy efficient nutrient uptake (Thapa et al., 2021). The seeding rate for the AR – HV mixture was 150% of the constituent monocultures, while the seeding rate for the CR – AWP mixture was 125% of the constituent monocultures, potentially influencing total belowground biomass and plant productivity (Table 5). However, the differences between grass-legume mixtures may also have been due to either greater facilitative interactions between the AR – HV mixture, or greater competitive interactions in the CR + AWP mixture. For example, quicker growth of AR compared to CR may have provided an advantage to the HV, such as improved temperature moderation to increase winter survival, increased plant architecture for HV growth, or increased competition for N, stimulating HV N fixation (Brainard et al., 2012; Bukovsky-Reyes et al., 2019; Hayden et al., 2014; Izaurralde et al., 1992; Maher et al., 2021; Tribouillois et al., 2016).

Overall, there was partial support for facilitative or synergistic interactions between cover crop mixtures. The AR – HV mixture had a significant effect on more categories of enzyme activity than either of its constituent monocultures and had higher EON levels than any other cover crop mixture (Figure 4, Figure 7, Figure 8, Figure 9, Figure 10, Figure 11, Figure 12). However, grass - legume mixtures were not uniformly more effective than monocultures. In contrast to the AR – HV mixture, the CR – AWP mixture did not have significantly more

enzyme activity in any category when compared to its constituent monocultures. Both the CR monoculture and the AWP monoculture had higher EON levels than the control on 11/9/2016 (70.3% and 54.8% higher than the control, respectively). However, there was no significant difference in EON between the CR – AWP mixture and the control, suggesting that the cover crop mixture was less successful than either of its constituent monocultures. Furthermore, the only cover crop treatments to reduce soil NO_3^- levels, indicating NO_3^- immobilization and conservation, were the CR monoculture and the HV monoculture, while the CR – AWP mixture resulted in higher soil NO_3^- values. Overall, there was a partial support for Hypothesis 2. The AR – HV mixture increased enzyme activity related to labile C and N compared to its constituent monocultures, however the CR – AWP mixture had less of an impact on soil microbial functions than its constituent monocultures. This could potentially be due to species level interactions in cover crop mixtures, or due to the significance of other functional traits beyond N fixation and N conservation, such as root architecture, plant growth characteristics, seasonal hardiness, or additional rhizosphere interactions involving root exudates and microbial communities (Bakker et al., 2014; Bardgett & Van Der Putten, 2014; Lavorel, 2013; Lynch, 1995; Steinauer et al., 2016; Turnbull et al., 2013).

Hypothesis 3: Functional diversity vs species diversity

I hypothesized that a mixture of four cover crops, two grasses and two legumes, would not increase soil functioning as compared to the paired mixtures of one legume and one grass, due to competition between the functionally similar grasses and legumes (Blesh et al., 2013; Dini-Andreote & van Elsas, 2013; Hooper et al., 2005; Polley et al., 2013; Tilman et al., 1997). I predicted that the functional traits of legumes and grasses would be more important than the overall number of cover crops species, or that functional diversity would be more important than

overall species diversity (Blesh et al., 2013; de Vries & Bardgett, 2016; Díaz et al., 2003, 2007; Drinkwater & Snapp, 2007; Finney & Kaye, 2017; Garnier et al., 2016; Hooper et al., 2005).

The belowground biomass of the four cover crop mixture was 31.4% lower than the AR – HV mixture, and 139.3% higher than the CR – AWP mixture, suggesting neither facilitative or competitive interactions between the four plant mixture (Table 20, Figure 14). While both two cover crop mixtures had significantly higher CBH activity than the control (38.6% higher for the AR – HV mixture; 24.5% higher for the CR - AWP mixture), the four cover crop mixture did not result in any significant increases in CBH enzyme activity (Figure 8). This suggests some disadvantage in the four cover crop mixture compared to both two cover crop mixtures, supporting Hypothesis 3. Similarly, on the sampling date of 8/17/2018, the AR – HV mixture had significantly higher PHOS activity than the control (46.3% higher), as did the CR – AWP mixture (32.6% higher), while the four cover crop mixture had no significant difference in PHOS activity compared to the control (Figure 21). In addition, while on the sampling date of 11/9/2016 the AR – HV mixture had 91.2% higher EON than the control, there was no significance difference between the four cover crop mixture and the control (Figure 4).

These results indicate not only a lack of increased benefits from the four cover crop mixture compared to the cover crop mixtures of one grass and one legume, but possibly competitive interactions between the four cover crop species. Competitive interactions or decrease performance of the four cover crop mixture may be driven by the poor performance of a single cover crop, or possibly competition due to the functional redundancy of two grasses and two legumes (Blesh, 2018; Blesh et al., 2013; Blesh & Ying, 2020; McDaniel, Tiemann, et al., 2014; Smith et al., 2014; White et al., 2017)

CONCLUSION

In some cases, cover crops significantly increased EEA and EON, and decreased soil NO_3^- levels compared to control treatments, indicating increased microbial activity and microbially mediated nutrient cycling (Figure 2, Figure 4, Figure 5, Figure 6, Figure 7, Figure 8, and Figure 10). However, cover crops did not have a significant effect on MBN and had either negative or no effects on MBC (Figure 5 and Figure 6). Furthermore, all cover crop treatments were not significantly different from the control. The inconsistency of cover crop effects point to partial support for Hypothesis 1, that cover crop treatments will increase microbially mediated nutrient provisioning services.

The cover crop mixture of AR – HV had significantly higher EEA compared to the control in more enzyme categories (BG, CBH, NAG, PHOS) than any monoculture (Figure 7, Figure 8, Figure 10, and Figure 12). However, the CR – AWP mixture did not significantly increase EEA more than the AWP monoculture (Figure 7, Figure 8, Figure 9, Figure 10, Figure 11, and Figure 12). This suggests that certain cover crop mixtures may have facilitative interactions that provide increased ecosystem services beyond their constituent monocultures, but that this is dependent on both functional traits and plant species interactions, in partial support to Hypothesis 2.

The four cover crop mixture did not provide increased ecosystem services in any of the categories measured beyond either two cover crop mixture, in support of Hypothesis 3. This may indicate competitive interactions between plant species, perhaps due to overlapping functional traits (Blesh, 2018; Blesh et al., 2013; Blesh & Ying, 2020; McDaniel, Tiemann, et al., 2014; Smith et al., 2014). Taken together, these results highlight the importance of functional diversity

in cover crop mixtures, but also indicate that species level interactions may either facilitate or inhibit cover crop mixture effectiveness.

Several measurements taken, including MBN, EOC, and soil NH_4^+, did not change due to cover crop treatments. EEA is commonly associated with microbial community size (Thapa et al., 2021a), however cover crop effects may be first detected in soil EEA before becoming apparent in changes to microbial biomass (Liang et al., 2014). Changes to MBC and MBN may become apparent in subsequent years. Indeed, many studies of cover crops have shown that several years may be necessary to observe changes in soil functioning (Feng et al., 2021; Kim et al., 2020). Microbial biomass is also affected by sampling date and season (Liang et al., 2014). It is possible that the sampling dates chosen did not fully capture the effects of cover crops on microbial activity.

The lack of further interactions between cover crop treatments and microbial biomass may have been partially due to the intensive management of the soil, including plowing to 15- 20 cm with a deep chisel plow to plant potatoes every other year. Tillage redistributes plant residues, and therefore the cover crop effects on microbial biomass will be redistributed throughout the ploughed layer (Poeplau & Don, 2014). While prior to potato planting the fields were deep chisel plowed to a depth of 15-20 cm, soil sampling was from the top 10cm of soil, potentially only partially accounting for the full effect of cover crops on microbial biomass. Tillage impacts microbial activity by increasing oxygen diffusion, physically breaking apart residues, and increasing soil to residue contact (Nevins et al., 2020). Therefore, the intensive tillage in this potato – corn system may have significantly decreased microbial activity, and impacted measurements of microbial biomass. In a comparison of cover crop treatments with tillage and non-tillage treatments, studies have found higher microbial activity in the no-till

treatment (Frasier et al., 2016; Nevins et al., 2020). Furthermore, it is possible that tillage reduced the microbial activity over all treatments, diminishing the relative effect of cover crops, or elongating the timeline needed to observe treatment effects on response variables sensitive to tillage such as microbial biomass. In a five-year cover crop experiment on a silty clay loam, Nivelle et al. found that cereal-legume cover crop mixtures had significant effects in no-till and no fertilizer treatments, but that these effects disappeared entirely in conventionally tilled fields with high fertilizer applications (Nivelle et al., 2016).

Yield did not increase under cover crop treatments but was significantly higher in the control plot (Figure 15). Crop yield has been shown to take longer than other metrics of soil function to change under cover cropping, therefore cover crop treatments may increase yields in subsequent years (dos Santos Cordeiro et al., 2021). Conversely, cover crop treatments may been poorly timed with potato N requirements, or in combination with fertilizer, supplied an excess of N to potatoes, causing a yield decline (Stark & Porter, 2005).

