Using Cover Crops and Arbuscular Mycorrhizal (AM) Fungi to Enhance the Sustainability of Hardneck Garlic Production in the Northeast

Alexandra Smychkovich
University of Massachusetts Amherst

Follow this and additional works at: https://scholarworks.umass.edu/dissertations_2

Recommended Citation

This Open Access Dissertation is brought to you for free and open access by the Dissertations and Theses at ScholarWorks@UMass Amherst. It has been accepted for inclusion in Doctoral Dissertations by an authorized administrator of ScholarWorks@UMass Amherst. For more information, please contact scholarworks@library.umass.edu.
Using Cover Crops and Arbuscular Mycorrhizal (AM) Fungi to Enhance the Sustainability of Hardneck Garlic Production in the Northeast

A Dissertation Presented
by
ALEXANDRA SMYCHKOVICH

Submitted to the Graduate School of the University of Massachusetts Amherst in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

September 2023

Plant and Soil Sciences Graduate Program
DEDICATION

For my grandparents, who first taught me about cover crops and with whom I first planted garlic.
ACKNOWLEDGEMENTS

I would like to thank my advisor, Masoud Hashemi, for his unwavering support and guidance over the past five years. He has encouraged me, without reservation, to pursue new goals and challenges. I am forever grateful for his careful mentorship and friendship, which have facilitated my growth both as a scientist and as a human. I would also like to extend thanks to my committee members, Wes Autio, Rob Wick, Ashley Keiser, and Dong Wang, who have persistently guided me through my academic journey and inspired my interest in new topics and disciplines.

I’d like to thank my lab managers, Sarah Weis and Samantha-Glaze Corcoran, for their mentorship, patience, and good humor, both in and outside of the lab. I have learned an immense amount from them and deeply appreciate their dedication to detail and the ingenuity that they bring to lab work. I owe a huge debt of gratitude to my research assistant, Olivia Larrivee, who has helped with nearly every aspect of this dissertation project and who has brought a spirit of teamwork and lightheartedness to the process. Appreciation is also due to Jasper Cowley for his help in the lab, particularly in developing a new protocol with me in the wake of a concussion, a process I imagine required significant resolve on his part.

I am deeply grateful for the help and hard work of Neil Woodard, Zack Zenk, Jim Cronk, and Keith Lilly, who have been instrumental in the implementation, troubleshooting, and crisis management of my field experiments. Thank you for your support and friendship, keeping me caffeinated during long days in the field, always being willing to help, and responding kindly when things didn’t go ‘according to plan.’
I am unconditionally grateful to my family and friends, who have consistently provided their warmth and kindness, keeping in me in good spirits, helping with harvests, and most importantly, keeping me well-fed in even the busiest of times. I will forever remember your endless support, love, and patience during this process.

Lastly, I would like to extend appreciation to my funding sources, without which I would not have been able to complete this project. The Lotta M. Crabtree Foundation, the Stockbridge School of Agriculture, Annie’s Homegrown, the Center for Agriculture, Food and the Environment, the Massachusetts Department of Agricultural Resources, Northeast SARE, and the UMass Graduate School have all provided financial support that allowed me to complete my dissertation. Additionally, I would like to thank the New England Vegetable and Berry Growers Association, the College of Natural Sciences, and the Stockbridge School of Agriculture for their support in sending me to complete a nematode identification course at Wageningen University in 2022, and Rob Wick, who not only made this trip possible, but instilled in me a passion for nematology that has changed my life forever.
ABSTRACT

USING COVER CROPS AND ARBUSCULAR MYCORRHIZAL (AM) FUNGI TO ENAHCE THE SUSTAINABILITY OF HARDNECK GARLIC PRODUCTION IN THE NORTHEAST

SEPTEMBER 2023

ALEXANDRA SMYCHKOVICH, B.S., UNIVERSITY OF MASSACHUSETTS AMHERST

Ph.D., UNIVERSITY OF MASSACHUSETTS AMHERST

Directed by: Professor Masoud Hashemi

Sustainable intensification has been proposed as a necessary means to ensure global food security by increasing agricultural production while protecting the environment. This dissertation evaluated an alternative garlic production system in which two major components of sustainable agriculture, cover crops and arbuscular mycorrhizal (AM) fungi, were integrated into production via a relay cropping system. Garlic was relay cropped into three species of annual, fall planted cover crops, planted in monoculture and as a mixture, and inoculated with a commercially available AM fungi inoculant at the time of planting. The three cover crop species chosen for evaluation in the alternative system were oat (Avena sativa), daikon radish (Raphanus sativus) and field pea (Pisum sativum). Rhizodeposition of fall cover crops provides soil organisms with a steady source of labile C until frost termination, after which nutrients are recycled via the decomposition of cover crop residues. Further, cover crops facilitate the development of AM fungi, which can improve crop nutrient uptake and soil structure via aggregate formation. Growers in the northeastern United States are confined by limited production acreage and a short growing season; thus, maximizing land use efficiency on farms is
critical to maintaining economic viability. Due to the exceptionally long cultivation period associated with hardneck garlic, garlic producers miss an opportunity to plant fall cover crops. The proposed system was developed to enhance soil health and nitrogen use efficiency, support soil biology, and facilitate nutrient cycling without taking land out of production. Overall, the alternative system showed promise to improve the sustainability of garlic production by increasing soil food web structure and improving nitrogen use efficiency (NUE), particularly when garlic was relay cropped into oat cover crops. Additionally, AM fungi improved iron (Fe) uptake in garlic in Fe limited soils. However, garlic bulb yield was negatively impacted by the relay crop system when cover crop biomass was high, suggesting that the economic viability of this system is dependent on cover crop biomass accumulation in the fall and that further research is required to ensure the feasibility of farmer adoption.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGEMENTS</td>
</tr>
<tr>
<td>ABSTRACT</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
</tr>
</tbody>
</table>

## CHAPTER

1. GENERAL INTRODUCTION .......................................................... 1

   1.1 Social, Economic, and Environmental Consequences of Intensive Agriculture .................................................. 1
   1.2 Sustainable Intensification and Soil Health ............................. 5

      1.2.1 Cover Crops .......................................................... 7
      1.2.2 Nitrogen Use Efficiency (NUE) .................................... 11
      1.2.3 Arbuscular Mycorrhizal (AM) Fungi ............................. 13
      1.2.4 Soil Food Webs and Ecosystem Function ......................... 16
      1.2.5 Relay Cropping ..................................................... 21

   1.3 Status of Soils and Food Systems in New England .................. 22
   1.4 Garlic Production in the Northeast ................................... 23
   1.5 Applied Research Knowledge Gaps and Project Objectives .......... 25
   1.6 Chapter References ..................................................... 27

