Cover Crops and Cover Crop Mixes: Stratification of Biological Effects

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COVER CROPS AND COVER CROP MIXES:
STRATIFICATION OF BIOLOGICAL EFFECTS

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THESIS

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A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Agriculture, Food and Environment
at the University of Kentucky

By

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Lexington, Kentucky

Director: Dr. Mark S. Coyne, Professor of Soil Science

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ABSTRACT OF THESIS

COVER CROPS AND COVER CROP MIXES:
STRATIFICATION OF BIOLOGICAL EFFECTS

The potential nutrient cycling benefits from legumes (e.g. N_2-fixation) and the high biomass potential of cereal rye are well known. Further studies are warranted to evaluate bi-culture mixtures and their effects on soil nutrient stratification and microbial enzyme activity because these two properties may be differently expressed (enhanced) by legume/grass mixes. The objectives of this study were: (1) show different cover crops and cover crop mixes containing grasses and legumes differentially stratify carbon and N; (2) show the change in microbial enzyme activity in soils planted with individual cover crops relative to cover crop mixes; 3) determine the persistence of any changes after a summer annual crop. Baseline samples were collected in fall 2016 at 0-15 and 15-30 cm depths after a seasonal fallow and a summer maize crop. Cover crop mixes were planted in fall 2016, terminated in spring 2017, and a summer hemp crop (*Cannabis sativa*) planted. After cover crop termination and hemp harvest, soils were sampled at 0-7.5, 7.5-15, and 15-30 cm depths. Total C, total N, total P, mineralizable N, POX_c (labile C), and four soil enzymes (phosphatase, sulfatase, glucosidase, and urease) were evaluated. Stratification ratios decreased following cover crops. Cover crop mixes stratified mineralizable N deeper than legumes alone in five of six instances. Enzyme activity increased following cover crops, but there was little significance due to cover crop type. Cover crop mixtures did not significantly increase measured variables more than single species did. This study did not demonstrate an advantage to using either an individual grass or legume or mixture in terms of enhancing soil quality parameters.

KEYWORDS: Cereal rye; Enzyme activity; Hemp; Legumes; Mineralizable N;
            Microbial communities

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COVER CROPS AND COVER CROP MIXES: STRATIFICATION OF
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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1. INTRODUCTION

1.1   Cover Crops in Agriculture

Cover crops are typically non cash crops used to improve some aspect of an agroecosystem during the absence of a cash crop rather than allowing fields to remain fallow. Cover crops have various benefits that can improve environmental and economic aspects of agricultural production systems (Clark, 2007). Cover crops can improve water infiltration, nutrient management, and yields. They can also reduce fertilizer runoff, erosion, and herbicide use. Cover crops such as cereal rye (*Secale cereal*) take up residual nitrate (NO_3^-) and add soil organic carbon. Through symbiotic N fixation legume cover crops such as clover (*Trifolium sp.*) and vetches (*Vicia sp.*) add mineralizable N to the soil for subsequent crops (Brozyna et al., 2013).

Cover crop acreage has steadily increased the last five to ten years (Cover Crop Survey Report, 2014). As of 2012, the United States Department of Agriculture (USDA) reported approximately 4,163,721 hectares (10,280,793 acres) planted to cover crops, with most cover crop users planting 81 to 166 hectares (USDA, 2012). Corn (*Zea mays L.*) and soybean (*Glycine max*) rotations are common in the Midwestern US (USDA, 2012) and comprise the bulk of production systems that incorporate some form of cover crop component (Fig. 1). Small grains are often incorporated into corn and soybean rotations (Fig. 1).

Due to factors such as increased management effort, cost, lack of success in nutrient cycling, poor establishment, and seed availability, cover crops have not been as widely adopted as one might expect despite being widely studied. (Pantoja et al., 2016). Because of recent increases in fertilizer costs, a greater awareness of the soil environment, and

1

increased interest by consumers in organic alternatives (free of pesticides, synthetic fertilizers, etc.) producers have become more interested in cover crop management systems to meet soil nutrient requirements (Hoorman et al., 2009; Pantoja et al., 2015).

Conservation practices such as no-tillage are common on farms in Kentucky. Cover crops planted in continuous no-till give producers a method of adding organic matter to soil, break up compacted soils, and reduce soil erosion (Cover Crop Survey, 2014). Cover crops are most commonly used in continuous no-till farms (Fig. 2) where soil aeration is valued due to the increased risk of compaction, because cover crops can help remediate compaction. Improving organic matter content is often the ultimate goal for farmers. Almost 74% of cover crop users who responded to the 2014 Cover Crop Survey report said their goal was to add organic matter to the soil.
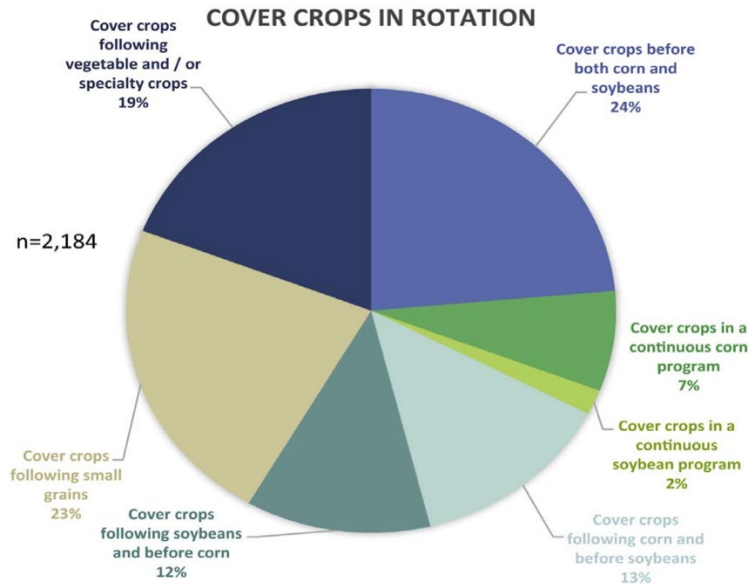
Figure 1. Type (and percent of respondents) of rotations used by farmers (Cover Crop Survey, 2014).
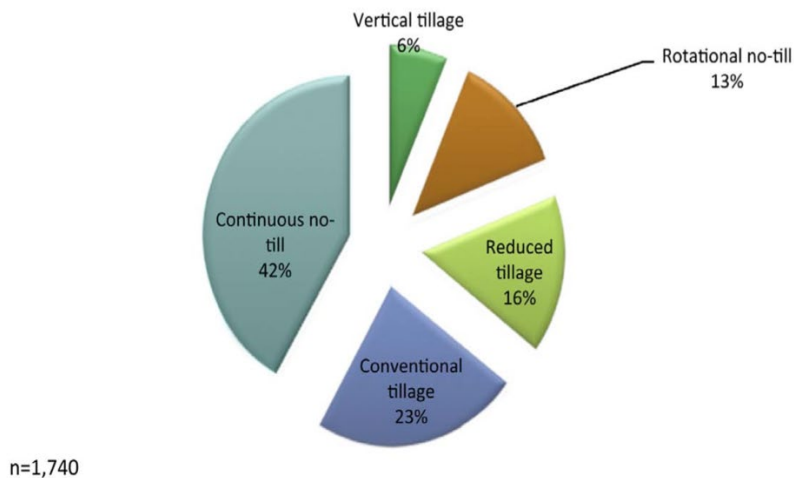
Figure 2. Tillage practices of cover crop users. 77% of all cover crop users employ some form of conservation tillage (Cover Crop Survey, 2014). Vertical tillage involves cutting the surface residue but no horizontal movement of soil to avoid burying the surface soil or residue and minimizing hardpan creation.

3

1.2   Cover Crops and Soil Health

Cover crop users typically plant one species in a field at a time. The most common species are grasses such as wheat (*Triticum aestivum*) and cereal rye (*Secale cereal*). Approximately 25% of cover crop users employ some sort of two-way mix when planting (Cover Crop Survey, 2014). The small proportion of farmers using cover crop mixtures is also representative of the literature, which lacks investigations on the effects of cover crop mixtures and their effect on soil health parameters.

The Natural Resources Conservation Service (NRCS) is a significant proponent of cover crop use. The NRCS National Soil Health and Sustainability Team advocates using cover crops as an important practice to improve soil health. The NRCS defines soil health as "the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans." Biological indicators of soil health include microbial biomass carbon (C) and nitrogen (N), mineralizable N, soil enzyme activity, and total organic C. Soils in agriculturally managed systems can become C-depleted approaching a 30-40% reduction compared to naturally vegetated soils (Poeplau et al., 2011), but cover crops can improve soil C sequestration (Table 1) (Hubbard et al., 2013).

Table 1. Soil organic C and N were significantly higher in cropping systems using cover crop rotations (from Hubbard et al., 2013).

Cropping system			Average 2003–2005[†]			Change 2003–2005[††]		
			Carbon (mg g^{-1})	Nitrogen (mg g^{-1})	C:N ratio	Carbon (mg g^{-1})	Nitrogen (mg g^{-1})	C:N ratio
A Sunn hemp	Crimson clover	Corn	7.25a	0.49a	15.4a	1.69	0.28[*]	−6.82[*]
B Sunn hemp	Fallow	Corn	7.24a	0.47a	15.9a	2.19[*]	0.34[*]	−7.50[*]
C Fallow	Crimson clover	Corn	5.48b	0.32b	17.9b	2.27[*]	0.30[*]	−8.36[*]
D Fallow	Fallow	Corn	5.07bc	0.28b	18.3b	2.06[*]	0.24[*]	−12.64[*]
E Fallow	Fallow	Fallow	4.69c	0.23c	20.6c	1.04	0.18[*]	−9.00[*]

[†]
 Where letters are different, means between treatments are significantly different at the 0.05 level.

[††]
 Change over time significant at the 0.05 level.

[*]
 Change over time significant at the 0.05 level.

Through soil C sequestration, cover crops can also reduce erodibility and reduce CO_2 emissions while increasing crop yield (Schipanski et al., 2014). Nitrogen from cover crops can improve soil health by increasing internal nutrient cycling in the soil ecosystem through processes such as N mineralization and N fixation (Schipanski et al., 2014). Enzyme activity can be used to measure or evaluate the intensity of biological and biochemical processes and is often one of the earlier indicators of changes in soil health (Tabatabai, 1996). Enzyme activity increases in continuous cover crop treatments

compared to fallow treatments; microbial biomass follows the same trend (Balota et al., 2014).

1.3   Overview of Common Cover Crops

Farmers often base their cover crop choices on availability, price, and long-term goals. Successful cover crop use in farming requires specific management practices and a dedicated timeline for harvesting, planting, and chemical application. In Kentucky, timely destruction of a cover crop is vital to the success of the subsequent cash crop. The cover crops listed below are species used in this study and represent cover crops commonly used by Kentucky farmers.

Cereal rye (*Secale cereal*): Cereal rye (rye) is the most commonly planted cover crop and is grown on millions of hectares annually (Clark, 2007). Rye has many benefits as a cover crop; one of the main reasons rye is so commonly used is because of its availability and low cost (Hayden et al., 2014). Rye has been the focus of many cover crop studies and is well known to positively affect agricultural soils and production. Most notably, rye is used to take up residual soil N (Chen and Weil, 2011). Rye is also used for its capacity to produce considerable biomass, suppress weeds, and serve as high quality forage (Duiker and Williams, 2005).

Hairy vetch *(Vicia villosa):* Hairy vetch is one of the most common legumes used to add N in cover crop systems. Few other legumes possess the same capacity to fix atmospheric N while producing as much surface residue (Clark, 2007). Due to the high amount of

viney biomass, hairy vetch can improve summer moisture retention in corn production

(Lichtenberg et al., 1994). Crop residue produced by hairy vetch can also improve winter

water recharge by creating macropores in the soil profile. Hairy vetch in grass mixes has

resulted in decreased surface ponding and soil crusting (Folorunso et al., 1992). Hairy

vetch also scavenges phorphorus; for example, vetch can acquire more residual soil

phosphorus than other legumes following poultry litter application (Clark, 2007).

Clover (*Trifolium)* species: Clovers represent many species used as cover crops. One of

the most common species is *Trifolium incarnatum,* or crimson clover. Crimson clover is a

good N source and a relatively high biomass producer that can be used for forage or

cover residue (Clark, 2007). Berseem clover (*Trifolium alexandrinum*) has been used in

Bermuda grass (*Cynodon dactylon*) forage production, resulting in a 3% yield increase

(Read et al., 2011). Red clover (*Trifolium pretense*) is a well-known forage plant in hay

production (Cover Crop Survey, 2014). Red clover is also commonly used as a cover

crop legume because red clover is readily available as seed and is relatively inexpensive

(Clark, 2007). Clovers offer N fixation as a benefit and also provide blooms that are used

by pollinators, therefore increasing biological diversity and beneficial insect presence

(McGraw and Smith, 1994).

Austrian Winter Pea *(Pisum sativum):* Field peas, similar to vetches, have trailing growth

habits and produce vining tendrils. The biomass produced from growth typically has a

C:N ratio below 15, and quickly decomposes to release available N. Austrian winter pea

is very cold hardy and grows rapidly in climates that are cool and moist (Clark, 2017).

1.4   Biological Activity and Stratification

Most soil microbial activity occurs within the surface 15 cm of the soil profile. In no-till systems, organic C and microbial biomass N tend to be more significantly stratified in the upper layers of the soil than in conventionally tilled systems (Balota et al., 2014). This is because the disturbance caused by tillage (in addition to mixing surface organic matter to deeper soil depths) results in organic matter oxidation and the degradation of soil aggregates, which are important structures that protect and mediate soil organic matter mineralization (Beare at al., 1994).

Balota et al. (2014) found that microbial biomass increased in winter cover crop treatments compared to the fallow treatment and that this increased enzyme activity (i.e. phosphatase and arylsulfatase) in the same soils. This study also found that after the long-term use of cover crops, organic C increased in no-till systems up to 126 % (62% in conventional tillage systems). This study did not focus on depth differences between treatments, but little significance was established between parameters measured and depth.

Organic N can be added to agricultural fields by incorporating plant biomass and leaf litter (Clark, 2007) and cover crops can produce large amounts of N-rich biomass. Mineralization is the process by which microorganisms produce inorganic N (in the form of NH_4^+) from organic N (Myrold and Bottomley, 2008). The multiple processes are mediated by biological activity that involves decomposing organic N compounds with microbial intracellular and extracellular enzymes (Myrold and Bottomley, 2008). High quality litter is often described as biomass that has a low C to N (C:N) ratio. A cover crop study with *Pueraria* showed that the high-quality leaf litter from the cover added soil

organic N, leading to a decrease in the soil C:N ratio, and an increase in net soil N mineralization (Pandey and Begum, 2010).

Litter that has a low C:N ratio decomposes faster than litter that has a high C:N ratio due to the greater availability of N which can be utilized to degrade organic matter. High C:N ratios result in immobilization of available N because there is insufficient N to meet microbial demand. Ratios of 15-30 are typical of grass species; ratios of 10-20 are typical of legume species (Balota et al., 2014).