The sandy loam soils at the Montcalm Research Center may respond slower to cover cropping effects than more silt and clay rich soils. I suggest that microbially mediated nutrient cycling may have been partially obscured by season, tillage, and fertilizer use, and that ecosystem services of nutrient provisioning may become more detectable over time. Given the sensitivity of potato systems to both excess and insufficient N, more research is needed on how cover crops influence the timing and quantity of N availability for farmers. Management recommendations should consider both cover crop functional diversity and species level interactions. Additional research would be helpful in elucidating the relationship between cover crop mixtures, plant functions, and microbially mediated nutrient cycling, in order to provide accurate fertilizer recommendations to complement cover crop effects on soil functions.

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APPENDIX A: TABLES

Table 1. Summary of precipitation (inches per month) recorded during the growing season at the Montcalm Research Center for 15 years

Year	April	May	June	July	August	September	Total
2016	2.25	2.77	1.33	3.42	5.35	3.05	18.17
2017	4.45	1.98	6.37	0.92	1.36	0.7	15.78
2018	2.04	5.51	3.64	1.19	7.73	2.65	22.76
2019	2.64	5.46	2.9	2.04	3.31	5.72	22.07
2020	3.49	4.75	1.4	4.07	2.21	3.12	19.04
5-Year Average	**2.974**	**4.094**	**3.128**	**2.328**	**3.992**	**3.048**	**19.564**

Table 2. Summary of average maximum and minimum temperature (°F) during the growing season at the Montcalm Research Center from 2006 to 2020

	April		May		June		July		August		Sept		Average	
Year	Mx	Mn	Mx	Mn	Mx	Mn	Mx	Mn	Mx	Mn	Mx	Mn	Mx	Mn
2016	53	32	70	45	78	53	82	60	85	60	78	54	74	51
2017	61	39	67	44	78	55	81	58	77	64	77	50	74	50
2018	55	33	81	46	84	58	88	64	84	63	76	52	78	53
2019	55	35	65	45	75	54	84	69	80	55	73	54	72	52
2020	56	29	76	35	77	54	81	68	78	60	70	48	73	49
5-Year Avg	56	34	72	43	78	55	83	64	81	60	75	52	74	51

Table 3. Agronomic practices for corn years: 2015, 2017, and 2019

Month	***Cultivation***	***Seeding, harvest***	***Fertilizer***	***Fungicide***
April	*Vertically disked to 2"*			
May		*34,000 seeds per acre; four row corn planter*	*100 lbs/ acre ammonium sulfate fertilizer (21-0-0-24S), 275 lbs/acre of dry granular fertilizer as urea (46-0-0), 1 quart/ton NDURE 2.0 nitrogen stabilizer*	*2.5 qt/acre Acuron (S-metolachlor 23.40%, Atrazine 10.93%, Mesotrione 2.60%, Bicyclopyrone 0.65%)*
June		*Cover crop seeds planted by hand*		
Sept		*Harvested with combine*		

Table 4. Agronomic practices for potato years: 2016, 2018, and 2020

Month	***Cultivation***	***Seeding, hilling, harvest***	***Fertilizer***	***Irrigation***	***Fungicide***	***Insecticide***
April	*Chisel plowed to 12", vertically disked to 2"*	*12" spacing within rows, 34" spacing between row; two row potato planter; (approximately 1,280 seeds per acre)*	*40 gallons/acre NPK, 50% (28-0-0) + 50% (10-34-0)*			
June		*Hilled*	*100 lbs/acre NPK as urea, dry granular fertilizer (46-0-0)*	*3.5"*	*32 oz/acre Echo 720*	*3.3 oz/acre Blackhawk*
July			*100 lbs/acre NPK as urea, dry granular fertilizer (46-0-0)*	*5.7"*	*2 lbs acre Mancozeb, 24 oz/acre Echo 720, 20 oz/acre Bravo,*	*9 oz/acre Besiege, 3 oz/acre Mustang Maxx*
Aug				*3.4"*	*2lbs acre Pencozeb Echo 720 48 oz/acre*	
Oct		*Harvested with a one row potato digger, Cover crop seeds planted by hand*				

Table 5. Cover crop mixtures and seeding rates

Treatment	***Seeding Rate (lbs/acre)***	***Percentage of Monoculture***
Control	*0*	*0%*
AR (AR)	*15*	*100% (monoculture)*
CR (CR)	*90*	*100% (monoculture)*
HV (HV)	*20*	*100% (monoculture)*
AWP	*70*	*100% (monoculture)*
AR + HV	*11.25 (AR) + 15 (HV)*	*75% + 75%*
CR + AWP	*45 (CR) + 52.5 (AWP)*	*50% + 75%*
AR + CR + HV + AWP	*7.5 (AR) + 22.5(CR) + 10(HV) + 35(AWP)*	*50% + 25% + 50% + 50%*

Table 6. Sampling dates for soil inorganic nitrogen, EOC, EON, MBC, MBN, EEA, root biomass, plant biomass, potato yield, and potato disease

Sampling Dates	***Soil nitrate, soil ammonium***	***EOC, EON***	***MBC, MBN***	***EEA***	***root biomass, plant biomass***	***potato yield, potato disease***
10/12/2016	*x*				*x*	
11/4/2016	*x*	*x*	*x*			
1/16/2017	*x*					
5/22/2017				*x*		
9/1/2017	*x*	*x*	*x*	*x*		
11/3/2017	*x*			*x*		
6/25/2018	*x*			*x*		
7/13/2018	*x*			*x*		
8/17/2018	*x*			*x*		
10/18/2019	*x*	*x*	*x*	*x*		
4/8/2019	*x*			*x*		
7/25/2019				*x*		
2/4/2020	*x*	*x*	*x*	*x*		
9/15/2020						*x*
10/7/2020	*x*	*x*	*x*	*x*		

Table 7. Type III ANOVA table of fixed effects for soil ammonium

Effect	df	F Value	P-value
treatment	*7*	*1.49*	*0.2032*
date	*10*	*20.96*	*<.0001*
*treatment*date*	*70*	*1.13*	*0.2596*

Table 8. Type III ANOVA table of fixed effects for soil nitrate

Effect	df	F Value	P-value
treatment	*7*	*2.72*	*0.0121*
date	*9*	*17.64*	*<.0001*
*treatment*date*	*63*	*1.90*	*0.0005*

Table 9. Type III ANOVA table of fixed effects for extractable organic carbon (EOC)

Effect	df	F Value	P-value
treatment	*7*	*0.89*	*0.5279*
date	*4*	*295.85*	*<.0001*
*treatment*date*	*28*	*0.57*	*0.9561*

Table 10. Type III ANOVA table of fixed effects for extractable organic nitrogen (EON)

Effect	df	F Value	P-value
treatment	*7*	*1.70*	*0.1478*
date	*4*	*103.92*	*<.0001*
*treatment*date*	*28*	*1.74*	*0.0260*

Table 11. Type III ANOVA table of fixed effects for microbial biomass carbon (MBC)

Effect	df	F Value	P-value
treatment	*7*	*2.21*	*0.0427*
date	*4*	*17.87*	*<.0001*
*treatment*date*	*28*	*0.96*	*0.5276*

Table 12. Type III ANOVA table of fixed effects for microbial biomass nitrogen (MBN)

Effect	df	F Value	P-value
treatment	7	1.66	0.1344
date	4	91.35	<.0001
treatment*date	28	1.10	0.3511

Table 13. Type III ANOVA table of fixed effects for BG extracellular enzyme activity

Effect	df	F Value	P-value
treatment	7	1.49	0.2423
date	10	17.3	<.0001
treatment*date	70	0.79	0.8013

Table 14. Type III ANOVA table of fixed effects for CBH extracellular enzyme activity

Effect	df	F Value	P-value
treatment	7	2.60	0.0291
date	10	16.38	<.0001
treatment*date	70	0.75	0.9215

Table 15. Type III ANOVA table of fixed effects for LAP extracellular enzyme activity

Effect	df	F Value	P-value
treatment	7	0.72	0.6557
date	10	239.24	<.0001
treatment*date	70	0.99	0.5251

Table 16. Type III ANOVA table of fixed effects for NAG extracellular enzyme activity

Effect	df	F Value	P-value
treatment	7	1.16	0.3538
date	9	10.71	<.0001
treatment*date	63	1.06	0.3820

17. Type III ANOVA table of fixed effects for OXIDASE extracellular enzyme activity

Effect	df	F Value	P-value
treatment	7	1.62	0.1937
date	9	239.5	<.0001
treatment*date	63	1.73	0.0698

Table 18. Type III ANOVA table of fixed effects for PHOS extracellular enzyme activity

Effect	***df***	***F Value***	***P-value***
treatment	*7*	*1.6*	*0.1750*
date	*10*	*32.71*	*<.0001*
*treatment*date*	*70*	*1.99*	*0.0001*

Table 19. Type III ANOVA table of fixed effects for above ground biomass

Effect	***df***	***F Value***	***P-value***
treatment	*6*	*13.61*	*<.0001*

Table 20. Cover crop aboveground and belowground biomass collected in fall of 2016; Potato yield measured in fall of 2020

	Aboveground biomass (kg/ha)	**Belowground biomass (g/kg soil)**	**Potato Yield (cwt/acre)**
No cover crop control	*N/A*	*0.75*	*226.6*
AR	*454.36*	*12.8*	*195.4*
CR	*321.96*	*4.89*	*201.4*
HV	*415.08*	*1.51*	*211.3*
AWP	*298.76*	*1.39*	*212.5*
AR and HV	*183.26*	*6.31*	*199*
CR and AWP	*202.18*	*1.81*	*199.7*
AR, CR, HV, AWP	*101.26*	*4.33*	*197.3*