2. ASSESSING THE ROOT AND SHOOT COMPOSITION, C CONTRIBUTION, DECOMPOSITION AND N MINERALIZATION TRENDS OF SINGLE SPECIES AND MIXED COVER CROPS ............................................. 45

   2.1 Abstract ................................................................. 45
   2.2 Introduction ............................................................ 46
   2.3 Materials and Methods .................................................. 50

      2.3.1 Experimental Site and Weather Conditions ..................... 50
      2.3.2 Experiment Layout ................................................ 51
      2.3.3 Preparation of Litter Bags and Data Collection ............... 51
      2.3.4 Sample Processing and Laboratory Analysis ................... 52
      2.3.5 Calculation of k, k_n, and k_c ................................. 53
2.3.6 Statistical Analysis ........................................................................ 54

2.4 Results ................................................................................................ 54

2.4.1 Weather at the Experimental Site ............................................. 54
2.4.2 Dry Weight Biomass Yield, N and C Accumulation, and C:N of Cover Crop Roots and Aerial Parts ........................................... 55
2.4.3 Moisture, Lignin, Cellulose, and Hemicellulose Content of Cover Crop Roots and Aerial Parts ............................................. 58
2.4.4 Spring Dry Matter Decomposition, N and C Release from Cover Crop Residues ........................................................................ 61
2.4.5 Differences among rate constants k, kc, and kn .......................... 70
2.4.6 Relationships between decomposition rate constants, proportion of litter decomposed, and litter quality parameters .......... 71
2.4.7 Changes in C:N of Cover Crop Root and Aerial Residues During Spring Decomposition ......................................................... 72

2.5 Discussion .......................................................................................... 73

2.5.1 Cover crop biomass, N and C yield, and residue quality .......... 74
2.5.2 Cover crop root and aerial residue decomposition .................. 78
2.5.3 Differences in residue decay rate, N and C release ................. 81
2.5.4 Relationships between litter quality and decay rate constants ... 82
2.5.5 Changes in root residue C:N during decomposition ............... 84

2.6 Conclusions ....................................................................................... 85
2.7 Chapter References ........................................................................... 87

3. EVALUATING THE IMPACTS OF ANNUAL, FALL PLANTED COVER CROPS AND ARBUSCULAR MYCORRHIZAL (AM) FUNGI ON GARLIC YIELD, BULB NUTRIENT ACCUMULATION, AND NITROGEN USE EFFICIENCY IN A RELAY CROP SYSTEM ................................................................. 102

3.1 Abstract ............................................................................................ 102
3.2 Introduction ...................................................................................... 103
3.3 Materials and Methods ................................................................... 110

3.3.1 Experimental Site and Weather Conditions ......................... 110
3.3.2 Experiment Layout and Management .................................... 110
3.3.3 Sample and Data Collection ..................................................... 112
3.3.4 Laboratory Analysis ................................................................. 114
3.3.5 Determination of Garlic Bulb NUE and NUtE ..................... 114
3.3.6 Statistical Analysis ................................................................. 115

3.4 Results .............................................................................................. 115
3.4.1 Weather Conditions at the Experimental Site .................. 115
3.4.2 Cover Crop Biomass and N Yield .............................. 117
3.4.3 Garlic Bulb, Aerial, and Root Dry Weight at Harvest ........ 122
3.4.4 Spring Biomass Accumulation and N Concentration of Garlic Bulbs, Aerial Parts, and Roots ............................. 123
3.4.5 Changes in Garlic Leaf Chlorophyll Concentrations During the Growing Period ................................................. 124
3.4.6 Garlic AE and NUEcrop ........................................ 125
3.4.7 Fluctuations in Spring Soil Nitrate Status ....................... 126
3.4.8 Nutrient Concentration of Garlic Bulbs at Time of Harvest .... 127

3.5. Discussion .................................................................. 130
3.6 Conclusions ............................................................ 142
3.7 Chapter References .................................................... 144

4. SHORT-TERM RESPONSE OF NEMATODE COMMUNITIES, PERMANGANATE OXIDIZABLE CARBON (POXC) LEVELS, AND BIOLOGICAL ACTIVITY TO COVER CROP RESIDUE DECOMPOSITION IN A GARLIC RELAY CROP SYSTEM .................................................. 157

4.1 Abstract ..................................................................... 157
4.2 Introduction ............................................................. 158
4.3 Materials and Methods ................................................ 164
   4.3.1 Experimental Site and Weather Conditions ...................... 164
   4.3.2 Experiment Layout and Management ............................ 165
   4.3.3 Sample and Data Collection ...................................... 166
   4.3.4 Laboratory Analysis ................................................ 167
   4.3.5 Statistical Analysis .................................................. 169

4.4 Results ....................................................................... 169
   4.4.1 Weather conditions and cover crop biomass, composition, and C input .......................................................... 169
   4.4.2 Biological Activity ................................................... 171
   4.4.3 Permanganate Oxidizable C (POXC) ............................ 172
   4.4.4 Nematode Faunal Analysis ....................................... 173
   4.4.5 Relationships between soil health indicators and NBIs ....... 177

4.5 Discussion .................................................................. 179
   4.5.1 Changes in Biological Activity and POXC levels ............ 179
   4.5.2 Nematode faunal analysis and soil food web response to cover crop amendments .............................................. 180
4.5.3 Relationships of NBIs to soil health indicators .......................... 187

4.6 Conclusions .................................................................................. 188
4.7 Chapter References ....................................................................... 189