Nutrient cycling properties are increased by cover crops and can help improve overall soil health. Nitrogen mineralization varies depending on cover crop species, biomass production, microbial community influence, and organic N input as factors influencing soil mineralization rate (Murungu et al., 2010). One can expect an increase in inorganic N from legume species and less so from grass species, which is due to the lower C:N ratio of legumes (Fig. 3).
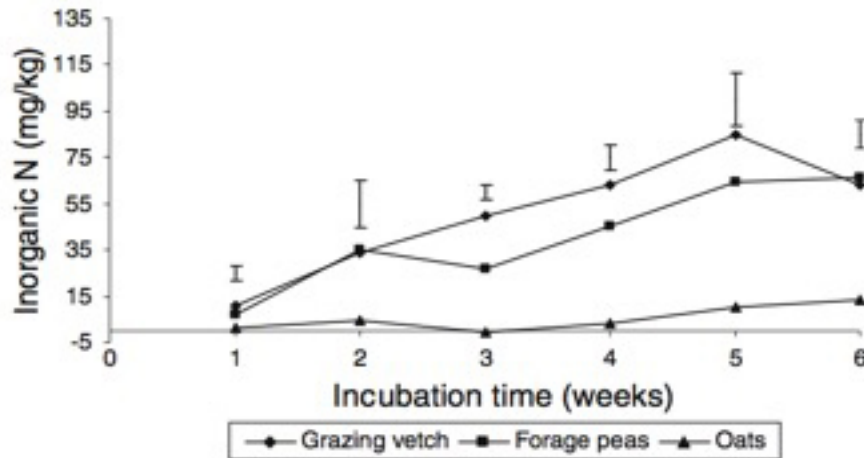
Figure 3. Inorganic N mineralized from various cover crops (Murungu et al., 2010). Vertical lines represent the standard error.

1.4.1　Enzymes in Agricultural Soils

Soil enzymes are an important aspect of soil nutrient cycling and can be used to indicate overall soil health. Soil enzymes respond to changes in soil management more rapidly than other soil health indicators, and can potentially be highly useful as indicators of biological shifts (Hai-Ming et al., 2014). Five soil enzymes dominate soil health studies: β -glucosidase, acid and alkaline phosphatase, arylsulfatase, and urease. β - glucosidase catalyzes the formation of glucose from cellulose, making β - glucosidase an important enzyme in the C cycle due to the role of glucose as an energy source for microbial metabolism (Tabatabai, 1996). Phosphatases release plant available inorganic P from organic P compounds. Acid and alkaline phosphatases are classified as such according to their optimum soil pH, with acid phosphatase being more active in soils with a pH below 7 and alkaline phosphatase being more active in soils with pH values higher

than 7. Acosta-Martinez et al. (2007) found that phosphatase activity increased in pasture soils, compared to cultivated soils due to the lack of tillage, increased root density, and availability of substrate. Arylsulfatase (sulfatase), catalyzes hydrolysis of organic sulfate esters, which releases plant-available SO_4^{2-} (Hai-Ming et al., 2014). Urease catalyzes urea hydrolysis to NH_3. Urease is widely distributed in soils and is produced by microorganisms, animals, and plants. Urease assays are used in many agricultural studies to evaluate and predict N mineralization in soils when there have been organic amendments or management changes (Dick, 2011).

In a study with cover crop residue management in rice fields, enzyme activity increased in all enzymes assayed due to the addition of organic matter from winter cover crop residue (Hai-Ming et al., 2014). This study also found that the differences in cover crop residues led to changes in microbial communities. For example, the number of fungi increased with Chinese milk vetch (*Atragalus sinicus L.).* Actinomycete numbers were also differentially affected by cover crop treatments (also increasing following Chinese milk vetch residue addition). This study shows that particular cover crop species can specifically influence microbial shifts. This warrants further investigation into the influences of cover crops and cover crop mixes on microbial communities because we do not know the specific shifts to expect or the processes that these changes affect.

1.4.2   Stratification of Soil Parameters

Stratification of microbial activity is well known in soils. For this thesis, stratification is defined as the arrangement and spatial separation of various soil properties by depth. Soil nutrient and organic matter stratification are widely observed;

particularly in no-till systems (Franzluebbers, 2002). Franzluebbers developed a method of assessing soil quality based on soil organic matter (SOM) stratification ratios. In this method, a ratio is created between the soil properties at 0-5 cm and the value of the same soil property at any other depth of interest. Franzleubbers found that in no-till systems there was a significant increase in soil organic C at the soil surface. Stratification ratios of potential C and N mineralization (based on 10-day aerobic mineralization) increased with greater cropping intensity, which was attributed to greater C inputs and reduced soil water availability that could be used for decomposition. Stratification ratio stresses the importance of soil biological indicators such as N and C mineralization due to the role that SOM plays in nutrient cycles, and emphasizes the importance of SOM and soil C at the soil surface.

For a study done in vineyard soils, Peregrina et al. (2014) found that covers with resident vegetation (several species of native grasses and forbs) had significantly more β-glucosidase and urease activity in the top 0-5 cm of soil than at lower depths. This resulted in significantly higher stratification ratios of the same soil properties when comparing the top 0-2.5 cm to depths below 5 cm. Conventionally tilled soils with no cover crop treatment had the lowest β-glucosidase and urease activities. Stratification ratios of these parameters in no-till systems under resident vegetation were always higher than ratios in conventional tillage and monoculture systems. Trends in microbial biomass C and soil organic C followed the same patterns, suggesting that a mixture or diverse planting of cover crop species beneficially influences soil biological properties.

My hypotheses will address the influence of cover crop mixes on biological activity. By evaluating significant differences in the level of stratification between

treatments and parameters, I will evaluate the effect of these mixes and individual species on biological activity at differing depths. I will try to use the stratification ratio as a measure of effect on differing soil depths. This has not been previously addressed in the literature.

1.5   Hypotheses

1.   Treatments containing a legume component will result in higher mineralizable N at lower soil depths than treatments not containing a legume.

2.   Cereal rye will deposit more C, deeper in the soil profile than legumes because of rye's ability to produce high amounts of biomass (Duiker and Williams 2005) and deeper root system.

3.   Enzyme activity will differ based on the cover crop treatment, as occurred in Hai-Ming et al. (2014).

4.   Treatments containing cover crop mixtures will have lower stratification ratios between depths than single species treatments, therefore indicating improved biological activity.

1.6   Specific Objectives

1.   Demonstrate the extent to which legume and non-legume cover crops and mixes cause differences in stratification of N, C, and enzyme activity.

2.   Determine if changes in biological activity persist after a period of summer hemp growth.

CHAPTER 2. MATERIALS AND METHODS

2.1   Site History and Characteristics

The plots were at the University of Kentucky's Maine Chance research farm in

Lexington KY (38° 7'16.20"N 84°29'11.86"W) on a Bluegrass Maury silt loam (70%

silt) with slope of approximately 6% (Soil Survey, 1968) (Figs. 4a,b). The Maury series

consists of well-drained upland soils that formed in material weathered from phosphatic

limestone. The top 0-36 cm are typically very friable silt loams, with the lower 36-97 cm

consisting of friable silty clay loams. The Maury series is medium to strongly acid and

has a high water holding capacity (Soil Survey, 1968). The plots have been in continuous

no-tillage for more than five years.

The site was previously planted with cover crops containing the same species

used for this study, but different treatment mixtures were used. Preliminary data

(Appendix 1) showed no significant differences in soil properties because of previous

research treatments. Prior to the start of this study, cover crops were present during

winter 2014-2015. No cover crop was present during winter 2015-2016. Maize (*Zea mays*

L.) was present during the 2015 and 2016 growing seasons.

2.2   Experiment Design and Management

The plots measured ~3.0 x 6.0 m and were separated by 2 m grass alleys (Fig. 5).

Individual plots were split in half along the short dimension to yield pairs of adjacent

plots 1.5 x 6.0 m long. The experiment design was a randomized complete block (RCBD)

with three replications. The cover crop species included cereal rye (CR), crimson clover

(C), hairy vetch (V), Austrian winter pea (P), and a weedy fallow as a control (CT).

Figure 4. Image of plots on North farm with soil series indicated in zones designated by

borders. The image dates to 2016 Web Soil Survey (https://websoilsurvey.nrcs.usda.gov).

Figure 4b. Soil series contained within the zones depicted in Figure 4a (From Web Soil Survey)
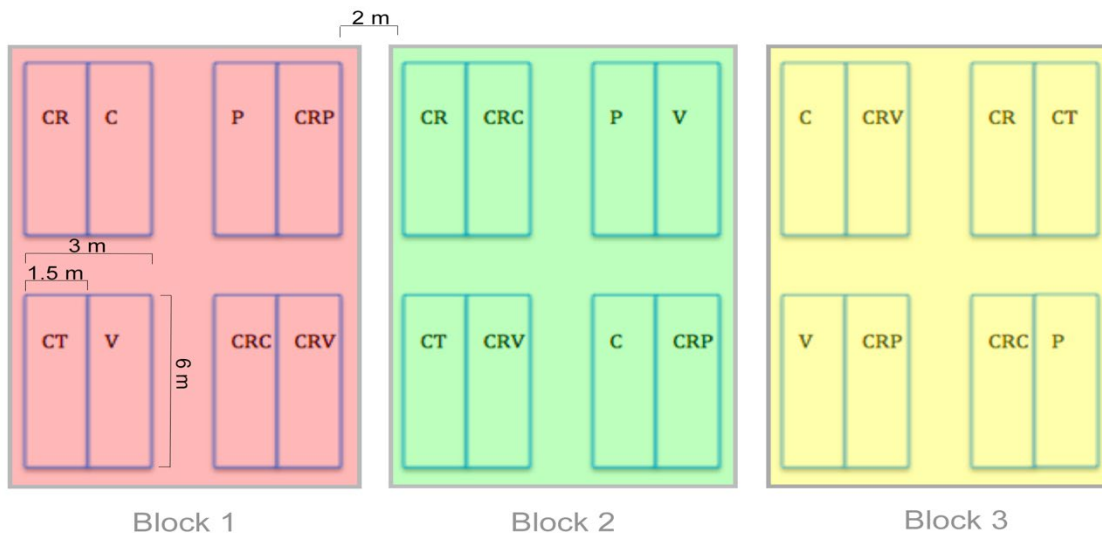
Figure 5. Treatment distribution and plot dimensions at the study site.

The cover crops were seeded by hand-broadcasting on 1 October 2016 at the following rates: cereal rye 0.118 kg plot^{-1}; clover 0.026 kg plot^{-1}; vetch 0.052 kg plot^{-1}; Austrian pea 0.110 kg plot^{-1}. The mix treatments were applied at a rate of half of each

individual species's rate (i.e. for a cereal rye/clover mix, 0.059 kg of cereal rye and 0.013 kg of clover were used). Prior to seeding, the summer annual maize residue was cut with a push mower and the residue retained on the plots. The cover crops were killed with glyphosate on 9 May 2016 by spraying a commercial mixture of Roundup® at a rate of 22 fl oz/A (1.6 L/Ha). On 18 May 2016 a mower was used to mulch the remaining residue, which was also retained on the plots.

Hemp variety "Santhica 27" was planted on 1 June 2016, using a Sukup seeder (Sheffield, IA). The hemp was planted at a rate of 45 kg ha^{-1} and drilled 0.64 cm (¼") into the soil. Urea fertilizer (46-0-0) was hand broadcast on plots at the time of seeding at a rate of 168 kg N ha^{-1}. Sod alleys and borders between the plots were continuously mowed throughout the year. See Table 2 for a complete timeline of plot management. The fall 2017 hemp harvest marked the end of my involvement with field activities for the purpose of this thesis.

2.3   Data Collection

2.3.1   Soil

Soil samples were collected three times during the study: prior to planting the summer annual maize in May 2016, after terminating the cover crop in May 2017, and after summer annual crop harvest (hemp) in September 2017. Samples collected in 2016 were taken at depths of 0-15 and 15-30 cm. Samples in 2017 were taken at depths of 0-7.5, 7.5-15, and 15-30 cm. In each case soil samples were removed with a 2.54 cm (1") soil probe and a composite sample was created from three different cores in each plot. The probe was marked at the three respective depth increments and soil from each

17

increment was collected into the composite for the corresponding depth, as marked on the soil probe. These samples were air dried and sieved through a 2 mm sieve to remove large clods and debris. Soil sampling following the termination of the cover crops was performed on 17 May 2017. Soil samples were similarly collected and processed on September 21 and 28 following the harvest of the hemp crop. All processed samples were stored at 4 C until analysis.

Table 2. Timeline of field management practices.

Season	2015	2016	2017
Spring	-Cover crops terminated	-Fallow	-Biomass harvested (5/02) -Cover crops terminated with glyphosate (5/06) -Soil sampled (5/17) -Hemp planted (5/31)
Summer	-Maize planted (no fertilizer applied)	-Maize planted (no fertilizer applied) -Chlorophyll and growth measured	- 168 kg N ha^{-1} as urea applied (6/11) - Hemp harvested (9/05)
Fall	-Maize harvested -No cover crops sown	-Maize Harvested -Soil samples taken (9/26) -Cover crops sown (10/1)	-Soil sampling (9/21, 9/28) - Hemp residue mowed (10/02) -Cover crops sown (10/12)
Winter	-Fallow	-Cover crops dormant	-Cover crops dormant

2.3.2   Cover Crop Biomass Sampling

Weed and cover crop biomass were reported on a dry weight basis. Part of each plot was harvested on 2 May 2017 to quantify total cover crop biomass. Samples from bi-culture mixtures were not separated because the total mixture as biomass was the focus of the study. Squares made of PVC pipe, 30.5 x 30.5 cm (1-foot square), were tossed randomly into each plot, brought to ground level, and all cover crop biomass above the PVC pipe collected (2.54 cm above soil surface). After collection, weed species were separated from each sample and the fresh cover crop and weed biomass were separately weighed for each plot. These samples were dried in an oven at 60 C for one week and reweighed. The dried samples were subsequently used to assess cover crop tissue nutrient content.

2.4   Measured Parameters

2.4.1   Cover Crop Tissue Analysis

Dried plant material harvested for cover crop biomass was analyzed for total N and P. The material was ground to a fine powder (1 mm) with a UDY mill (UDY Corporation, Fort Collins, CO). One hundred mg of the ground material was used for N and P analysis by acid digestion (James Crutchfield, University of Kentucky, personal communication). The 100 mg samples were placed into 25x200 mm glass ignition tubes. Five mL of concentrated sulfuric acid with 0.05 g mL^{-1} of salicylic acid were added and the tubes were incubated 1 hr at room temperature. Sodium thiosulfate (0.5 g) was added to each tube, which was placed in a block digester at 180 C for 1 hr. Potassium sulfate (1.8 g) was added with three selenized boiling chips, and the digestion continued an

additional 2.5 hours at 360 C. After the samples cooled, they were diluted to 50 mL with distilled water.

The samples were placed into polystyrene cups for colorimetric analysis. These analyses were performed with a dual Technicon System II Auto-analyzer at a wavelength of 660 nm. To measure ammonia, a modification of the Berthelot reaction was used (Chaney and Marbach, 1962). A solution containing 0.5 % sodium hydroxide and 0.042% sodium hypochlorite in distilled water (solution A), and a solution of 1.0% phenol and 0.02% sodium nitroprusside in distilled water (solution B) was used. The samples were introduced into a bubble segmented stream before the reagents were added. The reaction was contained within the instrument, and the resulting indophenol was passed through the colorimeter to determine ammonia concentration.

Phosphorus was determined by a modification of the Fiske and Subbarow method (1925). An ammonium molybdate solution in 1.92 N sulfuric acid was added to the sample in the segmented stream to make a hetero polyphosphomolybdate complex. This complex was reduced by adding a solution of 150 g sodium bisulfate, 5.0 g of sodium sulfite, and 2.5 g 1-amino-2-naphthol-4-sulfonic acid in 1000 mL distilled water and then heated to 95 C in an oil bath. This resulted in a blue color proportional to the phosphate concentration (James Crutchfield, University of Kentucky, personal communication) which was measured at a wavelength of 550 nm relative to calibrated standards.