Table 21. Type III ANOVA table of fixed effects for below ground biomass collected in fall of 2016

Effect	***df***	***F Value***	***P-value***
treatment	*7*	*2.88*	*0.0188*

Table 22. Type III ANOVA table of fixed effects for potato yield

Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
treatment	7	68	2.29	0.0369

APPENDIX B: FIGURES

Figure 1. Soil ammonium concentrations by cover crop treatments, across 11 sampling dates

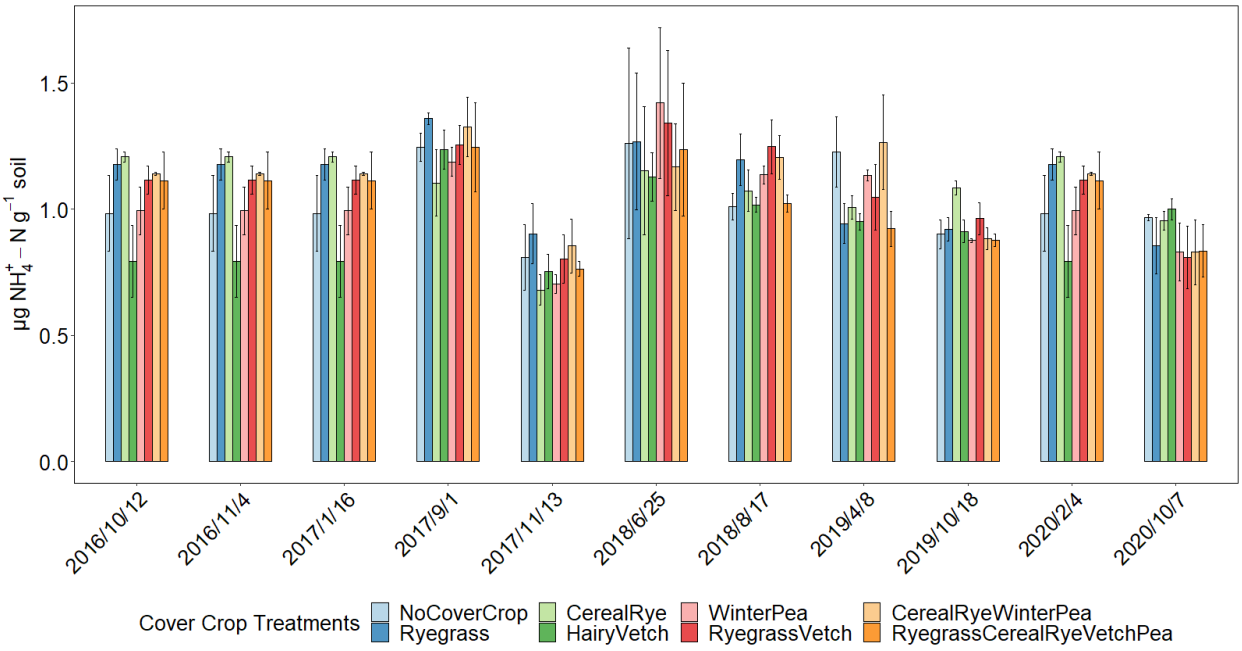

Figure 2. Soil nitrate concentrations by cover crop treatments, across 10 sampling dates