5. GENERAL CONCLUSIONS ................................................................. 200

BIBLIOGRAPHY .................................................................................. 204
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1 Regional weather conditions at the experimental site and deviation from the regional norm</td>
<td>55</td>
</tr>
<tr>
<td>2.2 Dry Matter Biomass, N and C Yield Cover Crops at Time of Winterkill</td>
<td>56</td>
</tr>
<tr>
<td>2.1. N and C Concentration of Cover Crops at Time of Winterkill</td>
<td>57</td>
</tr>
<tr>
<td>2.4. Influence of cover crop treatment on biomass, nitrogen and carbon exponential decay constants $k$, $k_c$, and $k_n$</td>
<td>70</td>
</tr>
<tr>
<td>2.5. Pearson correlation coefficients ($r$) between decay parameters $k$, $k_n$, $k_c$ and residue quality parameters (n=16)</td>
<td>71</td>
</tr>
<tr>
<td>3.1. Regional weather conditions at the experimental site and deviation from the regional norm</td>
<td>116</td>
</tr>
<tr>
<td>3.2. Influence of cover crop treatments on the dry weight (g) of garlic shoots, bulbs, and roots at time of harvest</td>
<td>122</td>
</tr>
<tr>
<td>3.3. Influence of cover crop treatments on the nutrient concentration (g kg$^{-1}$) of garlic bulbs at time of harvest</td>
<td>128</td>
</tr>
<tr>
<td>4.1 Regional weather conditions at the experimental site and deviation from the regional norm</td>
<td>170</td>
</tr>
<tr>
<td>4.2. Dry matter biomass of cover crops at time of winterkill</td>
<td>171</td>
</tr>
<tr>
<td>4.3. Cover crop residue composition, C:N, and C kg ha$^{-1}$ released during the experimental period</td>
<td>171</td>
</tr>
</tbody>
</table>
4.4. Nematode-based Indices (NBIs) associated with cover crop spring residue decomposition
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1 Mouthparts of nematodes of various feeding groups</td>
<td>19</td>
</tr>
<tr>
<td>1.2 Primary objectives, research activities, and outcomes associated with this dissertation project</td>
<td>26</td>
</tr>
<tr>
<td>2.1. Initial carbon: nitrogen (C:N) ratios of the cover crop aerial and root residues at time of winterkill</td>
<td>58</td>
</tr>
<tr>
<td>2.2. Percent initial hemicellulose, cellulose, and lignin composition of root and aerial residues</td>
<td>59</td>
</tr>
<tr>
<td>2.3 Dry matter (DM) remaining in cover crop aerial and root residues during spring decomposition</td>
<td>66</td>
</tr>
<tr>
<td>2.4. Percent N remaining in cover crop aerial and root residues during spring decomposition</td>
<td>67</td>
</tr>
<tr>
<td>2.5. Percent C remaining in cover crop aerial and root residues during spring decomposition</td>
<td>68</td>
</tr>
<tr>
<td>2.6 Total nitrogen (N) and carbon (C) released from cover crop residues during the experimental period</td>
<td>69</td>
</tr>
<tr>
<td>2.7. Changes in C:N of cover crop root residues during spring decomposition</td>
<td>73</td>
</tr>
<tr>
<td>3.1. Total dry matter (DM) biomass and N yield (kg ha(^{-1})) accumulated by cover crop treatments before winterkill in the two experimental years</td>
<td>118</td>
</tr>
<tr>
<td>3.2 Fresh weight (FW) garlic bulb yield (kg ha(^{-1})) as affected by cover crop treatments during the two experimental years</td>
<td>119</td>
</tr>
<tr>
<td>3.3 Effects of nitrogen (N) fertilizer on garlic growth</td>
<td>121</td>
</tr>
</tbody>
</table>
3.4 Patterns of garlic dry weight biomass accumulation (g plant\(^{-1}\)) and N concentration (g kg\(^{-1}\)) from April 1 – June 24……………………………………………………………………………….123

3.5. SPAD measurements of garlic shoots as affected by cover crop treatment, sample date, and N fertilizer application……………………………………………………………………………..125

3.6. Nitrogen Use Efficiency (NUE\(_{\text{crop}}\)) as affected by cover crop treatment and nitrogen (N) fertilization……………………………………………………………………………………………..126

3.7. Soil nitrate concentration (mg g\(^{-1}\) soil) as affected by cover crop treatment and sampling date during the garlic growth and residue decomposition period in the spring………………………………………………………………………………………127

3.8. Nutrient concentrations (g kg\(^{-1}\)) of garlic bulbs at time of harvest, as affected by rate of nitrogen (N) fertilizer application…………………………………………………………………………………..129

4.1 Microbial respiration (µl CO\(_2\) g\(^{-1}\) soil h\(^{-1}\)) as affected by sampling date during the experimental period………………………………………………………………………….172

Figure 4.2. Soil potassium permanganate (KMnO\(_4\)) oxidizable C (POX–C) (mg kg\(^{-1}\)) as affected by sampling date during the experimental period…………………………………….173

4.3 Indices of food web condition as determined by nematode faunal analysis and affected by sampling date during the experimental period……………………………………….175

Figure 4.4 Relationships between potassium permanganate (KMnO\(_4\)) oxidizable C (POXC) (mg kg\(^{-1}\)), microbial respiration (µl CO\(_2\) g\(^{-1}\) soil h\(^{-1}\)), and nematode-based indices of food web condition………………………………………………………………………………………………………………178
CHAPTER 1
GENERAL INTRODUCTION

1.1 Social, Economic, and Environmental Consequences of Intensive Agriculture

As world populations continue to increase and arable land is diminished annually, the food security of our planet grows increasingly unstable. More than 1 billion people in the world are food-insecure, with 462 million adults reported as underweight and approximately 45% of deaths among children under age 5 linked to malnutrition worldwide in 2014 (World Health Organization, 2020). It is predicted that global food supply will need to increase drastically to meet demand by 2050 (Lal, 2010). The most productive soils on the planet have already been exploited for agriculture and estimates predict that agricultural land degradation will continue to have negative effects on food production in the next half century (Hanson et al., 2008; Kendall & Pimentel, 1994). The steady depletion of non-renewable resources coupled with a rapidly changing climate further exacerbates the threat of food insecurity worldwide.

More than 99.7% of human caloric intake comes from commodities produced on land (Pimentel & Burgess, 2013). Therefore, land availability and the long-term productivity of agricultural soils are crucial to maintaining global food supply and supporting human life on this planet. Approximately half of the land on earth is devoted to agricultural production, with 38% used for cropping and grazing (Amundson et al., 2015; Foley et al., 2011; Pimentel & Burgess, 2013). However, accelerated rates of topsoil erosion combined with rapid urbanization are contributing to significant losses of production acreage and a decrease in the percentage of people employed in
agriculture around the world (Amundson et al., 2015; Hanson et al., 2008). Estimates suggest that urbanization will continue to increase, with an additional 1.5 million km\(^2\) predicted to be urbanized by 2030 (Seto, 2011).

Over the course of the last century, sustainable soil stewardship has been largely replaced with low-cost energy fertilizer inputs and machinery, reducing the need for agricultural labor and increasing reliance on fossil fuels and other non-renewable resources to replace nutrients displaced by intensive agriculture (Erisman et al., 2008; Lal, 2006). The energy intensive Haber-Bosch process as well as the mining and transport of other essential plant nutrients such as P and K is unsustainable in the long term (Erisman et al., 2008; Schipanski et al., 2014). Furthermore, there are no substitutes for rock-derived plant essential nutrients such as P, K, and Ca, which exist only in limited geological reservoirs. As the global supply of non-renewable nutrients is steadily exhausted, fertilizer prices increase, significantly impacting the economic stability of farmers and food security worldwide (Amundson et al., 2015).