2.4.2   Mineralization Incubation

A 7-d anaerobic mineralization incubation was performed on air dry, sieved (<2 mm) soil samples to assess residual and mineralizable N. This protocol was adapted from

Bundy and Meisinger (1996). The N availability was assessed by measuring the NH_4^+

produced after soils were anaerobically incubated at 40 C. Five g air dry soil was added

to each of five 16x150 mm threaded test tubes. One tube was set aside as a control. To

the remaining four tubes, 10 mL of distilled and deionized water was added and the tubes

sealed with threaded caps and incubated at 40 C. The control tubes were immediately

extracted with 2 M KCl to determine soluble inorganic N prior to mineralization. Tubes

were inverted to suspend soil residue and the suspension was quantitatively transferred to

50 mL plastic centrifuge tubes. The centrifuge tubes were made to volume with 2 M KCl.

Tubes were shaken on a reciprocating shaker for 30 min. After shaking, the contents were

allowed to settle for approximately 45 min before 1 mL of supernatant was transferred to

Eppendorf tubes and frozen at -20 C. The incubated samples were extracted similarly to

the control after 7-d. Samples were kept frozen until analysis of N as NH_4^+ and NO_3^- was

performed by colorimetric microplate method.

A colorimetric procedure was used for microplate determination of NH_4^+ (Chaney

and Marbach, 1962; Weatherburn, 1967). Twenty µL of standards ranging from 0 to 10

mg L^{-1} N as NH_4^+ in 1 M KCl were pipetted into the first two columns of a 96-well

microplate (well volume 360 µL). Twenty µL of sample were pipetted into duplicate

rows for a total of two replications for each sample. Reagent 1 (a solution of 1.0% phenol

containing 0.020% sodium nitroprusside)(100 µL) was added to each well followed by

100 µL of Reagent II (a solution of 0.5% sodium hydroxide containing 0.042% sodium

hypochlorite). An adhesive film was used to cover each of the plates to limit NH_3

volatilization. The plates were incubated for 30 min on a plate shaker. The NH_4^+

concentration was determined at 630 nm on a microplate reader, with the first two columns designated as standards.

To determine NO_3^-, the analysis was performed with a microplate method (Crutchfield and Grove, 2011). Initial NO_3^- (the control samples that were not incubated) and residual NO_3^- after incubation were measured by means of Cd reduction followed by the Griess-Ilosvay reaction (Crutchfield and Grove, 2011). Standards ranging from 0 to 10 mg L^{-1} N as NO_3^- were pipetted into the first two columns of a 96 well microplate plate (well volume 360 µL). Twenty µL of sample were pipetted into duplicate rows for a total of two replications for each sample. Ammonia buffer (pH 8.5, 200 µL) was pipetted into each well. Each well in the plate was in contact with a corresponding Cd prong and the plate was shaken for 45 min on a plate shaker. Cadmium prongs were cleaned in an ultrasonic bath with 1N HCl for 45-sec prior to use. Next, 5 mL of sulfanilamide solution (0.2 g of sulfanilamide in 5 mL 3 M HCl) was added to a 0.1% Napthylethylene diamine dihydrochloride solution to create a NED solution. Sixty µL NED was added to each well after shaking. After adding the NED solution the plates were shaken 5 min on a plate shaker. Nitrate was measured at 542 nm against the standards in the first two columns.

2.4.3   Permanganate Oxidizable Carbon

To determine the labile carbon content of the soil, a permanganate oxidizable C (POX_c) assay was performed. This procedure is based on the work of Weil et al. (2003). Two replicate samples of 2.5 grams of air-dried soil were added to labeled 50 mL screw cap plastic centrifuge tubes (Falcon, Fisher Scientific). To each tube, 20 mL of 0.02 mol L^{-1} $KMnO_4$ in 0.1 mol L^{-1} $CaCl_2$ was added. The tubes were sealed with threaded plastic caps and shaken on a reciprocating shaker at 180 rpm for 2 min. After shaking, the tubes

were allowed to settle for exactly 10 min, and 0.5 mL of the supernatant was transferred

to a separate labeled 50 mL centrifuge tube filled with 49.5 mL distilled H_2O. These

tubes were hand shaken, and an aliquot was transferred to a 1 cm square disposable

plastic cuvette. Absorbance change was measured by a Thermo Scientific Genesys 20

spectrophotometer at a 550 nm wavelength. The sample absorbance was compared to a

standard curve made with $KMnO_4$ concentrations varying from 0.005 to 0.02 mol L^{-1}.

Labile C was calculated in mg kg^{-1} by the following equation:

$$POXC(\text{mg kg}^{-1}) = [0.02 \text{ mol L}^{-1} - (a + bz)] \times (9000 \text{ mg C mol}^{-1}) \times \left(\frac{0.02 \text{ L solution}}{0.0025 \text{ kg soil}} \right)$$

in which 0.02 mol L^{-1} is the initial $KMnO_4$ concentration, a and b are the intercept and

slope of the measured standard curve, z is the absorbance of the sample, 9000 mg is the

amount of C that is oxidized by 1 mole of permanganate, 0.02 L is the volume of the

$KMnO_4$ solution, and 0.0025 kg is the mass of the soil used for the analysis.

2.4.4   Enzyme Activity

2.4.4.1   Acid Phosphatase

The protocol for analysis of soil phosphatase was adapted from Tabatabai and

Bremner (1969) and Acosta-Martinez and Tabatabai (2011). Because the measured pH of

all plots was less than 7, only acid phosphatase was measured. One g of air-dried soil for

each of two replicates was placed into a disposable 20 mL glass test tube. Toluene (0.2

mL) was added and the samples incubated at room temperature for 2 min. To this, 4 mL

of modified universal buffer (MUB) pH 6.5 (Acosta-Martinez and Tabatabai, 2011) and 1

mL of *p*-nitrophenyl phosphate (PNP) solution was added. The PNP solution was made

by dissolving 0.84 g of disodium *p*-nitrophenyl phosphate tetrahydrate in 50 mL of MUB,

pH 6.5. The tubes were capped with plastic caps and mixed with a vortex shaker for 5

sec. After mixing, the tubes were incubated at 37 C for 1 hr. On removal, the tubes were

amended with 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.5 NaOH, vortexed, and centrifuged at

2,000 rpm. Two mL of the supernatant was removed and placed into 1 cm square plastic

cuvettes, which were analyzed on a Thermo Scientific Genesys 20 spectrophotometer at

an absorbance of 405 nm. Reagent controls were made by adding the *p*-nitrophenyl

phosphate solution after the 1-hr incubation, and treated as previously described. A

calibration curve was created using standards containing 0 to 50 µg of *p*-nitrophenyl L^{-1}.

2.4.4.2 Sulfatase

The protocol for analysis of soil sulfatase activity was adapted from Tabatabai

(1996). Two replicate samples of 1 g of air-dried soil were placed in disposable 20 mL

tubes. Toluene, 0.25 mL, was added directly to the soil and the samples were incubated 2

min at room temperature. Four mL of acetate buffer, pH 5.8 was added, then 1 mL of *p*-

nitrophenyl sulfate (PNS) solution was added to each tube. The PNS solution was made

by dissolving 0.614 g of potassium *p*-nitrophenyl sulfate in 50 mL of acetate buffer, pH

5.8 (Klose et al., 2011). The tubes were capped with plastic caps and mixed with a vortex

shaker for 5 sec. After mixing, the tubes were incubated at 37 C for 1 hr. On removal

from the incubator, 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.5 NaOH were added to each

tube. The tubes were vortexed and centrifuged at 2,000 rpm. Two mL of the supernatant

was extracted and placed into 1 cm square plastic cuvettes that were analyzed on a

Thermo Scientific Genesys 20 spectrophotometer at an absorbance of 420 nm. Controls

were made by adding the *p*-nitrophenyl sulfate solution to unamended soil and treated as

previously described. A calibration curve was created using standards containing 0 to 50

μg of *p*-nitrophenyl L^{-1}.

2.4.4.3   β-D-Glucosidase

The protocol for analysis of soil glucosidase was adapted from Deng and Popova

(2011). Two replicate samples of 1 g of air-dried soil were placed into disposable 20 mL

tubes. Toluene, 0.2 mL, was added and the samples were allowed to sit for 15 min

(because this is an intracellular enzyme, the soil was allowed to sit longer with the

toluene to allow for membrane permeabilization). Four mL MUB, pH 6, then 1 mL *p*-

nitrophenyl β-D-glucoside (PNG) solution were added. The PNG solution was made by

dissolving 0.753 g of *p*-nitrophenyl β-D-glucoside in 50 mL MUB, pH 6 (Deng and

Popova, 2011). The tubes were capped with plastic caps and mixed with a vortex shaker

for 5 sec. After mixing, the tubes were incubated at 37 C for 1 hr. On removal from the

incubator, the tubes were amended with 1 mL of 0.5 M CaCl$_2$ and 4 mL of 0.1 THAM

buffer (Acosta-Martinez and Tabatabai, 1996), pH 12. The tubes were vortexed and

centrifuged at 2,000 rpm. Two mL of the supernatant was extracted and placed into

plastic cuvettes that were analyzed with a Thermo Scientific Genesys 20

spectrophotometer at an absorbance of 405 nm (Deng and Popova, 2011). Controls were

made by adding the PNG solution after incubation and amending the tubes as previously

described. A calibration curve was created using standards containing 0 to 50 μg of *p*-

nitrophenyl L^{-1}.

2.4.4.4 Urease

The protocol for analysis of soil urease was adapted from Tabatabai (1996). Air-dried soil (2.5 g) was placed into each of two 20 mL culture tubes. To this, 0.2 mL of toluene was added and the samples were incubated 15 min at room temperature. Afterward, 0.02 M phosphate buffer, pH 9 (4.5 mL), was added. The tubes were inverted to mix and then 1 mL of 0.2 M urea solution (in pH 9 phosphate buffer) was added to each tube. The tubes were capped with plastic caps and mixed with a vortex mixer for 5 sec. After mixing, the tubes were incubated at 37 C for 1 hour. After incubation, the tubes were removed and 4.5 mL of 2.5 M KCl was added. The tubes were vortexed and then cooled at -20 C for 5 min. Following this step, the tubes were centrifuged at 2,000 rpm and 0.1 mL of the sample supernatant was transferred to a 10 mL glass tube. To determine NH_4^+ content, the phenol-hypochlorite reaction was used (Ngo et al., 1981; Weatherburn, 1967). Weatherburn Reagent A (5 g phenol and 25 mg of sodium nitroprusside in 500 mL of H_2O)(2.5 mL) was added and the tubes were inverted. Next, 2.5 mL of Weatherburn Reagent B (2.5 g of sodium hydroxide and 4.2 mL of sodium hypochlorite in 500 mL of H_2O) was added and the tubes were inverted to mix (Weatherburn, 1967). This chemistry is similar to the protocol used for determination of NH_4^+ following anaerobic mineralization, but the reagents used differ slightly in their composition. The tubes were incubated for 30 min at room temperature for color development and the absorbance at 660 nm was measured on a Thermo Scientific Genesys 20 spectrophotometer. Controls were made by adding urea solution to a soil sample after incubation, followed by immediate extraction as previously described. A calibration curve was created with standards ranging from 0 to 10.5 μg mL^{-1} NH_4-N.

2.4.5 Statistical Analysis

For data analysis PC SAS was used as the primary program. Significance was set at $\alpha=0.05$. This alpha level was chosen because there is the potential for economic loss without a somewhat conservative alpha level. For analysis of cover crop characteristics (biomass and tissue N and P concentration) data was analyzed by the general linear model (Proc GLM). The data was analyzed as a randomized complete block design. Biomass was only harvested once during the study; therefore, this analysis did not include repeated measures. Species and block were treated as fixed effects. Species was treated as a fixed effect because the particular species were chosen for the specific study and were used over the course of several seasons in the same order. Tukey's least significant difference test was performed to show significance between species' means.

Proc GLIMMIX was used for analysis of soil parameters (N mineralization, POX_c, enzyme activity) using the Type I sum of squares. The data was treated as a randomized complete block design, and because data was produced several times during the study, time was introduced to account for the repeated measures with spring 2017 as Time 1 and fall 2017 as Time 2. Treatment (cover crop species), time, and depth were treated as fixed effects. The variance component structure (VC) was used to account for variance introduced by repeated measures and was determined to be adequate based on the error-variability in the dataset. A linear regression between cover crop tissue N concentration and mineralized N was performed in Microsoft Excel to show the correlation between the two variables. The same linear model was used for this regression. The means of each cover crop treatment were used to make the regression.

The time by treatment interaction was analyzed as a random effect with block being specified as the subject to assess homogeneity by block, by measurements over time, and by treatment. Normality of the data was assessed by use of a Proc MEANS statement to evaluate standard deviations and variance and then visual plots produced by SAS (box and whisker plots, histograms). No transformation was necessary. Any outlier values were excluded in the final analysis. Significant interactions between time and depth were analyzed by the use of least square means to make pairwise comparisons between Times 1 and 2 at each respective depth (sliced by depth). The only significant treatment interaction (treatment by depth) occurred in the β-D-Glucosidase analysis and was not analyzed further because of the small effect size (f=2), which was indicative of the effect of the control treatment that showed an unusual distribution and high numbers of outliers. Therefore, the interaction was disregarded.

Significance in time and depth were evaluated by least square means statements and the use of box plots generated in SAS to illustrate trends. The significant treatment effect in the β-D-Glucosidase had a small effect size (f=6), likely driven by only the control treatment. Stratification ratios were calculated by dividing parameter values from the upper depth by the lower depth value. Stratification ratios in spring 2017 and fall 2017 were calculated by averaging the measured variable between depths A and B (0-7.5 and 7.5-15 cm), and dividing that value by the same response variable at depth C (15-30cm). For fall 2016, values were simply calculated by dividing the measured variable at 0-15 cm by the same variable at 15-30 cm. The mean stratification ratios were analyzed in Proc GLM to establish a basic 2-way ANOVA with time and treatment as fixed

variables. Only time was significant in this analysis and was analyzed by least square means to show comparisons between fall 2016, spring 2017, and fall 2017.

CHAPTER 3. BIOMASS AND PLANT NUTRIENT COMPOSITION

3.1   Cover Crop Growth and Characteristics

3.1.1   Cover Crop Biomass

The cereal rye/clover, cereal rye/pea, and cereal rye/vetch treatments had the greatest dry cover crop biomass with values ranging from 3,500 to 3,590 kg ha^{-1}. These values were significantly higher than the vetch and cereal rye treatments ($p<0.05$), which had dry biomass totals of 1,610 and 1,430 kg ha^{-1}, respectively (Fig. 6). Control biomass is presented in Fig. 7.

The control treatment was a weedy fallow, therefore, all biomass was weed species. There were no measurable weeds in the biomass collected from the cereal rye/vetch treatments (Fig. 7). The control had the highest weedy biomass with a mean of 484 kg ha^{-1}. No treatments other than cereal rye/vetch were significantly different from the control treatment. Dried biomass for treatments clover, cereal rye/clover, cereal rye/pea, vetch, and cereal rye ranged from 251 to 323 kg ha^{-1} with cereal rye having the lowest value and cereal rye/pea having the highest.

The weed biomass was also calculated as a percent of the total biomass. The cereal rye treatment had the highest percent of weed biomass (14.5%) (Fig. 8). This was significantly higher than all other treatments.