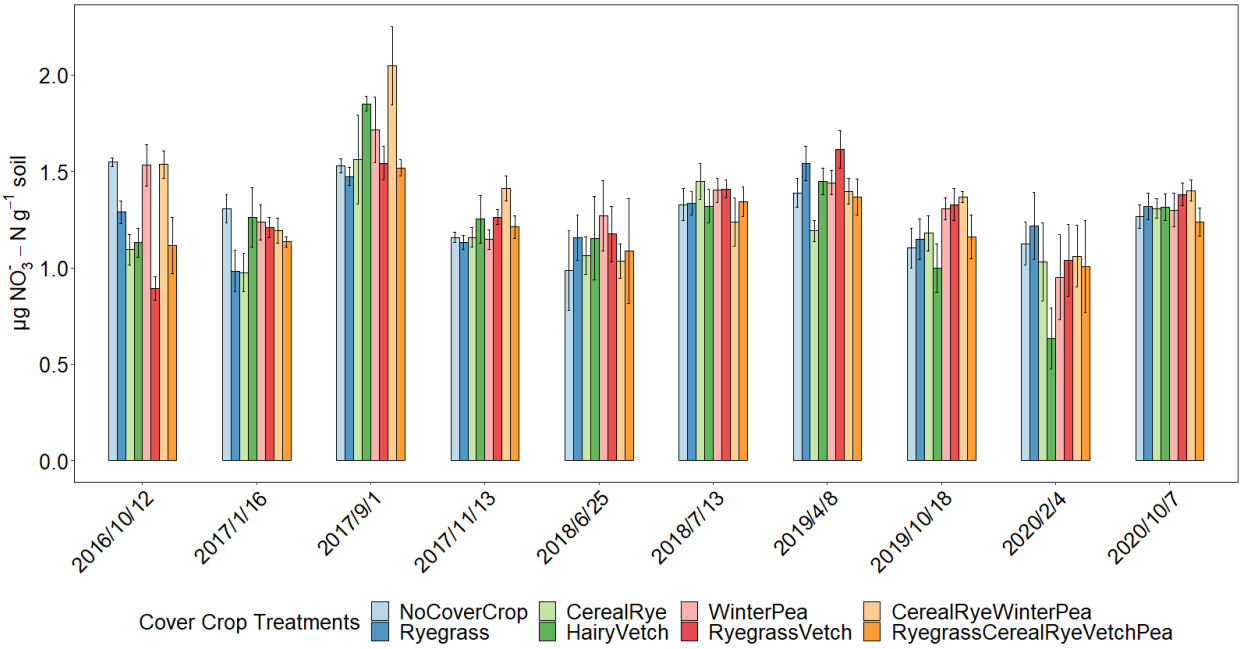

Figure 3. EOC by sampling date

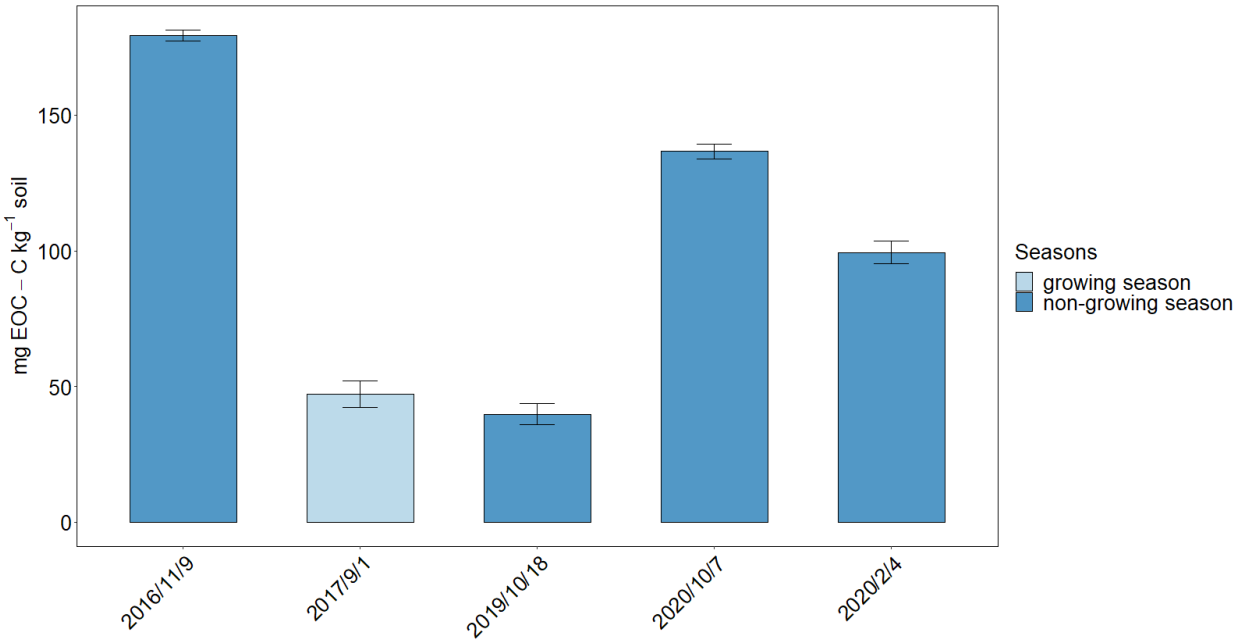

Figure 4. EON by sampling date and cover crop treatment

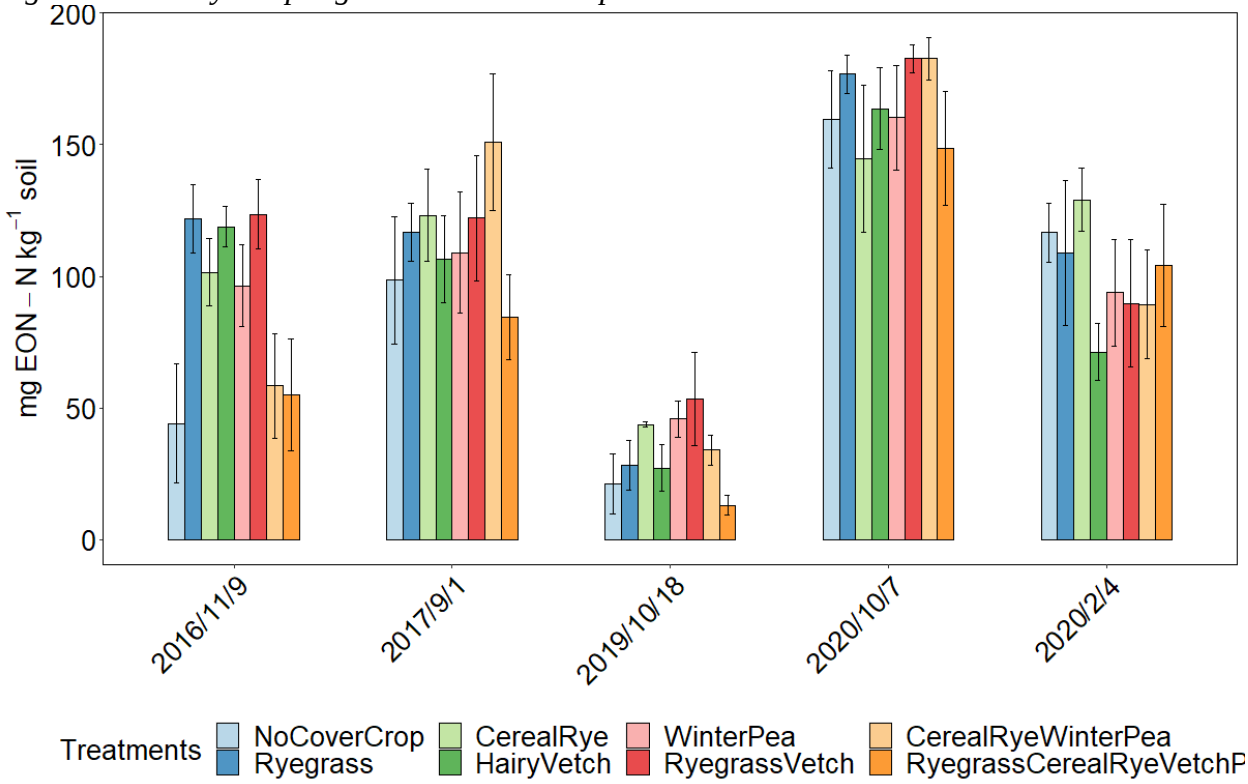

Figure 5. MBC by cover crop treatment

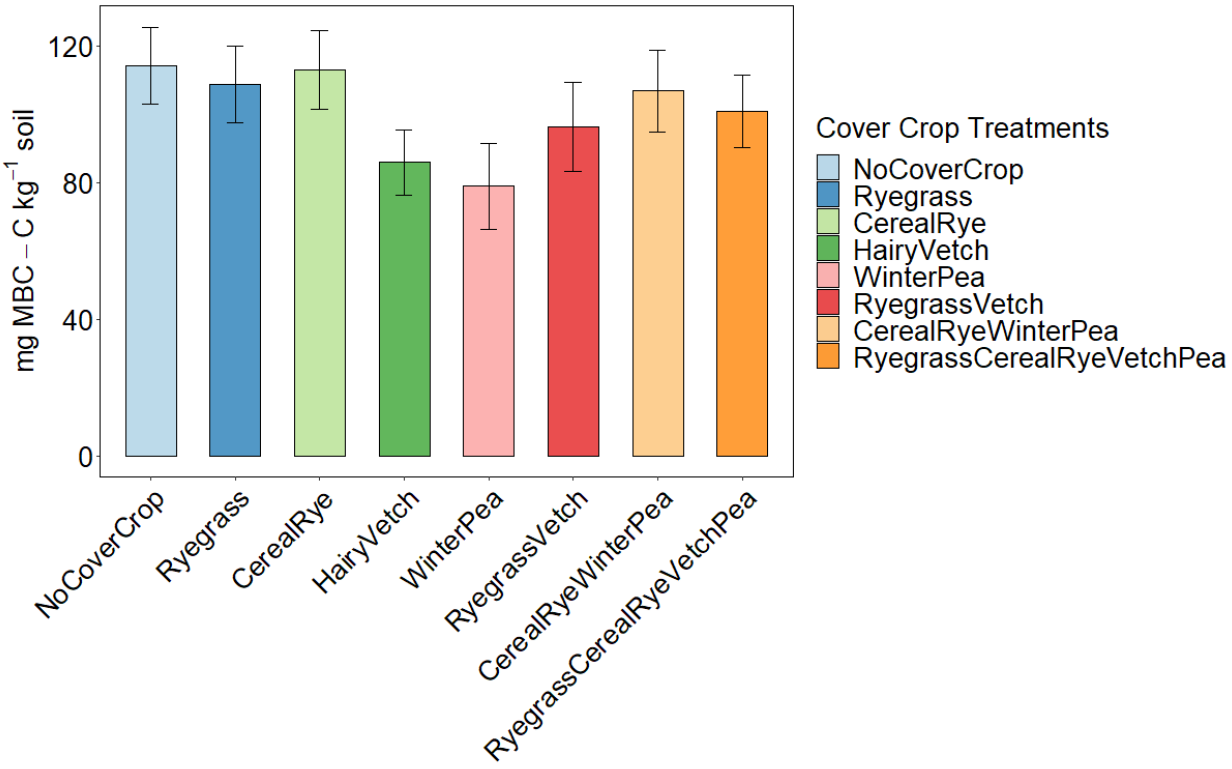

Figure 6. MBN by sampling date and season

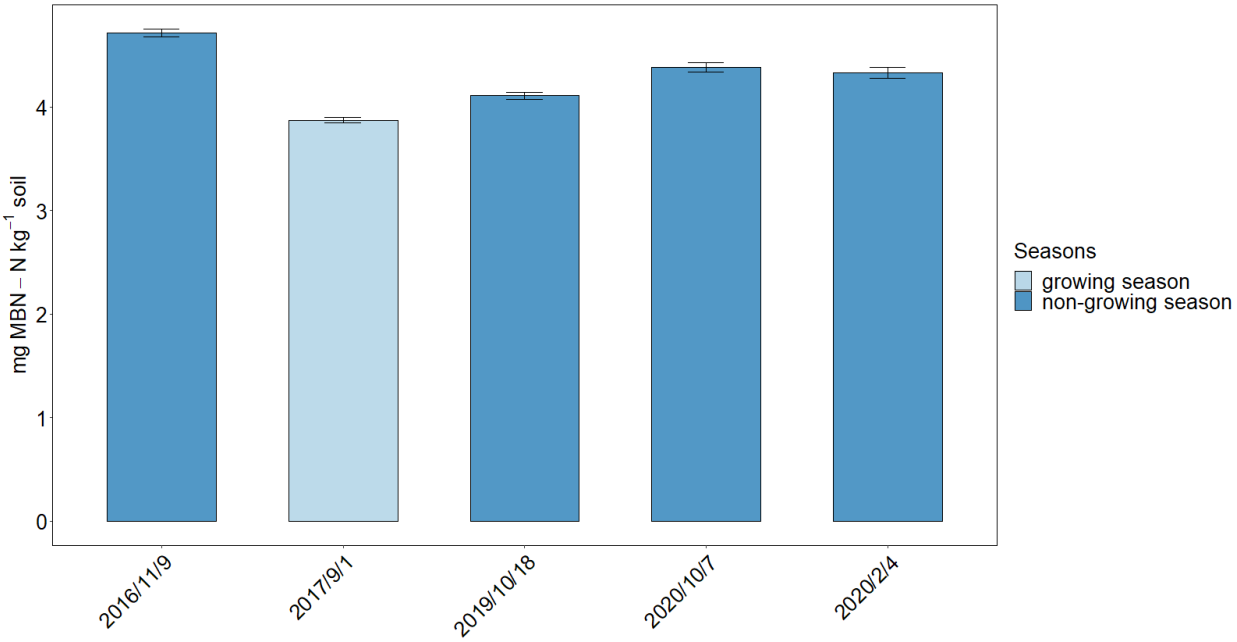

Figure 7. BG extracellular enzyme activity by cover crop treatment