Prioritizing soil conservation and rehabilitation in agricultural systems is necessary to support growing populations and avoid higher rates of malnutrition (Pimentel & Burgess, 2013). Erosion from agricultural systems accounts for approximately three-quarters of total soil erosion worldwide, occurring at an accelerated rate estimated to be 75 times greater than in natural forest areas due to inappropriate soil management practices and inconsistent vegetative cover (Lal, 2016a; Pimentel & Burgess, 2013; Telles et al., 2011). Erosion by wind and water alters soil chemical, physical, and biological properties, dramatically reducing soil fertility and causing decreased production and subsequent economic losses (Lal, 2006; Telles et al.,
Areas of land covered by vegetation or mulch are more resistant to erosion than bare soil because the layer of plant biomass serves as a protective barrier, holding together topsoil and dissipating water droplets and wind energy (Pimentel & Burgess, 2013). Intensive crop production often leaves land fallow for long periods of time between planting, exposing soil to erosion.

Fertile soil is displaced from agricultural systems at rates significantly greater than soil formation (Amundson et al., 2015; Eswaran et al., 2001; Lal, 2010; Pimentel & Burgess, 2013). Topsoil contains the bulk of plant available nutrients, averaging 1 to 6 kg of nitrogen (N), 1 to 3 kg of phosphorus (P), and 2 to 30 kg of potassium (K) per ton (Pimentel & Burgess, 2013). Soil organic matter found close to the soil surface contains approximately 95% of total soil N and 50% of total soil P (Pimentel & Burgess, 2013). Soil organic matter facilitates soil aggregation, improves soil structure and water filtration, and encourages microbial growth and diversity (Amundson et al., 2015). Soil removed from agricultural systems by erosion is estimated to contain 1.3 to 5 times more organic matter than the soil left behind (Pimentel & Burgess, 2013). Nutrient losses from agricultural systems by soil erosion contribute to poor soil fertility and encourage high fertilizer use on farms to meet crop production requirements (Eswaran et al., 2001; Pimentel & Burgess, 2013).

Lastly, water storage capacity and infiltration are reduced in heavily eroded soils, negatively impacting water use efficiency in agricultural systems. These changes hinder crop growth, limit the diversity of commodities that can be supported by an area of land and lead to higher production costs due to increased reliance on off-farm inputs (Pimentel & Burgess, 2013; Telles et al., 2011). These expenses include fertilizer and
irrigation costs as well as increased labor expenditures to offset the damage caused by accelerated topsoil removal (Telles et al., 2011). Loss in soil production potential threatens the overall economic security of farmers, can lead to drops in land value and land abandonment (Pimentel & Burgess, 2013; Telles et al., 2011).

In addition to the economic and food security consequences of poor soil stewardship, ecological concerns including the disturbance of soil microbial communities, increased emissions of greenhouse gases (GHGs), and pollution of waterways are growing (Erisman et al., 2008). One cubic meter of soil may support billions of microbes and tens of thousands of earthworms and arthropods (Pimentel and Burgess, 2013). The biodiversity and abundance of soil biota is decreased in eroded soils, resulting in less resilient agricultural systems (Pimentel & Burgess, 2013). About 60% of the soils eroded from U.S. croplands each year are deposited into streams and rivers (Pimentel et al., 1995). Nitrate (NO$_3^-$) losses from agricultural systems contaminate groundwater and are responsible for significant eutrophication and habitat destruction (Bergström et al., 2008; Erisman et al., 2008; Pimentel et al., 1995). Nitrous oxide (N$_2$O) and ammonia (NH$_3$) emissions from inorganic fertilizer and manure applications contribute to air pollution and global warming, while the vast energy demands of modern agriculture further accelerate climate change (Bergström et al., 2008; Erisman et al., 2008; Glendining et al., 2009).

Intensive agricultural practices and heavy reliance on fossil fuels have already disrupted the global carbon (C) cycle, intensifying per capita C emissions (Lal, 2010; Van Oost et al., 2007). Soil cultivation is responsible for a large portion of GHG emissions, releasing vast amounts of CO$_2$ into the atmosphere annually (Amundson et
al., 2015; Lal, 2006). C can be returned to the soil through the fixation of atmospheric CO$_2$ by plants (Lal, 2006). Reducing the amount of agricultural land left without vegetative cover can sequester atmospheric C and reduce the negative impact of agriculture on climate change.

1.2 Sustainable Intensification and Soil Health

In recent years, sustainable intensification has been proposed as a promising strategy to ensure global food security while mitigating the deleterious effects of food production on the environment (Garnett et al., 2013; Hunter et al., 2017; The Royal Society, 2009). Sustainable intensification identifies food production and environmental protection as “equal parts of agriculture’s grand challenge” (Hunter et al., 2017) and rejects the notion that increased food production should prioritize yield increase at any cost (Garnett et al., 2013). The development of sustainable production systems should prioritize meeting production and environmental goals simultaneously, which may be difficult or impossible in some systems (Garnett et al., 2013; Hunter et al., 2017). As such, sustainable intensification requires the development of practices that are context and location specific, and in some systems, reductions in yield may be a necessary consequence of sustainable production (Garnett et al., 2013; Hunter et al., 2017).

Sustainable agriculture prioritizes soil and natural resource conservation during production, with an emphasis on producing nutrient dense food that promotes human health without compromising the economic security of farmers (Bergström et al., 2008). Major goals of sustainable systems include maintaining vegetative cover on soil
at all times, reducing off-farm inputs (such as fertilizers, pesticides, and herbicides) by building natural soil fertility, and enhancing agroecological processes that support nutrient cycling by supporting soil microbe diversity and abundance (Hanson et al., 2008; The Royal Society, 2009). Soil conservation techniques incorporate cover crops, crop rotations and intercropping into production systems to increase biodiversity and land use efficiency on farms (Pimentel & Burgess, 2013). Other approaches, such as no-till or reduced tillage systems, are geared towards reducing soil disturbance to minimize erosion, maintain soil organic matter, and support the abundance and biodiversity of soil biota (Pimentel & Burgess, 2013; Telles et al., 2011). Improved soil stewardship can lead to greater nutrient use efficiency and natural soil fertility, minimizing the need for energy-intensive inputs and increasing productivity on farms. In this way, agricultural systems can reduce their reliance on fossil fuels, increase the soil organic carbon pool and mitigate the effects of climate change (Lal, 2010).

Soil is composed of an inorganic fraction of mineral matter (used to classify soil texture), organic matter, water, gases, and living organisms (Doran & Parkin, 1994). The status of these components and their interactions informs the health and quality of agricultural soils. Soil quality is primarily used to describe soil function and value as a substrate (Lal, 2016b). Soil health refers to the capacity of a soil to function as a dynamic ecosystem capable of sustaining biological productivity and maintaining environmental quality (Doran & Parkin, 1994; Frąc et al., 2018; Lal, 2016b). In recent years, the following four principles as being the most critical to maintaining soil health: 1) continuous soil cover, or ‘armor’ 2) minimized soil disturbance 3) plant and ecosystem biodiversity; and 4) the presence of continuous living roots (NRCS-USDA,
Soils that are considered healthy have good structure, are well aerated, contain high levels of both labile and stable organic matter, and retain a supply of nutrients sufficient to support plant and microbe productivity (Lal, 2016a).