29
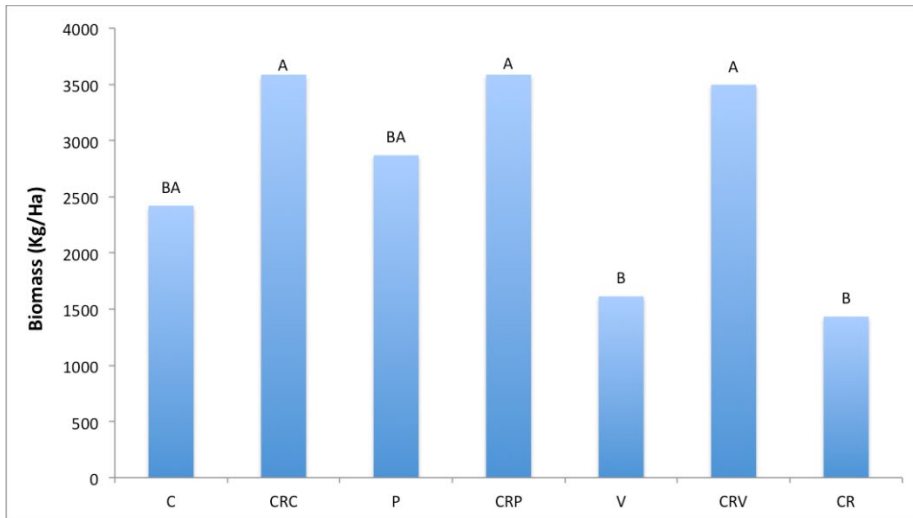
Figure 6. Dried cover crop biomass. Treatments with different letter groupings are significantly different ($p<0.05$). Clover (C), cereal rye/clover (CRC), Austrian winter pea (P), cereal rye/pea (CRP), vetch (V) cereal rye/vetch (CRV), cereal rye (CR).
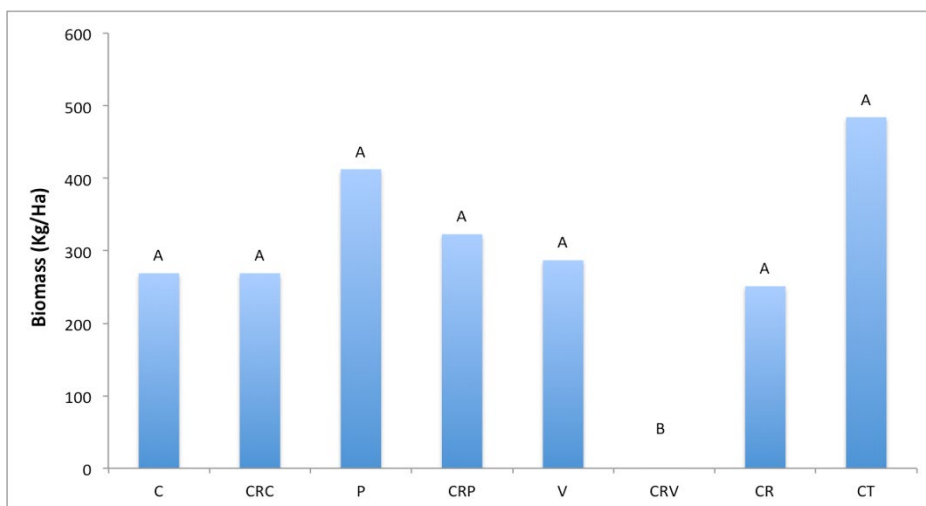
Figure 7. Dried weed biomass. Treatments with different letter groupings are significantly different ($p<0.05$). Clover (C), cereal rye/clover (CRC), Austrian winter pea (P), cereal rye/pea (CRP), vetch (V) cereal rye/vetch (CRV), cereal rye (CR) and control (CT-weedy biomass).
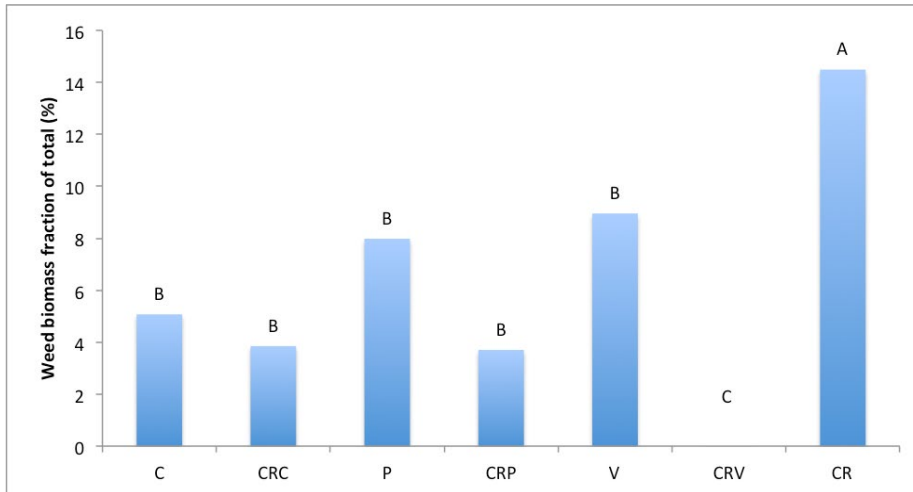
Figure 8. Weed biomass fraction. Treatments with different letter groupings are significantly different (p<0.05) Clover (C), cereal rye/clover (CRC), Austrian winter pea (P), cereal rye/pea (CRP), vetch (V) cereal rye/vetch (CRV), cereal rye (CR).

The weed biomass fraction, in clover, cereal rye/clover, and cereal rye/pea treatments ranged from 4% to 5%, but were not significantly different from the pea, vetch, or cereal rye/vetch treatments. The control treatment is not depicted in Fig. 8 because the control only contained weed species (100% of biomass).

3.1.2 Cover Crop Tissue Analysis

The P concentration in the cover crop tissue varied from 2.6 to 4.0 g kg^{-1}. The P concentration of vetch was significantly higher than all other treatments but Austrian pea. The cereal rye/pea and Austrian pea had tissue P concentrations of 3.5 and 3.8 g kg^{-1}, respectively. The cereal rye/vetch had 3.1 g kg^{-1} P and was significantly higher than the clover, cereal rye/clover, and cereal rye treatments, which had values of 2.6 g kg^{-1} P.
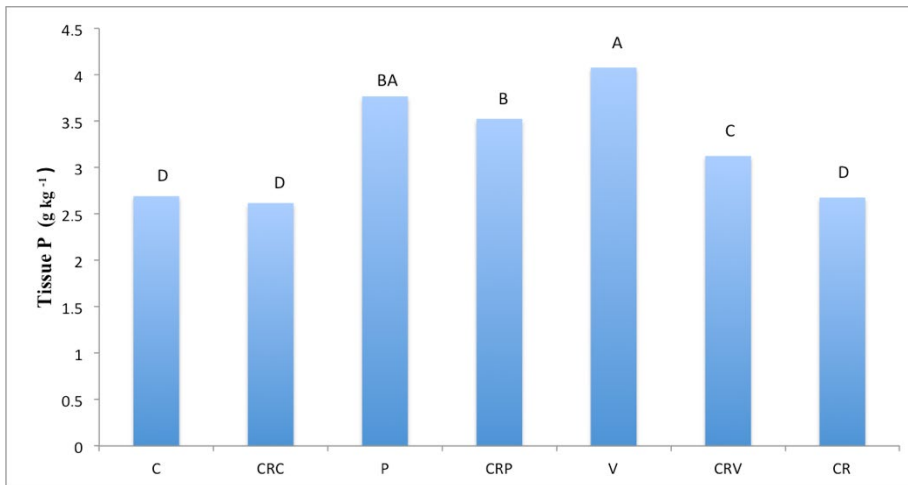
Figure 9. Cover crop tissue P. Treatments with different letter groupings are significantly different (p<0.05). Clover (C), cereal rye/clover (CRC), Austrian winter pea (P), cereal rye/pea (CRP), vetch (V) cereal rye/vetch (CRV), cereal rye (CR).

Cover crop tissue N concentration values ranged from 9 to 32 g kg^{-1}. Vetch had the highest tissue N concentration while cereal rye had the lowest tissue N concentration. The N concentration for vetch was significantly higher than all other treatments. The cereal rye and cereal rye/clover were not different from one another but were significantly lower than all other treatments. The N concentration for the remaining treatments ranged from 19 to 25 g k^{-1} (Fig. 10).

Part of the weedy biomass samples was also analyzed for tissue P and N concentrations. The P concentration in the weed tissue ranged from 3.8 to 5.7 g kg $^{-1}$. The weeds harvested from the cereal rye/pea treatment plots had the highest P concentration and the weeds from the cereal rye/clover treatment plots had the lowest P concentration. The weed portion of the cereal rye/pea treatment had significantly higher P than that of the vetch (3.8 g kg $^{-1}$) and cereal rye/clover treatments. The clover, control, cereal rye, and P treatments had weed P concentrations ranging from 4.2 to 5.5 g kg $^{-1}$ (Fig. 11).
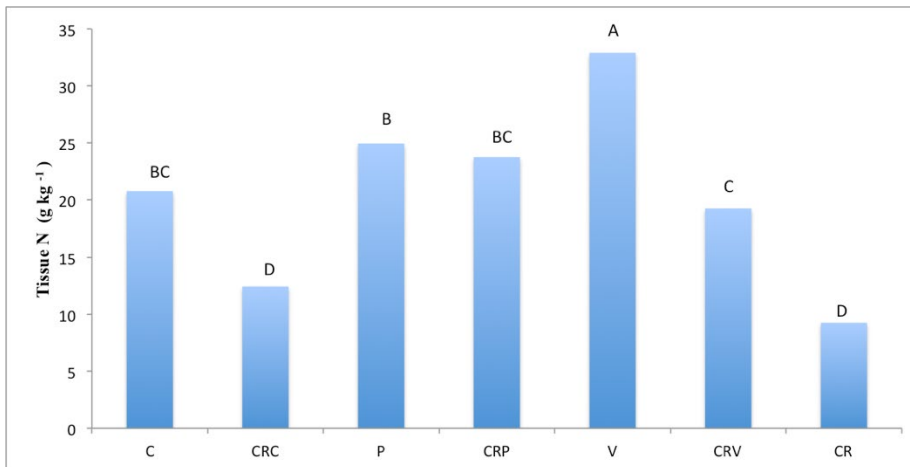
Figure 10. Cover crop tissue N. Treatments with different letter groupings are significantly different (p<.0.05). Clover (C), cereal rye/clover (CRC), Austrian winter pea (P), cereal rye/pea (CRP), vetch (V) cereal rye/vetch (CRV), cereal rye (CR).
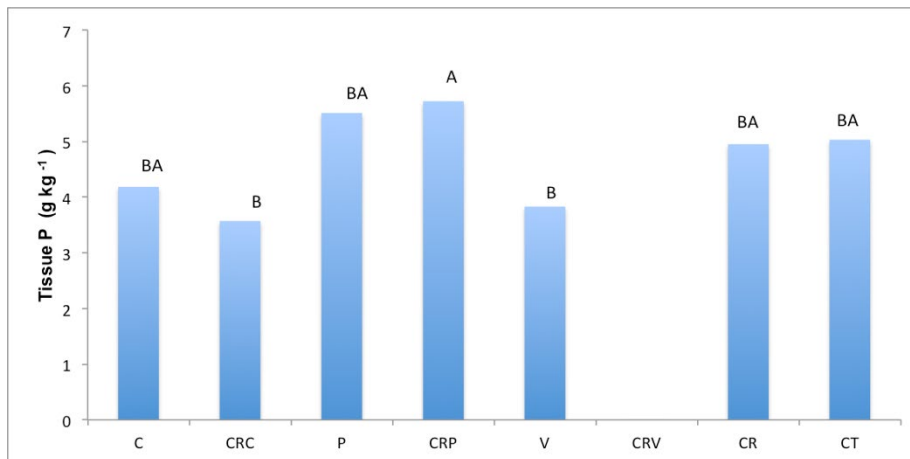
Figure 11. Weed tissue P. Treatments with different letter groupings are significantly different (p<0.05). Clover (C), cereal rye/clover (CRC), Austrian winter pea (P), cereal rye/pea (CRP), vetch (V), cereal rye/vetch (CRV), cereal rye (CR), control (CT-weedy Fallow). There was no weedy biomass in CRV.

The N concentration in the weed tissue ranged from 12 to 23 g kg $^{-1}$. The control treatment had the highest weed N concentration while the weed samples collected from the cereal rye/clover treatment had the lowest tissue N. The clover, pea, cereal rye/pea, vetch, and cereal rye treatments were not significantly different from one another and had values ranging from 16 to 22 g kg $^{-1}$. The control treatment N concentration was significantly higher than the cereal rye/clover, cereal rye/pea, and vetch treatments (Fig. 12).
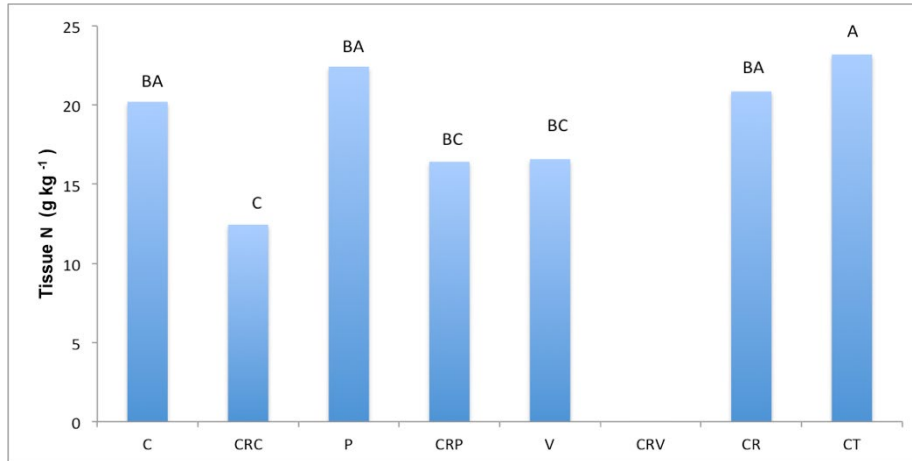
Figure 12. Weed tissue N. Treatments with different letter groupings are significantly different (p<0.05). Clover (C), cereal rye/clover (CRC), Austrian winter pea (P), cereal rye/pea (CRP), vetch (V), cereal rye/vetch (CRV), cereal rye (CR), control (CT-weedy Fallow). There was no weedy biomass in the CRV treatment.

CHAPTER 4. STRATIFICATION OF SOIL BIOLOGICAL PROPERTIES

Analysis for soil biological health parameters was performed on samples from fall 2016, spring 2017, and fall 2017. Statistical analyses were conducted on data from fall 2016 in isolation. The two sampling periods of 2017 were analyzed separately from 2016 because there were differences in sampling depths and prior treatments between the years, and the main focus of the study is centered on differences due to treatments, not year or season.

Samples from fall 2016 were analyzed based on past treatments and depth to assess if there were previous treatment effects. Therefore, data from fall 2016 were used as the baseline for parameters and cannot be statistically compared to data from 2017. ANOVA tables for 2016 and 2017 are in Table 3 and Table 4, respectively.

Table 3. Summary of ANOVAS for 2016 analyses. p values followed by 'ns' are not significant; p values with * indicate significance (p < 0.05).

Analysis	Treatment	Depth	Treatment*depth
Mineralizable N	0.08 ns	< 0.001*	0.49 ns
POXc (labile C)	0.43 ns	<0.001*	0.88 ns
Phosphatase	0.63 ns	<0.001*	0.07 ns
Sulfatase	0.23 ns	<0.001*	0.87 ns
β-glucosidase	0.56 ns	<0.001*	0.30 ns
Urease	0.33 ns	<0.001*	0.38 ns

Table 4. Summary of ANOVAS for spring and fall 2017 analyses. p values followed by 'ns' are not significant; p values with * indicate significance (p < 0.05). There were no significant three-way interactions.