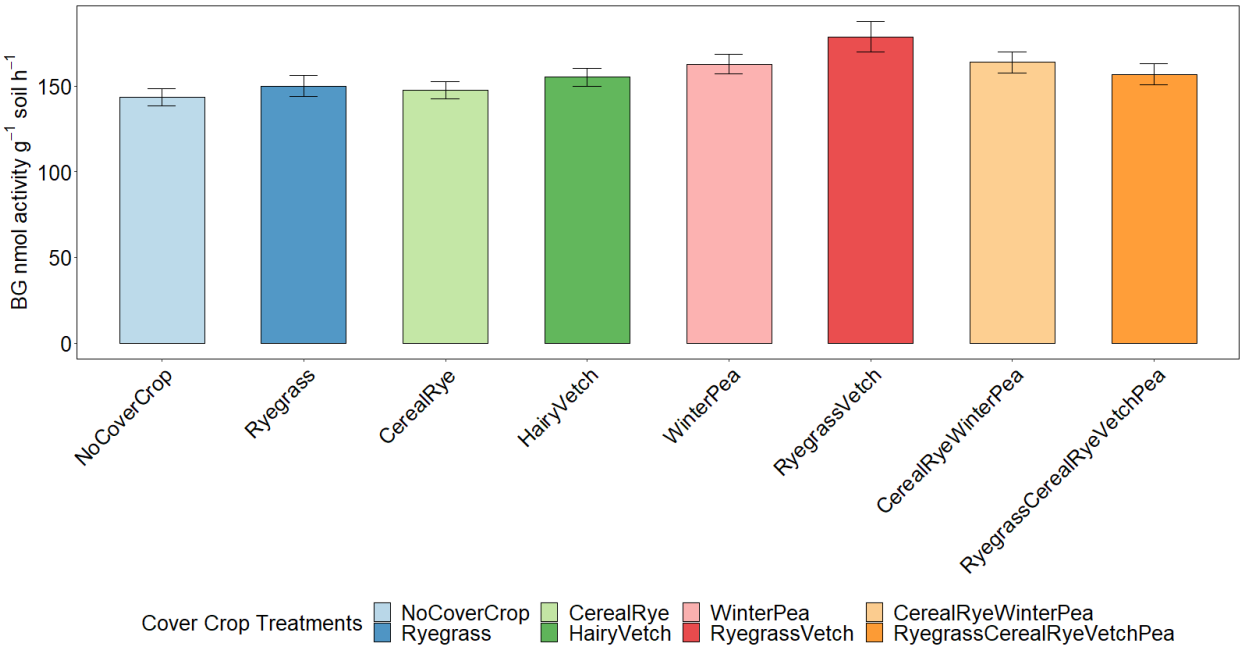

Figure 8. CBH extracellular enzyme activity by cover crop treatment

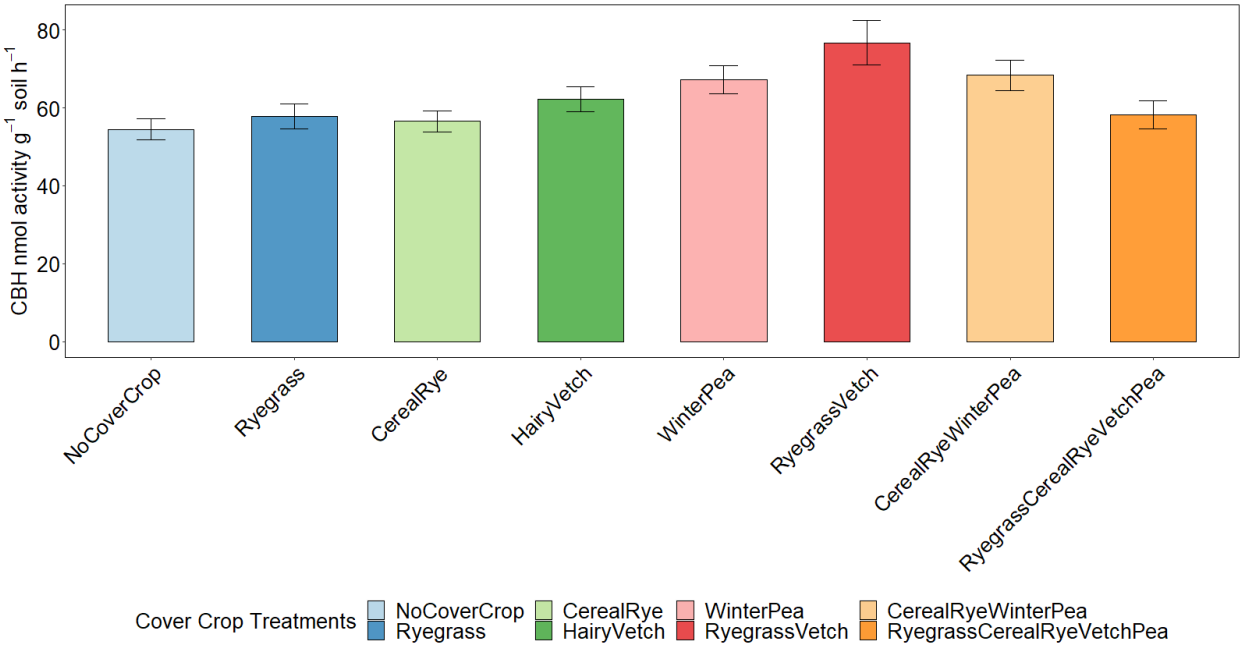

Figure 9. LAP extracellular enzyme activity by date and season

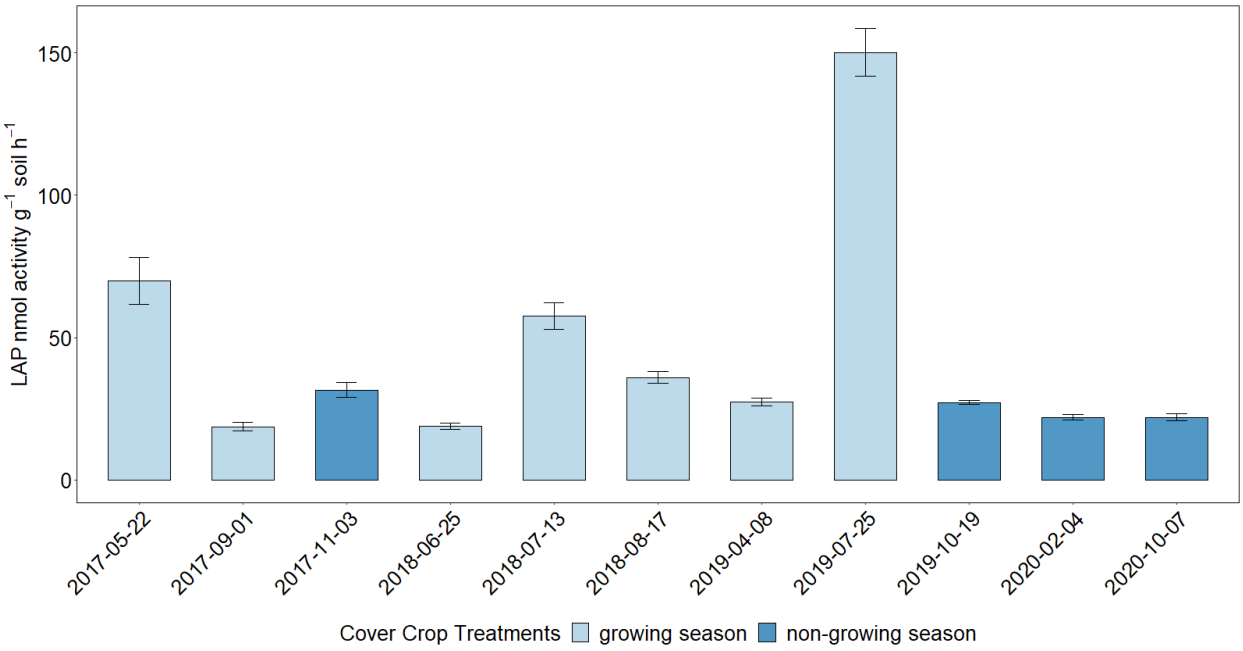

Figure 10. NAG extracellular enzyme activity by cover crop treatment

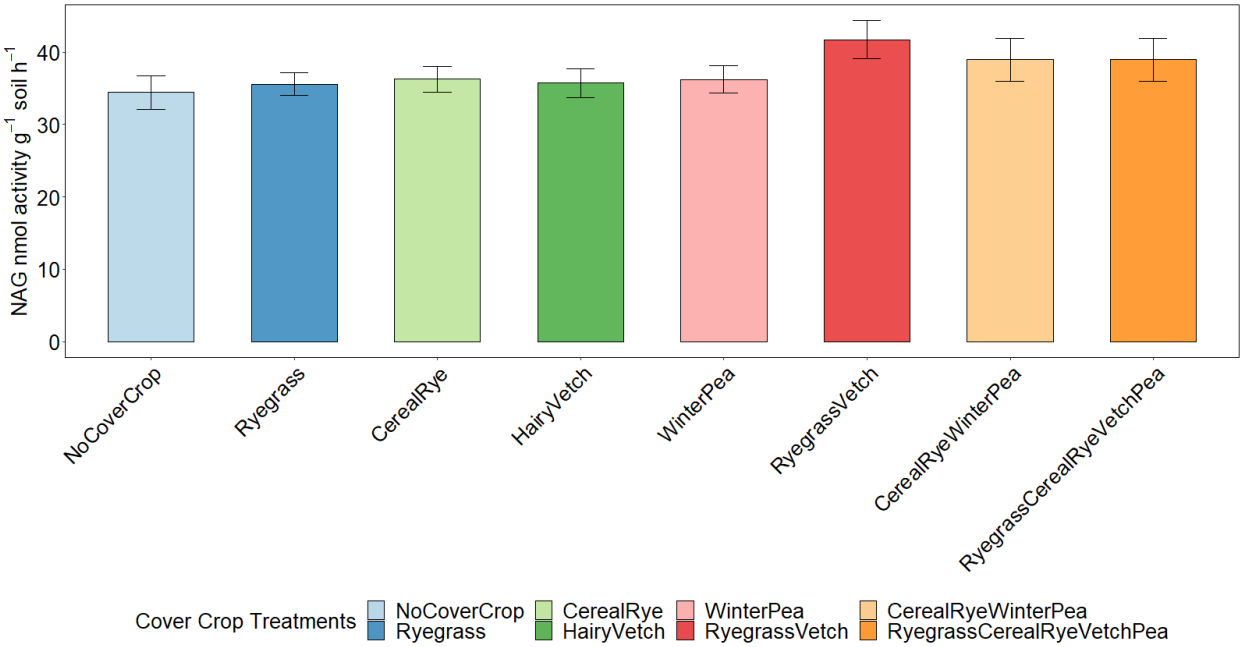

Figure 11. OXIDASE extracellular enzyme activity by sampling date and cover crop treatment