Appropriate management of agricultural systems is crucial to maximizing their soil health benefits. Tillage, fertilizer inputs, and the use of herbicides and pesticides disrupt soil microbial communities and negatively impact AM development and spore production (Frąc et al., 2018; Morriën, 2016; Verzeaux, Alahmad, et al., 2016; Verzeaux et al., 2017). A recent report indicated that the use of winter cover crops in conventional tillage systems can mitigate the negative effects of intensive N fertilization on soil microbial biodiversity and nutrient cycling (Verzeaux, Alahmad, et al., 2016). Combining practices that promote AM colonization, such as the use of cover crops, organic management, and reduced tillage can substantially improve on-farm soil health and increase microbial biodiversity (Bowles et al., 2016; Harkes et al., 2019; Nivelle et al., 2016; Verzeaux, Alahmad, et al., 2016). It is crucial that new and innovative management strategies are identified, researched and adopted by farmers to facilitate the rehabilitation of depleted land, protect soil from further erosion, and work towards an ecologically sound and food secure future. Introducing cover crops and AM fungi into production systems can amplify the effects of both on soil health and crop productivity (García-Gonzálvez et al., 2016).

1.2.1 Cover Crops

Cover crops, grown primarily for their contributions to soil health and environmental benefits rather than for harvest, are the backbone of sustainable agriculture (Delgado et al., 2007). Integrating cover crops into production systems can
improve soil health and quality by increasing water infiltration, improving overall soil structure, protecting topsoil from erosion, and feeding soil microbes via root exudates and residue decomposition (Dabney et al., 2001). Cover crop residues left on the soil surface dissipate the energy of raindrops, reducing water runoff and soil loss to erosion, and add organic matter to agricultural soils as they decompose (Fageria et al., 2005; Smith et al., 1987). Additions of organic matter enhance soil aggregation, resulting in improved structure that increases water infiltration and storage, hence reducing surface runoff (Fageria et al., 2005; Neher & Barbercheck, 2019; Smith et al., 1987).

Incorporating annual, fall planted cover crops into production systems can improve on-farm nutrient cycling, soil quality, and yields of subsequent crops while simultaneously reducing environmental pollution and decreasing reliance on external inputs (Dabney et al., 2001; Dean & Weil, 2009; Delgado et al., 2007; Dinnes et al., 2002; Tonitto et al., 2006; Wang et al., 2008; Wyland, 1996). Fall planted cover crops can accumulate up to 199 kg N / ha (178 lb N / ac) of residual N and reduce nitrate leaching by up to 70% compared to bare fallow systems (Delgado et al., 2007; Tonitto et al., 2006). The benefits of cover crops to cropping systems vary by crop type and management style. Brassica cover crops produce large amounts of biomass, which can enhance nutrient cycling and provide substantial weed control and yield benefits to subsequent crops (Lawley et al., 2012; Wang et al., 2008). Legumes supply successor crops with significant amounts of biologically fixed atmospheric N, and cereal crops scavenge for available soil nutrients, including residual nitrate, which they remove from the soil and store in organic form until frost terminated (Dabney et al., 2001; Dinnes et al., 2002; Fageria et al., 2005; Sainju & Singh, 1997; Tonitto et al., 2006). Although
monoculture cover crops may provide more of one specific benefit than multi-species mixtures, reports indicate that mixed plantings maximize the overall ecological services provided by cover crops (Brennan et al., 2009; Dabney et al., 2001; Fageria et al., 2005; Radicetti et al., 2016; Tribouillois et al., 2016). In the spring, decomposing cover crop residues release stored nutrients to subsequently planted cash crops, increasing yield and quality (Chen & Weil, 2011; Dinnes et al., 2002; Fageria et al., 2005; Wyland, 1996).

The timing of cover crop residue decomposition and associated N mineralization relative to successor crop uptake determines the magnitude of benefits provided by cover crops and must be taken into consideration to ensure appropriate on-farm nutrient management (Dabney et al., 2001; Doran, 1991; Shennan, 1992; Tonitto et al., 2006). The synchrony of frost terminated cover crop decomposition and crop nutrient uptake is influenced by many factors, including soil temperature, cover crop species composition, time of planting, and residue management (Dabney et al., 2001; Sainju & Singh, 1997).

The C:N of cover crop residues and method of their incorporation determines the fate of early season soil N (Doran, 1991). Cover crops with C:N lower than 25 encourage N mineralization, while residues with high C:N (greater than 35) can facilitate N immobilization and reduced N availability to subsequently planted cash crops (Dabney et al., 2001; Dinnes et al., 2002; Sainju & Singh, 1997). Cereal grain cover crops such as oats and rye have high C:N that can cause N immobilization (Aulakh et al., 1991; Dabney et al., 2001; Doran, 1991). Legumes, on the other hand, typically have C:N lower than 20 and as a result contribute significant amounts of N to
successor crops (Dabney et al., 2001; Smith et al., 1987). However, appropriate management is crucial to ensure the benefits of legume cover crops are realized by successor crops. Not only does $\text{N}_2$ fixation by annual legumes decline during physiological maturation, allowing legume cover crops to mature beyond flowering may tie up N in the seed and lead to slower mineralization (Dabney et al., 2001; Smith et al., 1987). On the other hand, the rapid decomposition of legume residues at early stages of maturity can provide poor synchrony to successor crops and increase N losses to the environment if managed poorly (Plaza-Bonilla et al., 2017; Sievers & Cook, 2018). The use of legume and cereal cover crop mixtures can ‘balance’ the C:N of residues, modifying N release and providing a combination of benefits to cropping systems (Dabney et al., 2001; Fageria et al., 2005; Fageria & Baligar, 2005; Lawley et al., 2012; Lawson et al., 2015; Tribouillois et al., 2016).