Analysis	Treatment	Time	Depth	Treatment * time	Treatment* depth	Depth* time
Mineralizable N	0.15 ns	0.02*	< 0.001*	0.32 ns	0.40 ns	0.17 ns
POXc (labile C)	0.90 ns	0.001*	< 0.001*	0.27 ns	0.10 ns	0.27 ns
Phosphatase	0.29 ns	0.16 ns	<0.001*	0.26 ns	0.48 ns	0.11 ns
Sulfatase	0.44 ns	<0.001*	<0.001*	0.77 ns	0.24 ns	<0.001*
β-glucosidase	<0.002*	<0.001*	<0.001*	0.19 ns	0.02*	0.16 ns
Urease	0.06 ns	<0.001*	<0.001*	0.40 ns	0.36 ns	0.22 ns

4.1 Anaerobic Mineralization

There was no significant treatment effect or treatment by depth interaction on net organic N mineralized to NH_4^+ following the 7-d incubation in 2016 (Table 3). Depth was significant ($p<0.001$) (Table 3). The average net mineralized N as NH_4^+ was 46 mg kg^{-1} at 0-15 cm and 14 mg kg^{-1} at 15-30 cm (Fig 13).
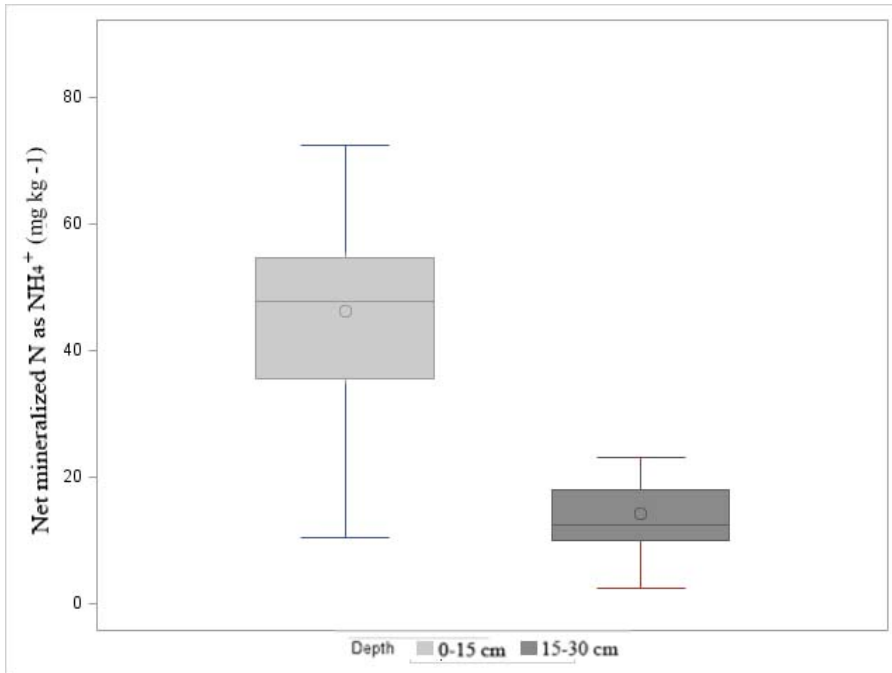
Figure 13. Fall 2016 mineralized N as NH_4^+ (mg kg^{-1}) across all treatments. Maximum and minimum values are represented by the whiskers and means are represented by the inner circles. Lines located within the boxes indicate the median value. The boxes represent the upper and lower quartiles.

In spring and fall 2017, treatment effects were not significant; only time (season) and depth were significant (Table 4). Net mineralized N as NH_4^+ was significantly higher in spring 2017 (85 mg kg^{-1}) than fall 2017 (74 mg kg^{-1}). There was no significant difference in the residual NO_3^- recovered after anaerobic incubation.

Depth was a significant factor for mineralized N as NH_4^+ ($p<0.05$) in spring and fall 2017. Among all treatments, mineralized N was highest at 0-7.5 cm, then decreased with increasing depth. This trend is shown in Figs. 14 and, which shows overall means for all depths in both spring and fall 2017. Values ranged from 53 to 120 mg kg^{-1} in spring 2017 and 48 to 106 mg kg^{-1} in fall 2017 across all depths.
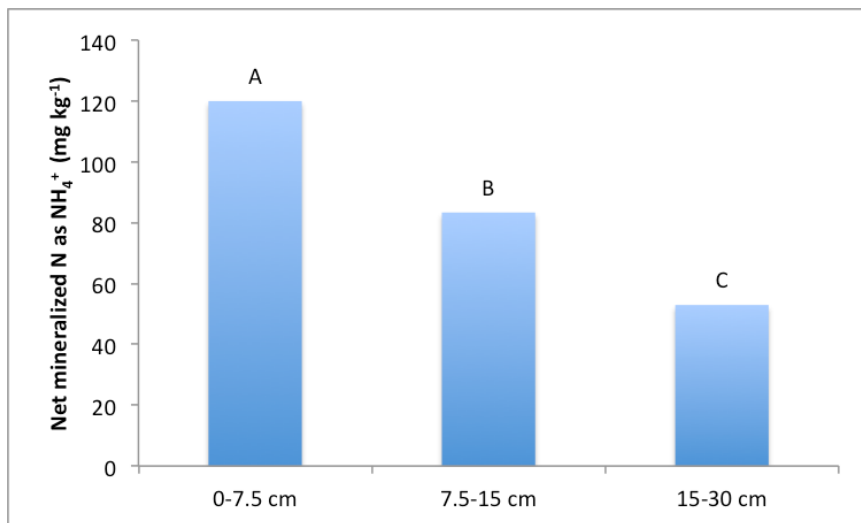
Figure 14. Mean net mineralized N as NH_4^+ (mg kg^{-1}) at each depth in spring 2017 – all treatments combined. Depths with different letters are significantly different ($p < 0.05$).

The data from the anaerobic mineralization incubation for spring and fall 2017 was fit to a regression with the tissue N concentration for each of the cover crop treatments (Fig. 16). The correlation was significant ($p = 0.04$) with an R-square for the regression of 0.70. As the N concentration in the cover crop tissue so did the capacity to mineralize N during the seven-day incubation. Vetch had the highest value of mineralized N as NH_4^+ in both spring and fall 2017 (108 and 87 mg kg^{-1} respectively) and the highest value of tissue N (32 g kg^{-1}).
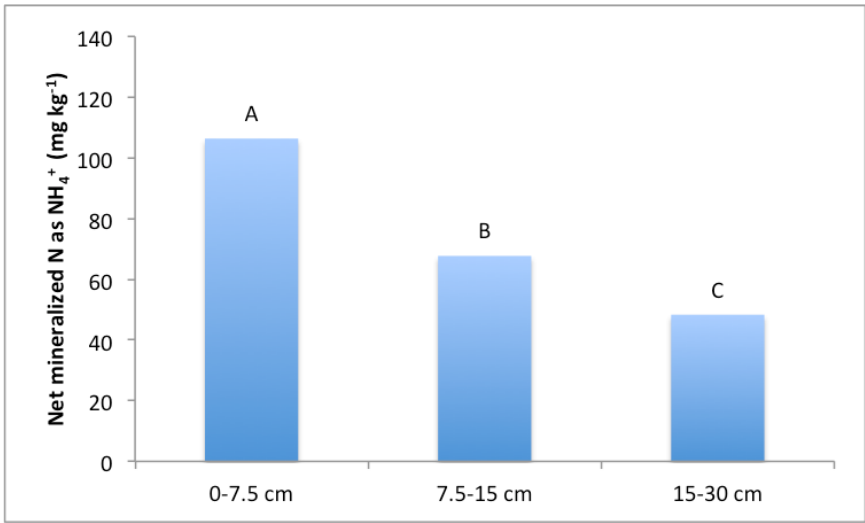
Figure 15. Mean net mineralized N as NH_4^+ (mg kg^{-1}) for each depth in fall 2017 – all treatments combined. Depths with different letters are significantly different ($p < 0.05$).
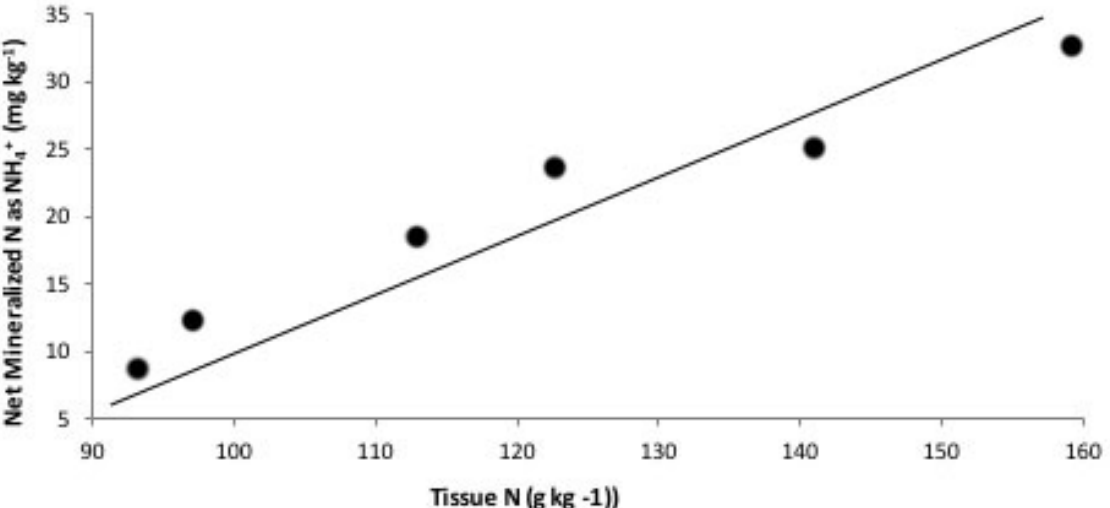
Figure 16. Regression of cover crop tissue N (g kg^{-1}) and net mineralized N as NH_4^+ (mg kg^{-1}) R-square-=0.70, p-level = 0.04.

4.2 Labile Carbon

Depth had a significant effect on labile carbon measured in fall 2016 (Fig. 18). The overall average for all 0-15 cm samples was 446 mg kg $^{-1.}$ The average labile carbon for all samples at 15-30 cm was 223 mg kg $^{-1}$. There was no significant difference due to cover crop treatment. The labile carbon due to treatment ranged from 495 to 341 mg kg^{-1} at 0-15 cm and 328 to 150 mg kg^{-1} at 15-30 cm.
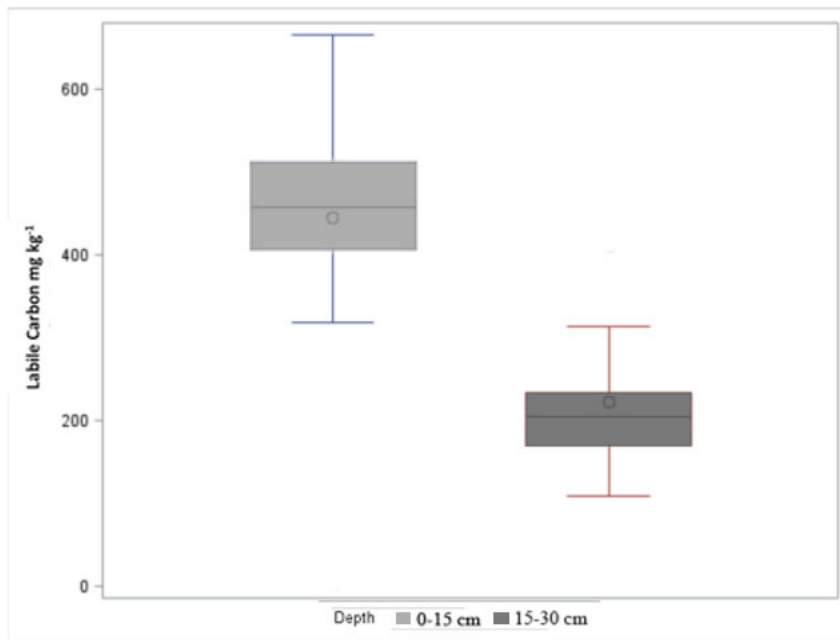
Figure 17. Labile carbon by depth for samples collected in fall 2016. Maximum and minimum values are represented by the whiskers and means are represented by the inner circles. Lines located within the boxes indicate the median value. The boxes represent the upper and lower quartiles.

There were no treatment effects on labile carbon in samples taken spring or fall 2017 (Table 4). Time had a significant effect on labile carbon (p=0.001) (Table 4); labile carbon was significantly higher in spring 2017 samples than fall 2017 samples (Fig. 18).

The mean labile carbon for samples taken in spring 2017 was 643 mg kg $^{-1}$. The mean labile carbon of samples taken in fall 2017 was 536 mg kg $^{-1}$.
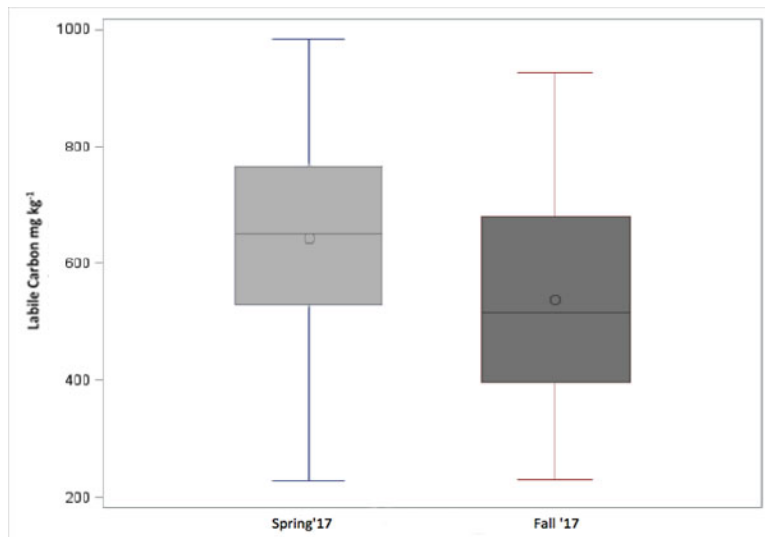
Figure 18. Labile carbon measured for spring and fall 2017. Maximum and minimum values are represented by the whiskers and means are represented by the inner circles. Lines located within the boxes indicate the median value. The boxes represent the upper and lower quartiles.

Overall, depth had a significant effect ($p<0.001$) on labile carbon (Fig. 19). For all treatments (save for cereal rye/pea in spring 2017) labile carbon decreased with increasing depth. At 0-7.5 cm the mean labile carbon was 754 mg kg $^{-1}$ in spring 2017 and 716 mg kg $^{-1}$ in Fall 2017.