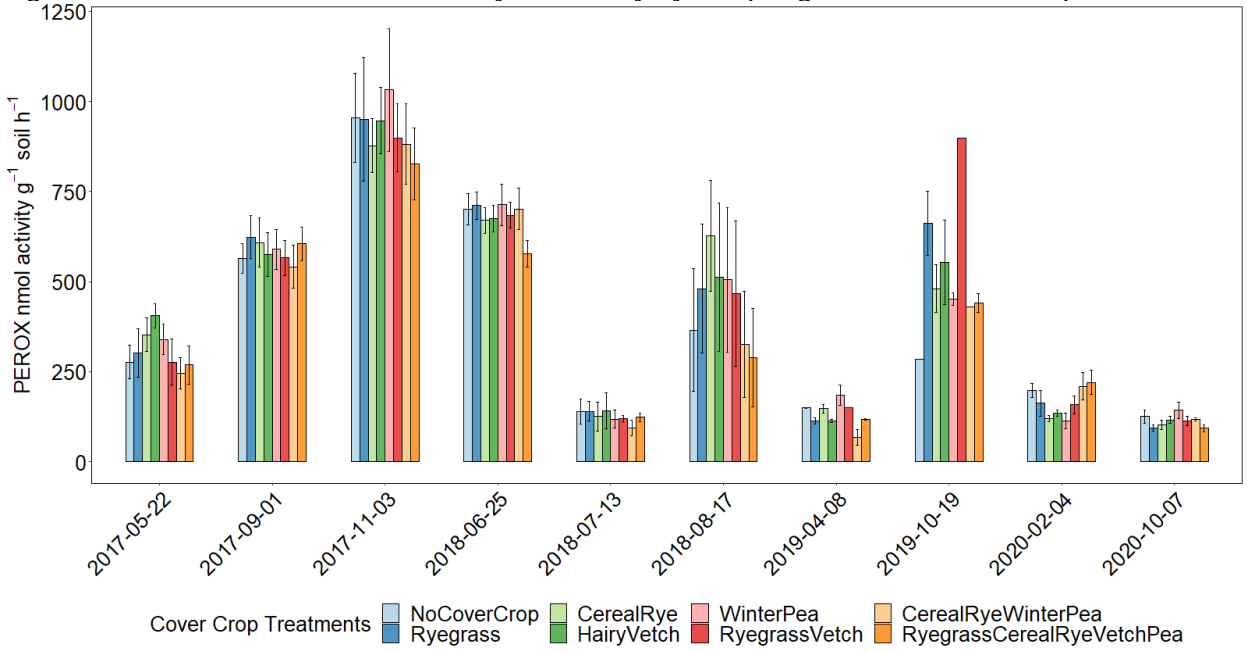

Figure 12. PHOS extracellular enzyme activity by cover crop treatment

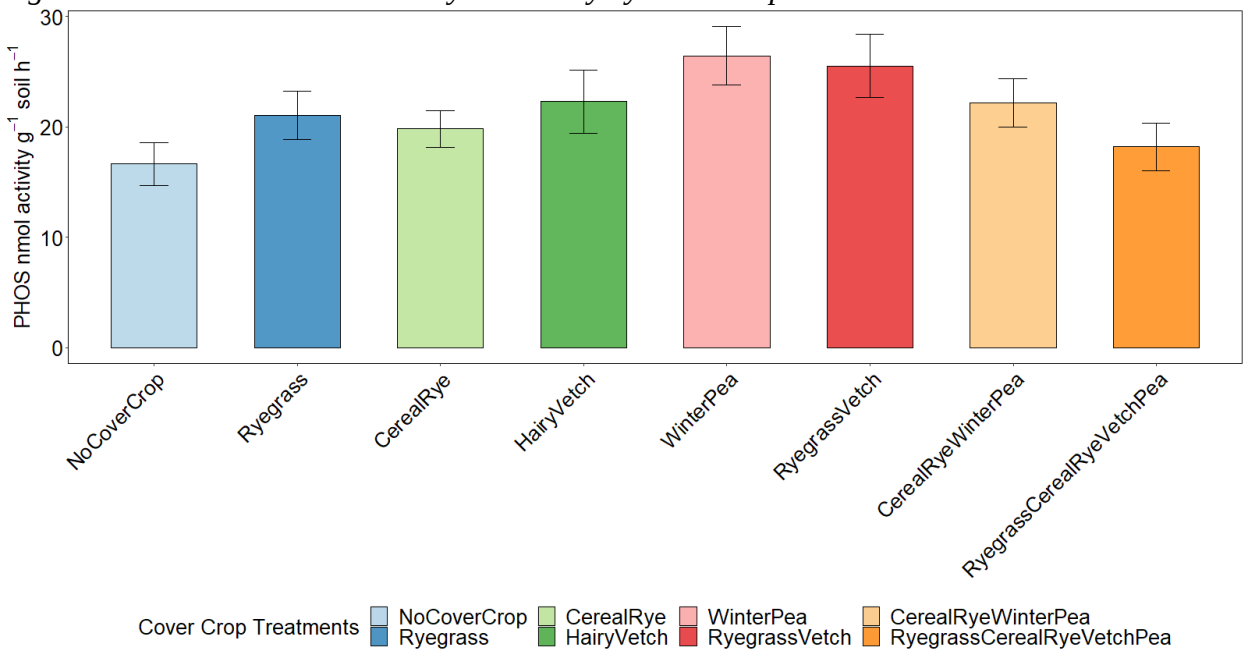

Figure 13. Cover crop above ground biomass in kilograms (kg) per hectare (ha)

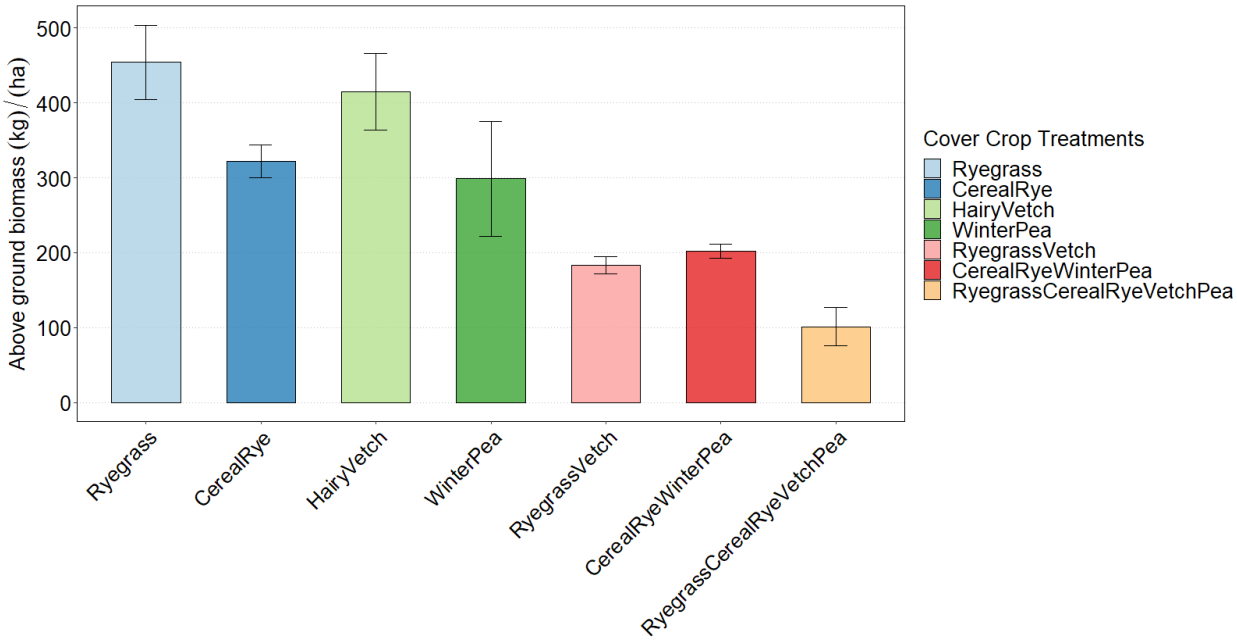

Figure 14. Cover crop below ground biomass in grams of below ground biomass per kilogram soil (g root/kg soil)