In addition to cover crop species composition, residue management heavily influences the rate of decomposition and nutrient release in agroecosystems. Residues left on the soil surface have significantly slower decomposition and mineralization rates than residues that are incorporated by disking or tilling (Etemadi et al., 2018; Jahanzad et al., 2016). The roots of cereal crops experience a lag in decomposition if left in place but begin decomposition immediately if incorporated (Malpassi et al., 2000). Legume roots decompose rapidly, regardless of whether they are incorporated 2018 (Jani et al., 2016; Sievers & Cook, 2018).
1.2.2 Nitrogen Use Efficiency (NUE)

The decline of natural fertility in agricultural soils worldwide has resulted in the overwhelming dependence of global agriculture on off-farm inputs to meet crop nutrient demands, exhausting global supplies of non-renewable resources, threatening food security and contributing significantly to environmental pollution (Delgado et al., 2007; Flora, 2010; Hawkesford & Griffiths, 2019; Tan et al., 2005). Because agronomic crops recover only a small portion of inorganic $N$ fertilizer, most of the $N$ applied to farming systems is immobilized in the soil or lost through leaching, denitrification, and gaseous emissions (Baligar et al., 2001; Delgado et al., 2007; Sainju & Singh, 1997; Tan et al., 2005; Tonitto et al., 2006). Transition to sustainable methods of crop production is the most effective approach to improve on-farm N use efficiency (NUE) and is crucial to reducing agricultural reliance on fossil fuel based fertilizers (Baligar et al., 2001; Hawkesford & Griffiths, 2019; Plaza-Bonilla et al., 2017).

Agricultural nitrogen use efficiency (NUE) can be influenced by many factors, including the time and method of fertilizer application, chemical composition of fertilizer or input, the physical, chemical, and biological properties of a soil, management techniques used by the farmer, and lastly, the efficiency of the crop species being grown (Baligar et al., 2001; Sheldrick et al., 2002; Tan et al., 2005). Improving crop NUE can prevent N over-fertilization, minimize reliance on off-farm inputs, increase growers’ income, and reduce the risk of environmental pollution (Delgado et al., 2007).

Although nitrate ($\text{NO}_3^-$) made available by the mineralization of organic matter and/or the application of inorganic fertilizers contributes significantly to meeting crop
N requirements, its high mobility makes NO\textsubscript{3} a major source of non-point source water pollution. NO\textsubscript{3} losses from farming systems are responsible for groundwater contamination, eutrophication, and habitat destruction (Bergström et al., 2008; Pimentel et al., 1995). Additionally, nitrous oxide and ammonia emissions from fertilizer applications contribute to air pollution and accelerated global warming (Bergström et al., 2008; Glendining et al., 2009).

Developing alternative management and production methods that improve NUE on farms is essential to ensure the sustainability of global food production and prevent further environmental degradation (Doran, 1991) (Baligar et al., 2001; Dean & Weil, 2009). Addition of organic amendments (e.g. compost, animal manures, and crop residues), effective use of proper crop rotations, and integration of cover crops can enhance the NUE of cropping systems by gradually releasing N, building soil organic matter, and improving soil structure (Baligar et al., 2001; Delgado et al., 2007). Other management practices that can improve NUE on farms and reduce NO\textsubscript{3} leaching include splitting N fertilizer applications to better synchronize with N uptake by crops, appropriately managing soil acidity via liming, and using proper irrigation techniques (Baligar et al., 2001; Delgado et al., 2007).

Fall planted cover crops can improve agricultural NUE by reducing N loss from agricultural systems via leaching and recycling N to successor crops via residue decomposition. Cover crops planted following summer cash crop harvest can reduce NO\textsubscript{3} leaching by up to 70% by scavenging for residual soil NO\textsubscript{3} that has accumulated in the soil from excess fertilizer applications or soil organic matter mineralization (Dinnes et al., 2002; Jackson et al., 1993; Wyland, 1996). N assimilated by cover crops before
frost termination is recycled to subsequently planted crops through residue decomposition in the spring (Dean & Weil, 2009; Dinnes et al., 2002; Francis et al., 1998; Sainju & Singh, 1997). In general, non-leguminous cover crops such as rye and forage radish (*Raphanus sativus* L.) are more effective scavengers with greater potential to reduce N pollution from agronomic systems (Dabney et al., 2001; Dinnes et al., 2002; Jackson et al., 1993; Sainju & Singh, 1997). Other nutrients taken up by cover crops are similarly recycled and released to successor crops (Delgado et al., 2007; Fageria et al., 2005; Wang et al., 2008).

Cover crops differ in their ability to recycle nutrients and provide benefits to successor crops. Legume cover crops enhance NUE by biologically fixing N and building soil organic matter reservoirs (Fageria et al., 2005; Tonitto et al., 2006; Verzeaux, Alahmad, et al., 2016). Because of the unique ways that various cover crops support nutrient cycling in agronomic systems, legume / non-legume cover crop mixtures often provide greater NUE improvements than monoculture plantings (Dabney et al., 2001; Fageria et al., 2005; Lawson et al., 2015; Thapa et al., 2018; Tribouillois et al., 2016). Cover crop mixtures combine the benefits of biological N fixation provided by legumes and the N scavenging abilities of cereal crops or brassicas, making more total N available to subsequent cash crops and reducing the amount of N leached from rapidly mineralized legume residues (Fageria et al., 2005; Lawson et al., 2015; Radicetti et al., 2016; Thapa et al., 2018; Tribouillois et al., 2016).

### 1.2.3 Arbuscular Mycorrhizal (AM) Fungi

Arbuscular Mycorrhizal (AM) fungi play an important role in soil aggregate
formation and stabilization of soil organic matter, both by providing a physical network of hyphae that stabilizes particles and through the production of a sticky, hydrophobic glycoprotein called glomalin (Bethlenfalvay and Schuepp, 1994; Gianinazzi et al., 2010; Gosling et al., 2006; Jeffries et al., 2003; Wright et al., 1999) (Bethlenfalvay & Schüepp, 1994; Gianinazzi et al., 2010; Gosling et al., 2006; Jeffries et al., 2003; Wright et al., 1999). The hyphae of AM fungi bind microaggregates into macroaggregates, improving the carbon and nutrient storage capacity of a soil (Bethlenfalvay & Schüepp, 1994; Daynes et al., 2013; Gosling et al., 2006; Rillig & Mummey, 2006). Moreover, the extensive mycelial network formed by AM fungi extends beyond the rhizosphere and transports C from host plants to microbial communities, directly contributing to soil C pools (Bethlenfalvay & Schüepp, 1994; Gosling et al., 2006; Jeffries et al., 2003). In addition to providing soil microbial communities with carbon, AM fungi form positive associations with microorganisms in the soil, including N-fixing bacteria (Gosling et al., 2006). These relationships increase the biodiversity of rhizosphere communities and can benefit cropping systems by providing benefits to both the soil and crops (Frąc et al., 2018; Gosling et al., 2006).