Labile carbon at 7.5-15 cm and 15-30 cm was significantly higher in spring 2017 than fall 2017. The mean labile carbon at 7.5-15 cm was 629 mg kg^{-1} in spring 2017 and 496 mg kg^{-1} in fall 2017. At the 15-30 cm depth, the mean labile carbon was 527 mg kg-1 in spring 2017 and 392 in fall 2017. In spring 2017, the labile carbon content ranged from 426 mg kg $^{-1}$ (cereal rye/vetch) to 854 mg kg^{-1} (control) across all depths. In fall 2017, the

labile carbon content ranged from 333 mg kg^{-1} (Austrian pea) to 802 mg kg^{-1} (vetch) across all depths. There was no significant cover crop treatment effect either year.
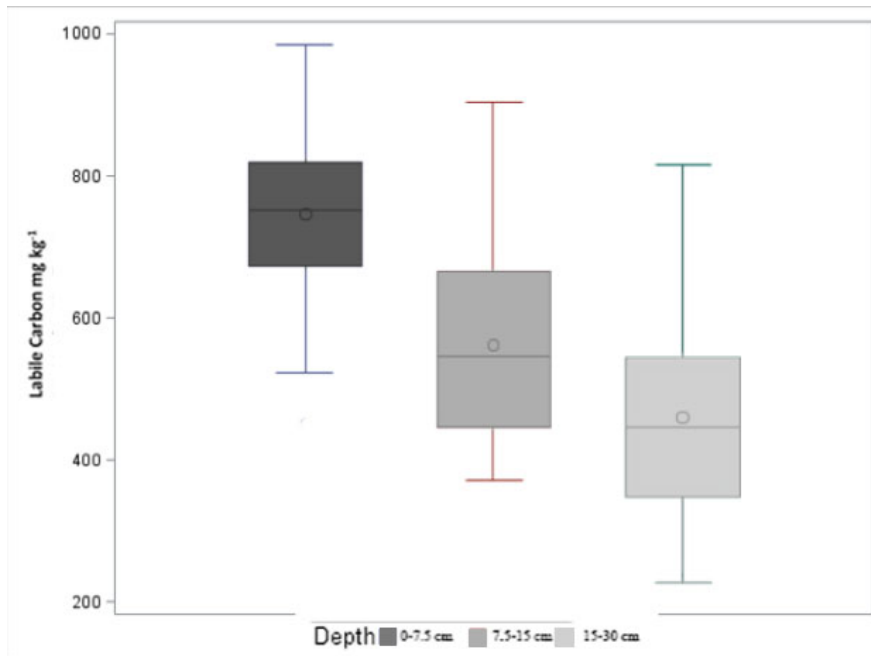
Figure 19. Labile carbon measurements by depth across all treatments averaged across spring and fall 2017. Maximum and minimum values are represented by the whiskers and means are represented by the inner circles. Lines located within the boxes indicate the median value. The boxes represent the upper and lower quartiles.

4.3 Enzyme Activity

4.3.1 Phosphatase Activity

There was a significant depth effect on phosphatase activity in fall 2016, but no significant treatment effect (Table 3). The mean phosphatase activity in fall 2016 was 478 mg P-nitrophenol (PNP) kg^{-1}soil h^{-1}at 0-15 cm and 210 mg PNP kg^{-1}soil h^{-1}at 15-30 cm (Fig. 20). The overall average phosphatase activity was 334 mg PNP kg^{-1}soil h^{-1} .

During spring and fall 2017, there was a significant depth effect on phosphatase activity (Table 4). As depth increased, the phosphatase activity decreased. There was no significant difference between treatments. The average phosphatase activity was 475 mg PNP kg $^{-1}$soil h $^{-1}$ at 0-7.5 cm, 302 mg PNP kg $^{-1}$ soil h $^{-1}$ at 7.5-15 cm, and 269 mg PNP kg $^{-1}$soil h^{-1} at 15-30 cm. Phosphatase activity ranged from 159 mg PNP kg $^{-1}$soil h $^{-1}$ (cereal rye/clover, 15-30 cm) to 509 mg PNP kg $^{-1}$soil h $^{-1}$ (cereal rye , 0-7.5 cm) in spring 2017. In fall 2017, phosphatase activity ranged from 255 mg PNP kg $^{-1}$soil h^{-1} (Austrian pea, 15-30 cm) to 532 mg PNP kg $^{-1}$soil h $^{-1}$ (cereal rye, 0-7.5 cm).
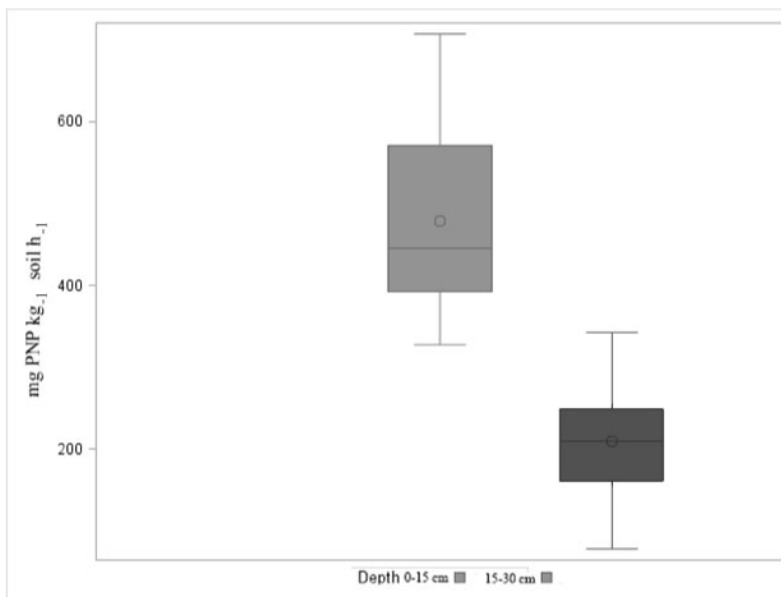
Figure 20. Fall 2016 phosphatase activity. Maximum and minimum values are represented by the whiskers and means are represented by the inner circles. Lines located within the boxes indicate the median value. The boxes represent the upper and lower quartiles.

4.3.2 Sulfatase Activity

Sulfatase activity in fall 2016 was significantly affected by depth (Table 3), but there was no overall treatment effect (p=0.23). As expected, sulfatase activity was higher at 0-15 cm than at 15-30 cm.

At 0-15 cm, sulfatase activity was an average of 57 mg PNP kg^{-1}soil h-1. At 15-30 cm sulfatase of 19 mg PNP kg^{-1}soil h^{-1} (Fig. 21).
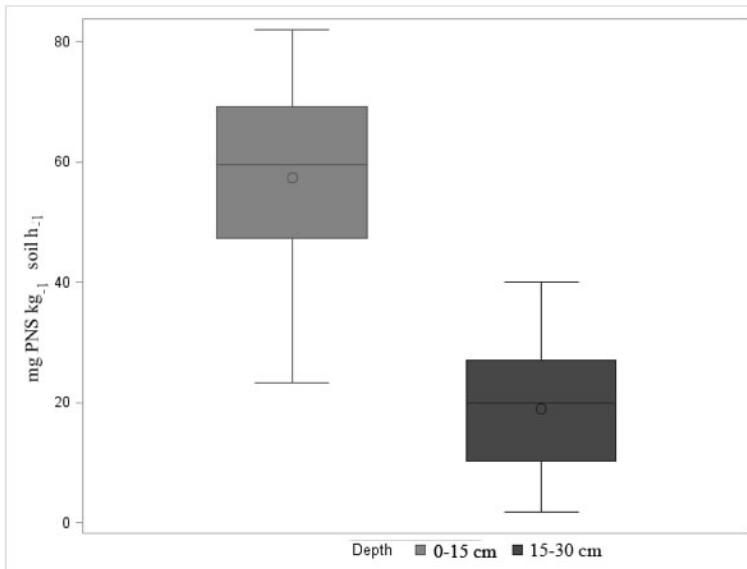
Figure 21. Fall 2016 sulfatase activity by depth, averaged across all treatments. Maximum and minimum values are represented by the whiskers and means are represented by the inner circles. Lines located within the boxes indicate the median value. The boxes represent the upper and lower quartiles.

Sulfatase activity in spring and fall 2017 was significantly affected by depth and time (p<0.05) but not by treatment (Table 4). Activity decreased as depth increased (Fig. 22). Sulfatase activity across all treatments and both seasons averaged 44 mg PNP kg^{-1}

soil h $^{-1}$at 0-7.5 cm, 28 mg PNP kg $^{-1}$soil h $^{-1}$at 7.5-15 cm, and 26 mg PNP kg $^{-1}$soil h $^{-1}$ at 15-30 cm (Fig. 22).
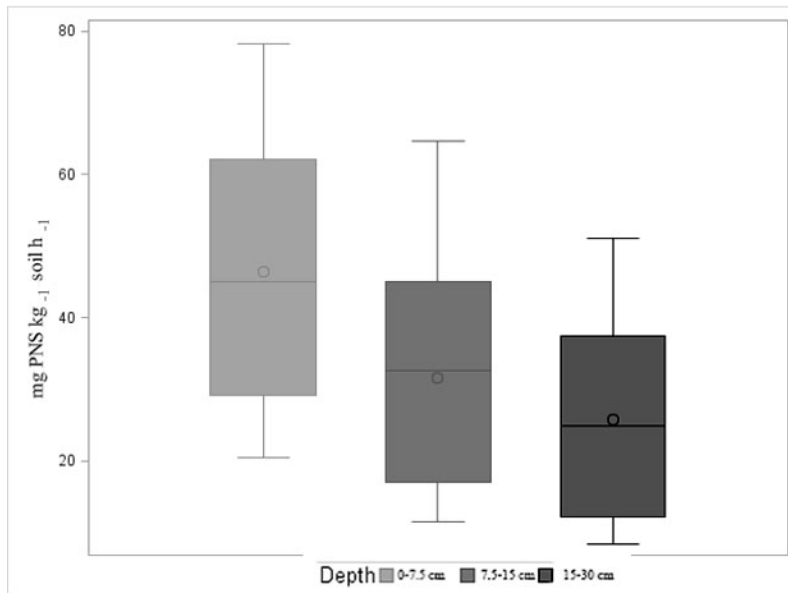
Figure 22. Spring and fall 2017 sulfatase activity by depth, averaged across all treatments. Maximum and minimum values are represented by the whiskers and means are represented by the inner circles. Lines located within the boxes indicate the median value. The boxes represent the upper and lower quartiles.

Time was highly significant for sulfatase activity (p<0.001) (Table 4) with activity greatly decreasing in fall 2017 (20 mg PNP kg $^{-1}$soil h^{-1}) compared to spring 2017 (45 mg PNP kg $^{-1}$soil h^{-1}) . There was a significant time by depth interaction. At 0-7.5 cm, sulfatase activity was significantly higher in spring than fall. This trend was the same for 7.5-15 cm and 15-30 cm. At 7.5-15 cm, the average sulfatase activity was 38 mg PNP kg^{-1} soil h^{-1} in spring and 18 mg PNP kg^{-1} soil h^{-1} in fall. At 15-30 cm, the average sulfatase activity was 39 mg PNP kg^{-1} soil h^{-1} in spring and 13 mg PNP kg^{-1} soil h $^{-1}$ in fall.

In spring 2017, activity ranged from 24 mg PNS kg^{-1} soil h^{-1} (control, 7.5-15 cm) to 64 mg PNP kg^{-1} soil h^{-1} (cereal rye/pea, 0-7.5 cm). In fall 2017, activity ranged from 11 mg PNP kg^{-1} soil h^{-1} (Austrian pea, 15-30 cm) to 34 mg PNP kg^{-1} soil h^{-1} (vetch, 0-7.5 cm).

4.3.3   β-D Glucosidase Activity.

In fall 2016, there was no effect of prior treatment on β-D Glucosidase activity. There was a significant depth effect (Table 3). Activity averaged 403 mg PNP kg^{-1} soil h^{-1} at 0-15 cm and 184 mg PNP kg^{-1} soil h^{-1} at 15-30 cm (Fig. 23).
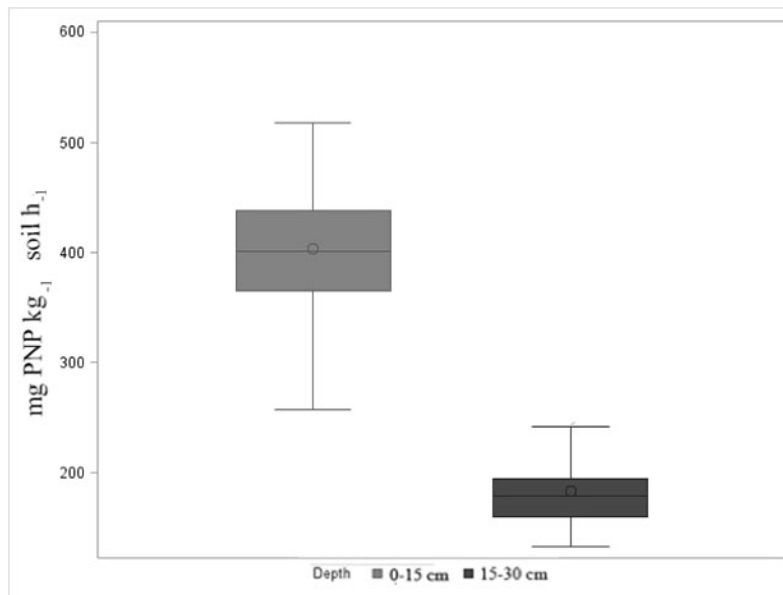
Figure 23. β-D Glucosidase activity by depth averaged, across all treatments in fall 2016. Maximum and minimum values are represented by the whiskers and means are represented by the inner circles. Lines located within the boxes indicate the median value. The boxes represent the upper and lower quartiles.

In spring and fall 2017, treatment, depth, and time were significant for β-D Glucosidase activity. There was also a significant treatment by depth interaction.
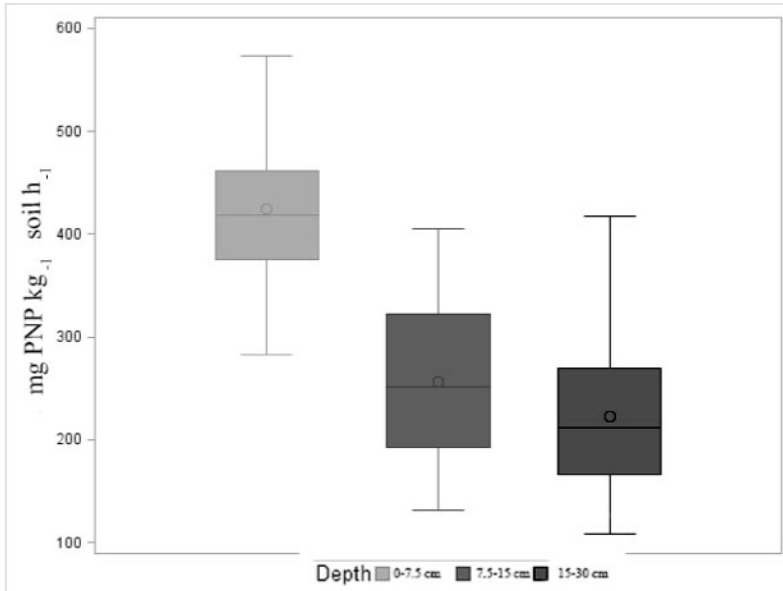
Figure 24. β-D Glucosidase activity averaged across all treatments and both seasons for each depth. Maximum and minimum values are represented by the whiskers and means are represented by the inner circles. Lines located within the boxes indicate the median value. The boxes represent the upper and lower quartiles.

Figure 24 shows the β-D Glucosidase activity across all treatments and both seasons for each depth. At 0-7.5 cm, the average activity was 403 mg PNP kg^{-1} soil h^{-1}, nearly twice as much as at lower depths. Activity at 7.5-15 cm averaged 237 PNP kg^{-1} and was 222 PNP kg^{-1} at 15-30 cm. Activity was significantly higher during spring 2017 than fall 2017. The average activity during spring 2017 was 317 mg PNP kg^{-1} soil h^{-1} and the average β-D glucosidase activity during fall 2017 was 258 mg PNP kg^{-1} soil h^{-1}.