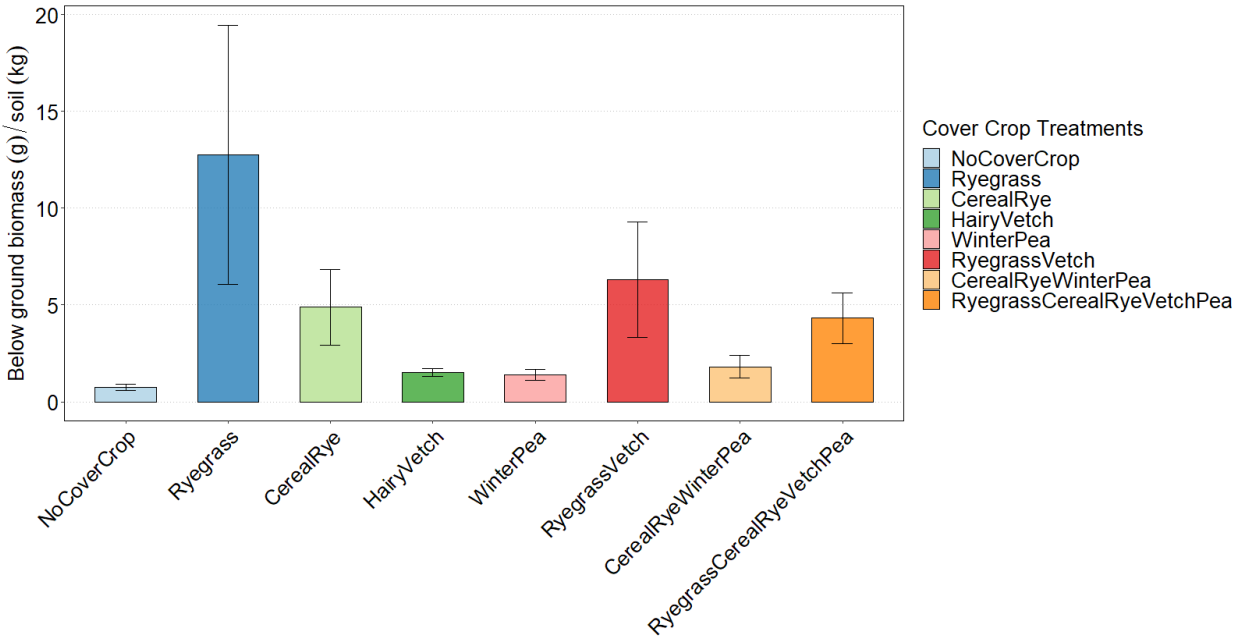

Figure 15. Potato yield in hundredweight (cwt) per acre

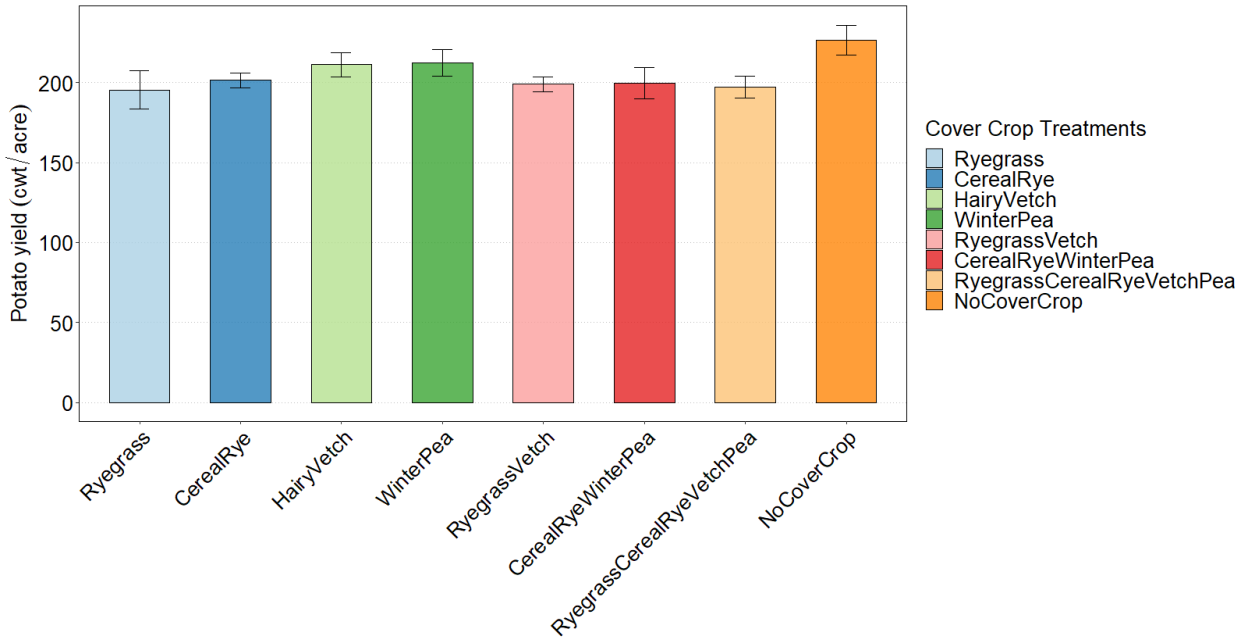

Figure 16. Potato scab rating averaged across treatment, rating both scab coverage and severity, with 0 being the lowest (none) and 5 being the highest

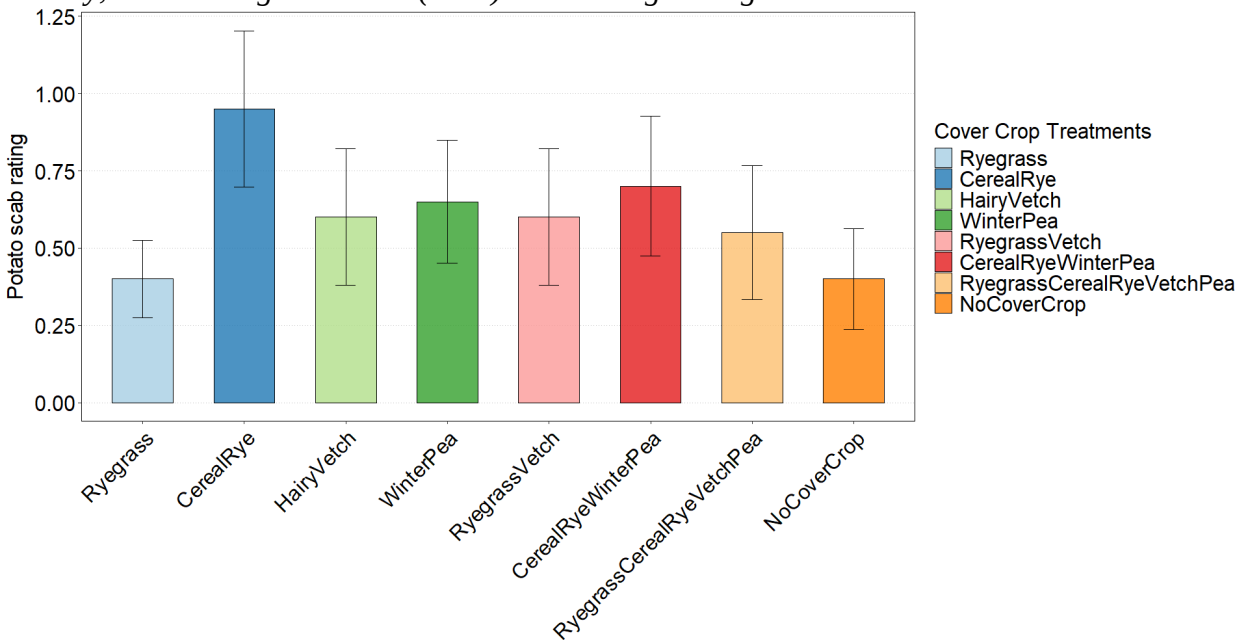

Figure 17. Potato hollow heart prevalence averaged across treatment, rating both coverage and severity, with 0 being the lowest (none) and 5 being the highest

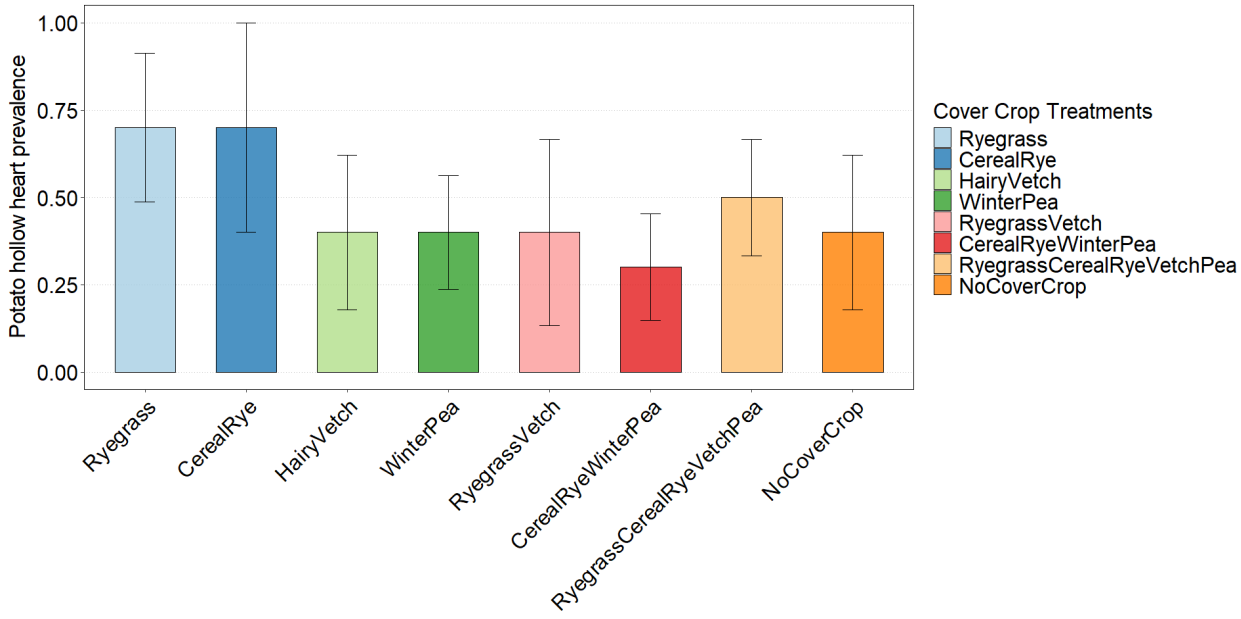

Figure 18. Potato brown center prevalence averaged across treatment, rating both coverage and severity, with 0 being the lowest (none) and 5 being the highest