Associations with AM fungi can increase the yield and nutritional quality of many agronomic crops by enhancing access and uptake of nutrients (Mishra et al., 2018; Verzeaux, Roger, et al., 2016). *Allium* species have high mycorrhizal dependency relative to other crops, and previous studies have demonstrated that inoculation with AM fungi can increase the yield, bulb diameter, and flavor of garlic grown in field conditions (Al-Karaki, 2002; Borde et al., 2009; Hart & Forsythe, 2012; Koch et al., 1997). Inoculation with *Claroideoglomus etunicatum, Glomus intraradices*, and
*Funneliformus mosseae*, three species of AM fungi commonly found in commercial inoculants and selected for use in this project, significantly increased yields of spring onion (*Allium* sp.) in a 2007 study (Guo et al., 2007). *Glomus intraradices* has consistently increased nutrient levels of *Allium* species in soils with adequate nutrient supply (Hart & Forsythe, 2012). Leeks inoculated with *Glomus intraradices* and *Funneliformus mosseae* removed more P from the soil and had increased biomass compared to non-inoculated plants (Jansa et al., 2008).

Incorporating winter cover crops into cropping systems can mitigate the negative effects of intensive N fertilization, promote mycorrhizal colonization of successor crops and increase AM abundance in soil (García-González et al., 2016; Kabir & Koide, 2002; Lehman et al., 2012; Murrell et al., 2019; Plenchette et al., 2005; Rillig et al., 2002; Rosner et al., 2018; Verzeaux, Alahmad, et al., 2016). The degree of mycorrhizal colonization and nutrient uptake by successor crops is significantly influenced by cover crop species and the level of soil disturbance, as conventional tillage practices are detrimental to AM development and spore production (Bowles et al., 2016; Frąc et al., 2018; García-González et al., 2016; Morriën, 2016; Murrell et al., 2019, 2019; Verzeaux et al., 2017). Reduced tillage and continuous cropping systems enhance the colonization potential of AM fungi in agricultural soils (Hirel et al., 2011).

Fall planted cover crops, particularly mycorrhizal species, can enhance the role of AM fungi in agricultural systems by providing plant hosts during an otherwise fallow period, increasing overwintering potential, host root colonization, and AM abundance in the soil (Dabney et al., 2001; García-González et al., 2016; Kabir et al., 1997; Kabir & Koide, 2002; Lehman et al., 2012; Magdoff, 2001; Murrell et al., 2019;
Plenchette et al., 2005; Rillig et al., 2002; Rosner et al., 2018; Verzeaux, Alahmad, et al., 2016). Studies have reported that oat cover crops are particularly effective in increasing mycorrhizal inoculum potential of the soil and providing benefits to successor crops (Kabir & Koide, 2002; Lehman et al., 2012). Mixtures of fall planted cover crops have demonstrated increased AM colonization and higher yields of subsequently planted cash crops as compared to cover crop species planted individually (Kabir & Koide, 2002; Lehman et al., 2012). Forage radish, one of the cover crops chosen for this experiment, does not associate with AM fungi and can in fact inhibit spore germination and colonization due to the production of glucosinolates. However, fall planted forage radish does not inhibit mycorrhizal colonization of subsequently planted crops and may improve the NUE of AM inoculated garlic (Murrell et al., 2019; White & Weil, 2010). Incorporating AM fungi and fall-planted cover crops into garlic production has the potential to significantly improve garlic yield and nutritional quality.

1.2.4 Soil Food Webs and Ecosystem Function

Organisms that make up the living fraction of soils influence both soil physical properties and the rate of biological processes in agronomic soils, including mineralization and N fixation (Fageria & Baligar, 2005; Frąc et al., 2018; Magdoff, 2001). The soil food web, which documents the relationships between soil organisms and environmental processes, can provide valuable insight into measures of ecosystem condition. The organisms interacting within this system redistribute minerals through space and time and directly influence soil fertility through the mineralization and immobilization of important plant nutrients. Additionally, soil organisms modify soil
structure, decompose organic matter, degrade pollutants, regulate pest populations and sequester C (Bongers & Ferris, 1999; Ferris et al., 2001). Assessing soil food web response to soil enrichment and disturbance can enhance the sustainability of farming systems by tailoring cropping systems to improve nutrient management (Melakeberhan et al., 2018).

The primary consumers of the soil food web are bacteria, fungi, and plant-parasitic nematodes. Plant-parasitic nematodes feed on living roots, while fungi and bacteria assimilate plant root exudates and decompose organic matter such as plant residue or manure (Ferris & Matute, 2003). These organisms in turn become food and energy sources for organisms higher in the food web, with the amount of C accumulated by each organism decreasing with each trophic interchange (Ferris & Matute, 2003). Thus, C inputs reflect the size potential of food webs – limited carbon availability decreases the amount of trophic interchanges that can occur in a food web, resulting in resource-poor conditions that are unable to support soil organisms at higher trophic levels and leading to lower rates of microbial turnover and mineral immobilization (Dinnes et al., 2002; Ferris & Matute, 2003).

Assessing soil food web condition can be useful when evaluating the resilience of agroecosystems and overall soil health, particularly during transition to sustainable management practices. Because of the complexity of soil food webs and fluctuations in microbe populations, this analysis can be difficult if not impossible to perform by examining the diversity and abundance of all organisms present. An easier way to examine food web structure, function and resilience is to measure the presence and abundance of indicators that selectively feed on functional guilds (Ferris et al., 2001;
Ferris & Matute, 2003). Nematodes are used as bio-indicators in terrestrial systems to assess soil food web conditions and function.

Nematodes, the most abundant metazoans, play an important role soil nutrient cycling and serve as effective bioindicators of terrestrial ecosystems, as their community composition is correlated to important ecological processes in the soil (Neher, 2001). Using nematode faunal analysis to assess food web structure can be more effective than microbial analysis because of nematodes’ longer generation time and higher occupancy in the food chain (Neher, 2001). Assessment of nematode community structure can provide useful information about the current soil food web condition and serve as a tool to evaluate the status of environmental remediation or degradation (Bongers & Ferris, 1999). As nematodes occupy key positions as primary and intermediate consumers in the soil food web, their abundance reflects the relative availability of their energy sources (Bongers & Ferris, 1999; Ferris et al., 2001; Ferris & Matute, 2003; Neher, 2001). In addition, nematodes are relatively easy and inexpensive to extract from the soil and are easily identified by morphological and anatomical features (Ferris et al., 2001; Neher, 2001).

Nematodes can be easily classified by feeding type through the observation of morphological structures under a microscope (Neher, 2001). Figure 1.1 shows an illustration of the various mouthparts observed in nematodes with various feeding habits: plant-parasitic, fungal-feeding, bacterial-feeding, omnivorous, or carnivorous. Generally, nematodes within the same genera have the same feeding preferences; however, some nematodes exhibit inconsistent feeding behavior throughout their
developmental stages and others have displayed evidence of multi-channel omnivory (Bongers & Bongers, 1998; Kadota et al., 2002; Okada et al., 2005).