The significant effect of the treatment and treatment by depth interaction was driven by one treatment - the control. At 15-30 cm, the control had significantly higher β-D glucosidase activity than all other treatments (Fig. 25). This difference drove the interaction. The mean activity for the control treatment at 15-30 cm was 326 mg PNP kg^{-1} soil h^{-1} and the rest of the treatments all fell below 234 mg PNP kg^{-1} soil h^{-1}. The mean β-D glucosidase activity across all depths and seasons was significantly higher in the control vs. the C, cereal rye/clover, pea, and cereal rye/vetch treatments (Fig. 26). The mean activity for the control was 336 mg PNP kg^{-1} soil h^{-1}. The activity for the C, cereal rye/clover, pea, and cereal rye/vetch treatments all fell below 279 mg PNP kg^{-1} soil h^{-1}.
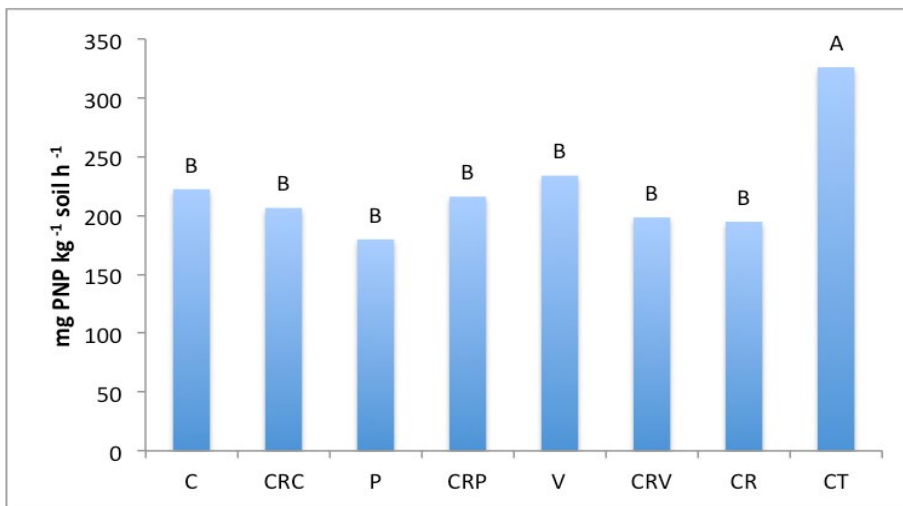
Figure 25. β-D Glucosidase activity at 15-30 cm averaged across spring and fall 2017. **Treatments** with different letter groupings are significantly different from one-another . Clover (C), cereal rye/clover (CRC), Austrian winter pea (P), cereal rye/pea (CRP), vetch (V), cereal rye/vetch (CRV), cereal rye (CR), control (CT-weedy fallow).
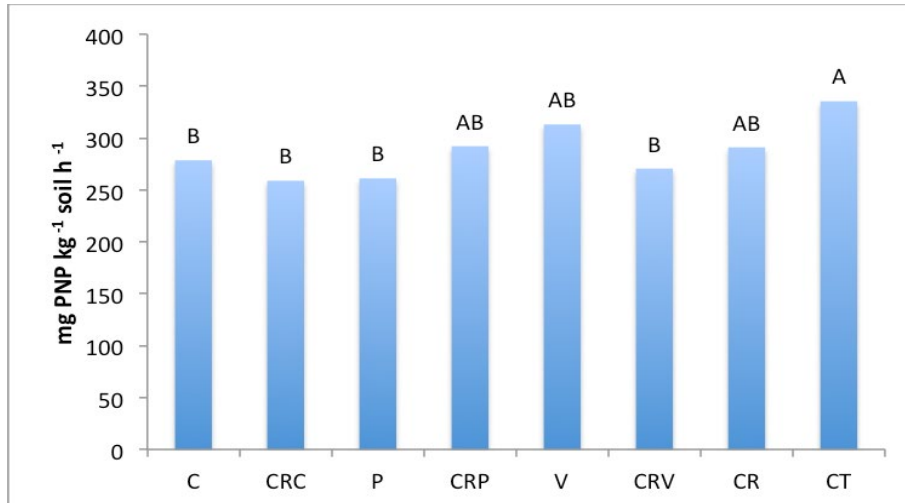
Figure 26. Fall and spring 2017 β-D Glucosidase means across all depths. **Treatments** with different letter groupings are significantly different from one-another. Clover (C), cereal rye/clover (CRC), Austrian winter pea (P), cereal rye/pea (CRP), vetch (V), cereal rye/vetch (CRV), cereal rye (CR), control (CT-weedy **f**allow).

4.3.4   Urease Activity

Depth was significant for fall 2016 urease activity (Table 3). At 0-15 cm, the mean urease activity was 1.33 mg NH_4^+ kg^{-1} soil h^{-1}, and at 15-30 cm the mean urease activity was 0.91 mg NH_4^+ kg^{-1} soil h^{-1}.

The urease activity measured in spring and fall 2017 was significantly affected by depth and time.
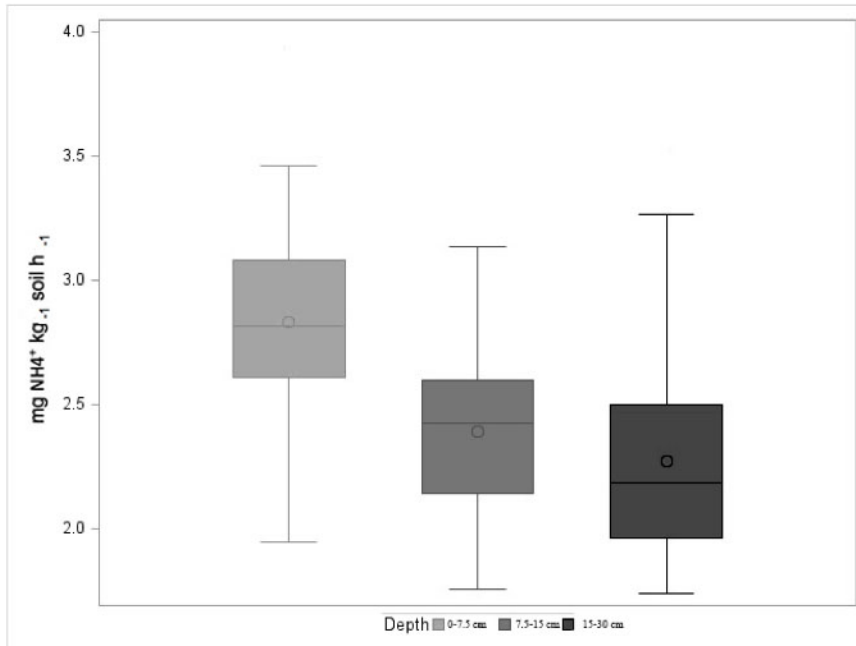
Figure 27. Urease activity by depth, averaged across all treatments for fall and spring 2017. Maximum and minimum values are represented by the whiskers and means are represented by the inner circles. Lines located within the boxes indicate the median value. The boxes represent the upper and lower quartiles.

Urease activity at 0-7.5 cm averaged 2.83 mg NH_4^+ kg^{-1} soil h^{-1}, 2.39 mg NH_4^+ kg^{-1} soil h^{-1} at 7.5-15 cm, and 2.27 mg NH_4^+ kg^{-1} soil h^{-1} at 15-30 cm. Urease activity at 0-7.5 cm was significantly higher than activity at 7.5-15 cm and 15-30 cm. Activity in spring 2017 was significantly higher than fall 2017. Mean urease activity across all depths and treatments in spring 2017 was 2.71 mg NH_4^+ kg^{-1} soil h^{-1} and in fall 2017 was 2.28 mg NH_4^+ kg^{-1} soil h^{-1}.

4.4 Stratification Ratios

Stratification ratios were calculated for each soil quality parameter in each respective season. ANOVA analysis is in Table 5. Cover crop treatment was not significant, so all cover types were combined for analysis of time effects. Time was highly significant for all stratification ratios. Fall 2016 always had a greater stratification ratio than any sampling time in 2017 ($p < 0.05$) (Table 6).

In fall 2016 stratification ratios ranged from 1.48 to 3.63. There was no significant difference between stratification ratios measured in spring and fall 2017 (Table 6). In most cases there was a slight (7-28%) numerical increase in stratification ratio from spring to fall.

Table 5. ANOVA of stratification ratios. p-values followed by ns are not significant, p-values with * indicate significance ($p<0.05$).

Analysis	Time	Treatment
N mineralization	0.001*	0.78 ns
POXc	0.001*	0.99 ns
Phosphatase	< 0.001*	0.17 ns
Sulfatase	< 0.001*	0.71 ns
β-D Glucosidase	< 0.001*	0.39 ns
Urease	< 0.001*	0.38 ns

Table 6. Stratification ratios for soil quality variable. Times with different letters within a row are significantly different ($p < 0.05$).

Assay	Fall 2016	Spring 2017	Fall 2017
N mineralization	3.29A	1.96B	1.84B
POX$_c$	2.11A	1.35B	1.56B
Phosphatase	2.47A	1.67B	1.46B
Sulfatase	3.63A	1.43B	1.83B
β-D Glucosidase	2.2A	1.36B	1.63B
Urease	1.48A	1.12B	1.20B

CHAPTER 5. DISCUSSION

5.1 Cover Crop Growth and Characteristics

Overall, the legume/cereal rye mixtures produced more biomass than the single species cover crops. Legume treatments had higher tissue N and P than the mixes. Cover crop growth was good following the summer maize crop, except for the cereal rye. The cereal rye produced much less biomass (1430 kg ha^{-1}) than is typical of a cereal rye cover crop with no fertilizer (Duiker and Curran, 2005; Ruffo et al., 2004;). Some studies have measured cereal rye biomass production of up to 7,000 kg ha^{-1}. The cereal rye could have experienced poor germination due to the heavy maize residue from the previous summer (resulting in low seed to soil contact).

All legume/cereal rye mixes tended to outperform their single species legume counterparts in terms of biomass. The cereal rye/vetch treatment produced significantly higher biomass than vetch alone, and all mixes significantly outperformed the cereal rye treatment. This is a common observation. In other studies evaluating legume/grass cover crops, mixtures typically produce more biomass than the single species treatments (Brainard et al., 2012; Finney et al., 2016; Shelton, 2015; Snapp et al., 2005). The biomass of the mixtures in my study was comparable to what was found in other studies. For example, Shelton (2015) reported a vetch/wheat mixture produced biomass of approximately 4900 kg ha^{-1}.

The main reason for the higher biomass production in the cereal rye/vetch treatment compared to the vetch and cereal rye treatments is largely due to poor biomass production of the cereal rye alone. The cereal rye was unable to compete with existing weeds as well as the vining and larger leafed vetch could. The vetch treatment produced a more clumping habit that seemed to better suppress weeds, but distribution of the vetch growth over the plot was not uniform. A potential driving factor in the cereal rye/vetch biomass production could be a synergistic effect between the plant growth types. The cereal rye provided a support for the vetch to bind and more evenly grow across the plots, therefore producing more biomass in a more uniformly distributed manner. This effect and synergism due to plant architecture is noted by Brainard et al. (2012).

The N benefit from the legumes can also promote greater cereal rye growth (Shelton, 2015; Snapp et al., 2005), and it is thought that due to the N scavenging abilities of grass species like cereal rye, nodulation and N fixation is promoted in legume/grass

mixtures. This may have affected overall vetch and cereal rye biomass in the cereal rye/vetch treatment.

No weed biomass was measured in the cereal rye/vetch treatment. The uniform growth of the vetch in the cereal rye/vetch treatment was able to suppress weed species better than other treatments. This could be due simply to higher biomass production, or the ability of the vetch to grow more uniformly across the plot when paired with cereal rye, relative to other treatments.

Cover crop tissue P was highest in the vetch treatment, significantly higher than all treatments other than monoculture pea. Vetch is known as a P scavenger (Alsup et al., 2002; Clark 2007) so this is not surprising. The weed tissue P values from the vetch and cereal rye/clover treatments were significantly lower than that for the cereal rye/pea treatment. Further analysis would be needed to identify why this occurred because there was ample soil P as shown from the Mehlich III analysis (Appendix I). Differences in weed species composition may have affected tissue P. Cereal rye/pea had among the lowest percentages of weedy biomass, but weed species diversity was not measured.

Cover crop tissue N was higher in legume treatments alone compared to the legume/rye mixes. This was expected due to the ability of legumes to fix atmospheric N and the overall lower C/N ratio of legumes vs. grasses. The N concentration of the legume/grass mixes was diluted due to the low N content of the cereal rye across all treatments (Figure 10). The vetch and clover treatments had significantly higher tissue N compared to the cereal rye/vetch and cereal rye/clover mixes, whereas pea monoculture treatment tissue N was not significantly higher than that for cereal rye/pea treatment. Due to the greater presence of weeds in the pea monoculture treatment, more of the fixed N in

pea may have been scavenged by the weeds. This possibility is supported by the weed tissue N concentration, which was highest in the pea treatment compared to all other cover crop treatments. The pea monoculture and cereal rye/pea treatments still had higher tissue N levels compared to the cereal rye/clover treatment. Therefore, the lack of significance between the pea and cereal rye/pea treatments may be due to differences in the legume proportion of biomass. The cereal rye/pea treatment may have had a higher proportion of pea dry matter relative to cereal rye, resulting in a relatively high concentration of tissue N.

The vetch treatment had significantly higher levels of tissue N compared to all other treatments. Vetch is well known to be a vigorous grower that fixes large amounts of N, so this result is typical. Clover is typically outperformed by vetches and peas, so this could explain the tissue N results.

5.2   Anaerobic Mineralization

There was no significant cover crop treatment effect on mineralizable N at any depth or sample period. Therefore, the first hypothesis, that there would be less difference in mineralizable N between depths in cover crop mixes than individual cover crops, was not supported. This hypothesis was based on the idea that mixes would influence biological activity to a greater depth in the soil profile than individual species. Stratification ratios for mineralizable N were not significantly affected by cover crop treatment and therefore the presence or absence of mixtures did not differentially affect this biological activity at different depths.

The overall difference in mineralizable N between depths significantly decreased from fall 2016 to spring 2017 (evident from stratification ratios) and persisted through the summer hemp crop. The average mineralizable N in the plots in fall 2016 was around 30 mg kg^{-1} and the average the following 2017 seasons was approximately 80 mg kg^{-1}, which is consistent with other work done in the midwest or mid-south regions (Chu et al., 2017). This also implies some contribution of mineralizable N from the 2016 maize crop residue over winter.

The stratification ratios were significantly lower in spring and fall 2017, compared to all 2016. While lower stratification ratios typically suggest lower soil health, my study argues the opposite. The ratio seems to illustrate a more uniform distribution of mineralizable N throughout the soil profile, which does not indicate greater mineralizable N in one treatment compared to another. Franzluebbers (2002) argued that using organic matter stratification ratios was an indicator of soil quality, and that large ratios indicated higher soil quality because there was increased importance for organic matter at the soil surface. The limitations of this idea to my study include the higher ratios for parameters prior to cover crop use, due to much lower values in the subsurface depths. Increasing values in the lower depths tended to shrink these ratios; not resulting in a loss of soil health but rather an increase in biological activity at lower depths.

As is typical of most no-till systems, with time there is a much greater accumulation of organic matter and microbial activity at the soil surface compared to conventional tillage systems (e.g. Balota et al., 2014; Franzleubbers, 2002; Mbuthia et al., 2015). This was evident when the plots were first sampled; the average SOM stratification ratio was 3.3. Due to the accumulation of organic matter from the previous

years of no-till management, the mineralizable N at the upper depths was many times greater than that of the mineralizable N at deeper depths.