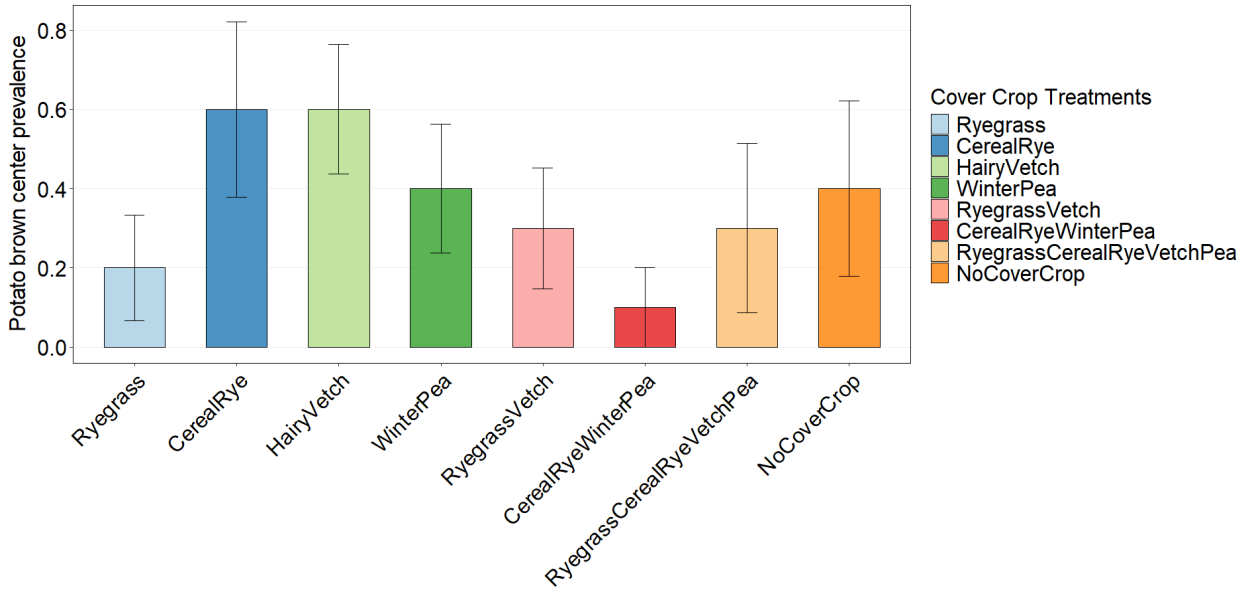

Figure 19. Potato viral disease prevalence averaged across treatment, rating both coverage and severity, with 0 being the lowest (none) and 5 being the highest

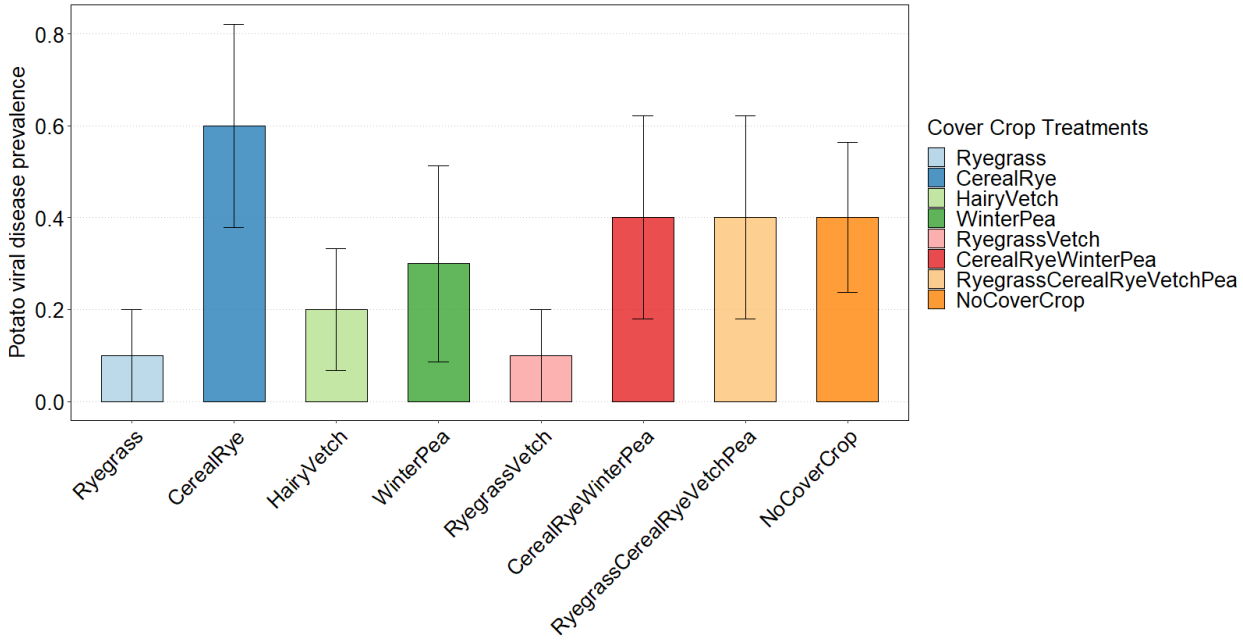

Figure 20. Microbial biomass carbon by sampling date

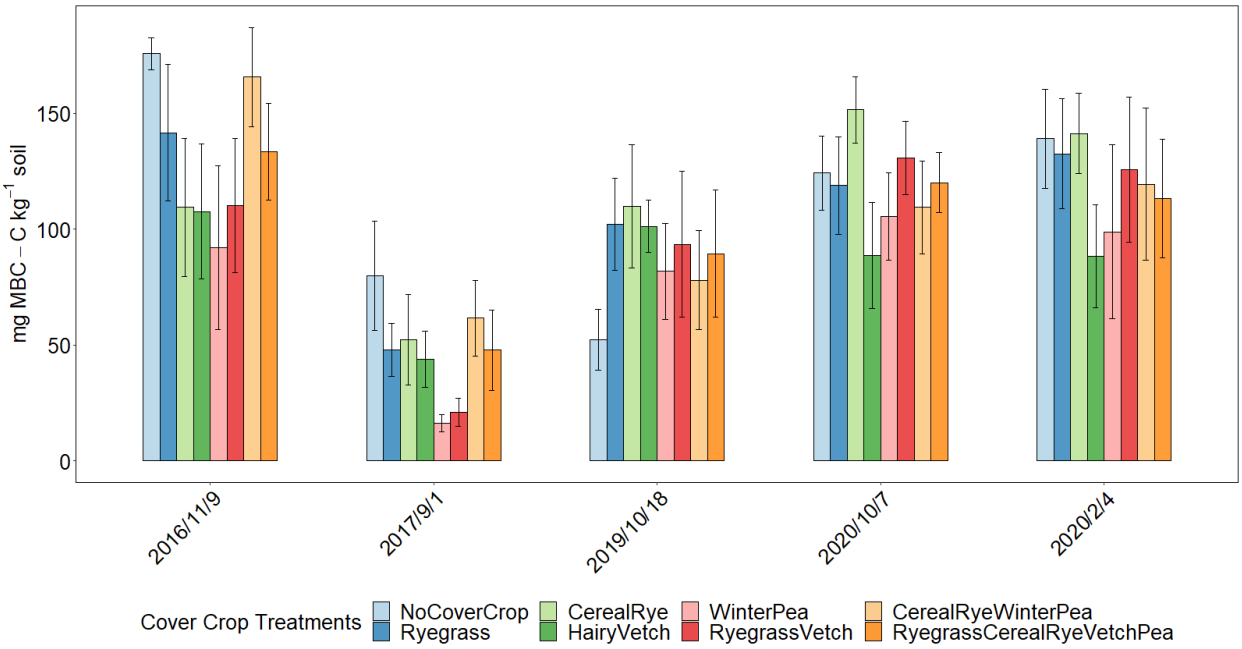

Figure 21. PHOS extracellular enzyme activity by cover crop treatment and date