The cover crop tissue N was positively correlated with mineralizable N. As cover crop tissue N in the cover crop treatments increased, so did mineralizable soil N. This is likely due to a lower C:N ratio in the presence of legumes. The C:N ratio was not determined for cover crop residue, but because legume/grass mixes contained a cereal rye component and had significantly lower overall N tissue concentration, it is likely that they had a higher C:N ratio, which would have resulted in a greater probability for immobilization and less available mineralizable N. Ultimately, there was more mineralizable N in fall 2017 than fall 2016, which may reflect the inability of the summer hemp crop in 2017 to adequately scavenge available N from the mineralizing cover crop biomass during the growing season.

5.3   Labile Carbon

The second hypothesis, that mixes containing legumes would cause differences in labile carbon stratification differentially depending on legume species, was also not supported by the data. In spring 2017, immediately following cover crop termination, there was no difference in labile carbon between treatments (there was also no treatment effect in fall 2017). Changes in soil organic C are generally not noticeable in the first few years following cover crop establishment (Blanco-Canqui et al., 2015). Lewis et al. (2011) found that the cover crop treatment did not have an effect on labile carbon after use of a cereal rye and hairy vetch cover crop for one season. Their study found tillage to have greater effect on labile carbon than cover crops.

57

There was a significant increase in labile carbon from pre-cover to post-cover, with the pre-cover average at 446 mg kg^{-1} and the post-cover average at 740 mg kg^{-1}. Both pre- and post-cover values are similar to those found in other studies looking at labile carbon (Lewis et al., 2011; Weil et al., 2003). This significant increase in labile carbon is due to the high input of high-quality biomass from the cover crop. The pre-cover samples were taken following the summer maize season, a point at which plant available nutrients and labile carbon were likely lowest due to the maize crop.

The significant time by depth interaction was driven by differences in the lower two depths between fall and spring 2017. The mean labile carbon at 7.5-15 cm was 629 mg kg^{-1} in spring 2017 and 496 mg kg^{-1} in fall 2017. At the 15-30 cm depth, the mean labile carbon was 527 mg kg^{-1} in spring 2017 and 392 mg kg^{-1} in fall 2017. Following the growing season, the labile carbon that had existed at lower depths was used by the microbial biomass. Failure to recover any residual NO_3^- from anaerobic mineralization incubations was evidence that this labile pool could be utilized by microbial activity.

The hypothesis, that cereal rye would cause increased C deeper in the soil profile than the legumes, was also not supported by the data. In the literature, there is little to no data showing the effect that cereal rye has on labile carbon at differing depths, and there is little data showing how cereal rye affects carbon dynamics at depths below the soil surface. In a study done on cereal rye cover crops in corn and soybean rotations, Moore et al. (2014) found no changes in organic matter pools at depths greater than 5 cm following cereal rye cover crops in a no-till system. Even though cereal rye produces an extensive rooting system, most of the added organic C is concentrated in the surface because it is not being incorporated in no till systems (Moore et al., 2014). Root exudate C from the

cereal rye was not readily available and therefore did not show up in the POXc data. The

the lack of difference in treatments with cereal rye can be attributed to the low amount of

cereal rye biomass produced.

Stratification ratios were significantly lower in spring and fall 2017 compared to

2016. This difference was driven by the increase in labile carbon in both the upper and

lower depths from 2016 to 2017. Also possible is that seasonality and climate played a

role in buildup of labile carbon. In spring 2017, existing C pools were being mineralized

and stimulated by not only the existing cover crop biomass and climate (Chu et al., 2017)

but also by the existing weeds in the control treatment.

5.4 Enzyme Activity

5.4.1 Acid Phosphatase

Phosphatase activity was only significantly affected by depth. Phosphatase

activity had similar values to those found in studies done in Kentucky and the

midwest/south (Acosta-Martinez et al., 2011; Mullen et al., 1998). The limited length of

the study may not have provided enough time for changes in acid phosphatase activity to

occur. Baseline soil samples sent to Regulatory Services also had extractable Mehlich III

soil test P values of approximately 204 kg ha^{-1} of soil test P at 0-15 cm and 162 kg ha^{-1} at

15-30 cm (Appendix I). These values are high for soil test P especially soils without

receiving P input. This is expected in the Maury soil series, which is noted in the Fayette

County soil survey for "being formed in material weathered from phosphatic limestone

and [being] high in phosphate" (Soil Survey, 1968). There was probably no change in

acid phosphatase activity due to the high amount of inorganic P already present in the

59

soil. High amounts of P tend to inhibit phosphatase activity (Nannipieri et al., 2011). Cover crop treatments likewise did not significantly affect phosphatase activity because of the elevated inorganic P levels in the soil.

Stratification ratios for phosphatase activity were significantly lower during spring and fall 2017 compared to fall 2016. This was because of the biomass that came from all treatments. The weed and cover crop biomass provided enough substrate to stimulate biological activity at lower depths, resulting in lower stratification ratios.

5.4.2 Sulfatase

There was no significant treatment effect on sulfatase activity but there was a significant time by depth interaction. Sulfatase activity decreased with increasing depth, which is a well-known trend (Tabatabai, 1996). Sulfatase activity significantly decreased between spring 2017 and fall 2017 and at each depth sulfatase activity was significantly lower in fall 2017 than in spring 2017. These differences show that any potential effect of the cover crops on sulfatase activity did not persist through summer 2017 and into fall 2017.

Sulfate esters can comprise up to 75% of the organic sulfur (S) pool and there been suggestions that arylsulfatase (sulfatase) activity is strongly linked to the S supply in soils, due to the large portion of sulfate that comes from the hydrolysis of the organic fraction (which is largely comprised of the sulfate esters) (Dick, 2011; Hai-Ming et al., 2014). The high quality (low C/N) biomass added to the plots following the termination of the cover crops in spring 2017 likely resulted in greater organic S available for mineralization. Throughout the summer, the organic S supply was likely mineralized and the resulting sulfate utilized by the summer hemp crop and the large amount of weeds

present in the summer hemp crop. During summer growth at sampling in fall 2017, the summer vegetation had not yet completely senesced and most of the S was still residing within plant tissue.

The values in spring 2017were similar to those found in other studies in the region (Mullen et al., 1998). The activity of sulfatase in fall 2017, however, was markedly lower than observed by Mullen et al. (1998). Soil pH was not measured in fall 2017, but it is possible that following the summer application of urea N at a rate of 168 kg N ha^{-1} the pH of the soil further decreased and sulfatase activity was further suppressed (Mullen et al., 1998). Stratification ratios of sulfatase activity were significantly lower during spring and fall 2017 compared to fall 2016. This is because of the biomass that came from the cover crop treatments and also from the weedy fallow treatment. The existing weed biomass and cover crop biomass provided enough substrate to stimulate more biological activity at lower depths, resulting in a lower stratification ratio.

5.4.3   β-D-Glucosidase

β-D-Glucosidase activity in the study was similar to that found in the Acosta-Martinez et al. (2011) study in Kentucky, with the average glucosidase activity in this study being highest at 317 mg p-nitrophenol kg^{-1} soil h^{-1} averaged across depths. This value is higher, however, than the Mullen et al. (1998) study done in Tennessee, but this difference could be due to their use of moist soil in the enzyme assay, which has been shown to potentially alter measured enzyme activity in samples (Dick, 2011; Eivazi and Tabatabai, 1990). Samples for enzyme analysis were air dried before use in this study.

There was a significant treatment by depth interaction, which was driven by the control treatment, which had much higher glucosidase activity in the lower two depths than other treatments. The glucosidase data collected on the control treatment was very narrowly distributed with many outliers (which were not included in the statistical analysis), that may have affected the analysis.

There were differing levels of biomass coming from the treatments that may have influenced glucosidase activity, but these results are in conflict with other studies (Acosta-Martinez et al., 2001; Mullen et al.1998; Peregrina et al., 2014), which found that adding more organic matter or cover crop biomass resulted in higher glucosidase activity. Glucosidase hydrolyses glucose that has come from cellulose (Dick, 2011; Tabatabai, 1996), and this makes it an important enzyme in the C cycle. The control treatment had less aboveground biomass input due to a lack of cover crop but had the highest glucosidase activity in the deepest depth. The importance of quality C (low C:N ratio) in the activity of glucosidase is stressed (Acosta-Martinez et al., 2011). With no significant differences between the control and other treatments in any other measured parameter, the biomass would be the only driving factor, but is the opposite of what the literature suggests for glucosidase activity under cover crop treatment. Therefore, more time is needed to see if this is a consistent result in the plots. In addition, examining rooting depth of weed species and weed species composition would be warranted because this is the only biomass being added to the control treatment other than the annual hemp crop.

Stratification ratios for glucosidase activity were significantly lower during spring and fall 2017 compared to fall 2016. This was because of the biomass that came from the

cover crop and also from the weedy fallow treatments. The existing weed and cover crop biomass provided enough substrate to stimulate more biological activity at deeper depths, resulting in a lower stratification ratio.

5.4.4 Urease

The urease activity measured in the collected soil samples was low compared to reports from other studies (Dick, 2011; Nannipieri et al., 2002; Hai Ming et al., 2014). This difference in activity was likely due to the method used for the urease analysis. There are many buffers used to determine urease activity, although most in the literature tend to use a borate buffer or a modified THAM (tris-hydroxymethyl aminomethane) buffer. The method of Tabatabai (1996) uses a THAM buffer and a 2-hour incubation time. The use of the phosphate buffer can produce lower urease activity values, in the range of 4-5 mg NH_4^+ kg soil^{-1} (Dick, 2011). The phosphate buffer was still elected for use, rather other buffer systems, because it does not interfere with the Weatherburn reagents used for colorimetric determination of NH_4^+.

Time and depth were significant for urease activity, with activity decreasing with depth, which is a common trend (Tabatabai, 1996). Urease activity significantly decreased from spring to fall 2017. This difference can be linked to the higher amounts of labile carbon in spring 2017 versus fall 2017. Peregrina et al. (2014) make a strong correlation between increased labile C and urease activity. With increased amounts of labile carbon, there was increased urease activity. There was no significant treatment effect on labile carbon, or mineralizable N, which likely resulted in there being little difference in urease activity between treatments as well. The lack of difference between treatments may also again be due to the assay used. Toluene is typically added to the

63

solution and not directly to the soil in urease assays, but it was added directly to the soil in this study.

5.5   Stratification Ratios

In Franzleubber's (2002) original study assessing stratification ratios, evaluation was performed on organic matter at the surface depths (0-2.5 cm) divided by subsequent deeper depths. Franzleubber stressed these ratios as indicators of soil health due to the importance of organic matter at the soil surface. Therefore, higher values were argued to indicate a greater degree of soil health. In opposition to this, my study found that the stratification ratios following the implementing of any cover crop shrank the ratio from fall 2016 to spring and fall 2017.

While Franzeubbers's approach might say this indicates lower soil quality, my study argues the opposite. Through cover crop use, the labile carbon and mineralizable N increased from fall 2016 to spring and fall 2017. Comparisons of stratification ratios from 2017, compared to 2016 ratios, show these numbers decreased significantly. However, this was due to the increased activity at deeper soil depths following cover crop treatment, which should not be associated with decreased soil health.

Stratification ratios, from the results of this study, did not indicate greater soil health in one particular treatment over another as shown by the lack of significant differences due to treatments. These ratios, rather, show how the cover crops as a whole affected biological activity at deeper soil depths, compared to having no previous cover crop.

While this ratio begins to explain some changes caused by cover crop use, they can be rather convoluted in their interpretation because overall increases in biological activity can be overlooked when using stratification ratios. Franzleubbers stressed the importance of organic matter and surface activity, but I believe he missed the mark in disregarding the importance of biological activity at lower depths. This study shows that increasing activity at lower depths can significantly affect stratification ratios.

CHAPTER 6. CONCLUSION

This study found there were not differing effects on microbial parameters at various soil depths following the use of different cover crops and cover crop mixes. Most of the results indicate significant effects due to time and depth, but not treatment. The results are consistent with prior work regarding enzyme activity and N dynamics (e.g. Balota et al., 2014; Pandy and Begum, 2010; Peregrina et al., 2014). However, few studies have evaluated cover crop species and their effects on microbial communities and parameters at different sampling depths, particularly with a focus on enzyme activity. Cover crops serve to stimulate biological activity at deeper depths, which is evident from the lower stratification ratios in 2017. The weed population in the control treatment, however, also provided this biological stimulation at deeper depths. The effects of the cover crop and weedy fallow treatments persisted through the summer hemp crop, therefore showing that cover crops can produce season-long benefits even from a singular growth cycle.

Cover crop mixes compared to single species do not produce more biological benefits based on the treatments in this study. While more time may produce significant results, the single species treatments produced similar results to that of mixes.

65

Stratification ratios were not a reliable method of measuring soil biological activity. They can be valuable in assessing soil organic matter or health contained in the profile. The biological assessments done in this study are good measures of biological activity and show the extent to which specific biological processes are occurring in the soil. This study would benefit from more targeted assessments, such as phospholipid fatty acid analysis to provide specific amounts of microbial biomass and specific microbial community size. The hemp crop did not produce any consequence to the benefits of cover crops.

The research increases the basic knowledge regarding cover crops and their interactions with microbial activity, especially when evaluating their impact on microbial enzyme activity and stratification ratios.

APPENDICES

Depth(cm)	0-15					15-30				
Treatment	K kg/ha	P kg/ha	Mg kg/ha	Total N %	Total C %	K kg/ha	P kg/ha	Mg kg/ha	Total N %	Total C %
Clover	488	204	335	0.15	3.15	237	163	265	0.08	1.65
Control	346	186	321	0.17	3.40	161	154	246	0.07	1.56
Pea	483	235	343	0.11	2.90	248	168	268	0.05	1.44
RC	376	178	289	0.14	2.95	201	151	237	0.08	1.50
RCP	404	187	266	0.17	3.31	238	178	264	0.08	1.57
RCVP	398	187	297	0.16	3.17	225	155	245	0.09	1.72
RP	478	225	330	0.12	2.96	249	156	271	0.06	1.43
RV	333	194	263	0.15	3.16	195	150	239	0.08	1.42
RVC	374	216	310	0.11	2.84	191	161	265	0.06	1.48
RVP	326	204	298	0.11	2.87	186	174	266	0.05	1.38
Rye	369	203	314	0.15	3.07	184	159	228	0.08	1.57
Vetch	441	229	289	0.17	3.35	239	167	242	0.08	1.57
Grand Total	402	204	304	0.14	3.10	214	162	254	0.07	1.53

APPENDIX 1: SOIL CHEMICAL PROPERTIES PRIOR TO COVER CROP PLANTING IN SPRING 2017. THE PREVIOUS YEARS' TREATMENTS SHOW NO SIGNIFICANT DIFFERENCES BETWEEN TREATMENTS OR PLOTS. SAMPLES WERE ANALYZED BY THE UK REGULATORY SERVICES SOIL TESTING LAB.

Average of Yield Bu/A	Column1
Treatment	Total
Clover	38.6
Control	43.6
Pea	50.8
RC	40.9
RCP	46.2
RCVP	47.6
RP	46.2
RV	42.0
RVC	43.7
RVP	34.4
Rye	36.9
Vetch	47.9
Grand Total	43.2
STDEV	4.701

APPENDIX 2: MAIZE YIELD FROM SUMMER MAIZE 2016. THERE WAS NO STATISTICALLY SIGNIFICANT DIFFERENCE BETWEEN PRIOR TREATMENTS OR PLOTS.

APPENDIX 3: RESEARCH PLOTS IN FALL 2016 IMMEDIATELY FOLLOWING COVER CROP PLANTING.

APPENDIX 4: TOP LEFT- P TREATMENT NEXT TO CEREAL RYE/PEA
TREATMENT.
TOP RIGHT- CLOVER BLOOMING NEXT TO CEREAL RYE/PEA
TREATMENT.
BOTTOM LEFT- VETCH NEXT TO CERAL RYE/VETCH.

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71

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