![Mouthparts of nematodes of various feeding groups.](image)

Figure 2.1 Mouthparts of nematodes of various feeding groups. a) bacterial feeder b) fungal feeder c) plant parasite d) predator e) omnivore; (Adapted from Zaborski et al., 2020).

Because the results obtained from analyzing nematode communities based solely on feeding type are too broad to be useful in most contexts, nematodes are further divided based on their response to food web enrichment or environmental disturbance (Ferris et al., 2001). Nematode families are distributed along a colonizer-persister (c-p) continuum based on their life strategy characteristics and response to disturbance and enrichment, and further characterized by functional guild based on the combination of feeding type and c-p value (Bongers & Bongers, 1998; Ferris et al., 2001; Neher, 2001) Nematode functional guilds are used to evaluate nematode-based indices (NBIs) of food web condition, which include the maturity index (MI), plant-parasitic index (PPI), basal index (BI), structure index (SI), and channel index (CI) (Neher et al., 2004). Each of these indices provides unique insight into the structural and functional characteristics of soil food webs, including the rate of N cycling, dominant decomposition channel, and the ability of soil to suppress plant pathogens.
Although detecting food web response to disturbance may take several weeks, due to the lag period between resource availability and population response, this is considered a relatively short response time (Ferris et al., 1996).

Management practices that promote ecosystem biodiversity enhance the beneficial roles of nematodes in agroecosystems (Ferris, 2010). Cover crops enhance soil food web services by supplying soil organisms with a continuous flow of photosynthetic C inputs, which is necessary for supporting higher trophic level organisms in food webs and enhancing food web function (Ferris, 2010; Zhang et al., 2017). Moreover, including cover crops in crop rotations can increase the abundance of bacterial feeding enrichment opportunists (c-p 1), which contribute significantly to N mineralization in soils by dispersing bacteria and excreting excess N from feeding in mineral (NO$_3^-$) form (Ferris et al., 1998, 2004, p. 2; Ingham et al., 1985). Both the roots of living cover crops as well as decomposing crop residues impact microbial community composition and soil food web structure (Ferris & Matute, 2003; Morriën, 2016). Fine root turnover and root exudates of actively growing cover crops provide microbes with carbon to sustain growth and metabolism, resulting in increased populations of soil microbial communities even after cover crops are terminated (de Vries & Caruso, 2016; Fageria et al., 2005; Finney et al., 2017; Verzeaux, Alahmad, et al., 2016). Additionally, roots of living cover crops form symbiotic relationships with soil microbes, boosting crop productivity by increasing nutrient uptake efficiency, enhancing photosynthetic capacity, and promoting growth (Harmon & Uphoff, 2019). Incorporating cover crops into garlic production via relay cropping can enhance soil
food web structure by providing additional resources throughout the winter and spring, resulting in higher trophic level interchange, enhanced nutrient cycling, and greater food web resilience.

1.2.5 Relay Cropping

Relay cropping, the practice of planting a secondary crop into an already established ‘primary’ crop, increases crop productivity per unit land area and enhances resource use efficiency by staggering the growth phase and harvest of the two component crops (Tanveer et al., 2017). The practice of relay cropping contributes to soil health by reducing soil N loss via leaching, increasing the density and duration of living roots in soils, and diversifying agricultural systems, which can help mitigate pest and disease pressure and improve system resilience to climate change (Gesch et al., 2023; Tanveer et al., 2017).

In the United States, over 60% of farms produce only one or two commodities, while less than 14% of farms produce over four commodities (Hanson et al., 2008). Incorporating cover crops into cropping systems via relay cropping can diversify production, contributing to soil health, while simultaneously increasing cash crop yields (Amossé et al., 2014; Martens et al., 2001). Further, as increasing agricultural land area is environmentally unsustainable and, in many cases, spatially unfeasible, improving land use efficiency is a critical challenge of sustainable intensification (Garnett et al., 2013). For farmers limited by small acreage or short growing seasons, taking land out of cash crop production to plant cover crops is unrealistic; thus, a relay crop system provides a solution to integrate cover crops into crop rotations without taking land out of production.
Although it has been practices for many decades, relay cropping is poorly adopted
worldwide, as this system must be carefully tailored to meet the production requirements
of its component crops (Gesch et al., 2023; Lamichhane et al., 2023; Tanveer et al.,
2017). Garlic is an excellent candidate for a relay crop system, as it is planted in the fall
but doesn’t begin vegetative growth until the following spring, limiting competition for
resources. Relay planting garlic into standing cover crop residues can provide a
sustainable intensification method by which fall cover crops are integrated into garlic
production.

1.3 Status of Soils and Food Systems in New England

According to the 2017 Agricultural Census, the number of small farms (1-9 acres)
in Massachusetts has been increasing annually over the last twenty years. Farms
operating on such small acreage must maximize their land use efficiency while keeping
soils productive to ensure that fertility requirements of all crops are met. To do this,
farmers must take measures to build natural fertility, protect their soil by minimizing
erosion, and continuously enhance the resiliency of their systems by promoting
agroecosystem biodiversity.

As the number of small farms in New England increases and growers become
progressively concerned with soil health and the environmental impacts of agricultural
practices, many farms are transitioning to no-till or reduced-till systems. The
implementation of projects such as the “Massachusetts Healthy Soils Action Plan”
suggests that soil health and responsible land stewardship are at the forefront of
diversified vegetable production. The revision of current agronomic practices and
introduction of new methods that promote sustainable resource use and biodiversity are essential to the long-term sustainability of food production. Appropriate research is needed to effectively transition to better methods of production without compromising crop yield or quality.

The move towards increasing on-farm sustainability and resiliency extends to communities and consumers, who are becoming increasingly conscious of the source and quality of their commodities. Over the last eight years, direct sales from farms to consumers have doubled in Massachusetts (USDA, 2017). Consumers are interested in sourcing locally, supporting sustainable practices, and purchasing high quality, nutritionally dense food. A study conducted in 2009 revealed that consumers in New England prioritize freshness, taste, and nutritional quality when purchasing produce. Many New England consumers prefer to buy from local farmers, recognizing the value of supporting their local economy and rejecting the harsh realities of industrialized agriculture, including detrimental environmental impacts and poor labor practices (Berlin et al., 2009). Improving the sustainability of farming practices can increase crop quality and encourage active community support and engagement (Hanson et al., 2008; Pimentel et al., 1995).

1.4 Garlic Production in the Northeast

Garlic (*Allium sativum*) has been cultivated for thousands of years around the world and is an important economic crop in the northeastern United States. Known for its pungent flavor, high nutrient density, and anti-microbial properties, it is revered both as a culinary staple and for its medicinal uses. In the northeast, the relatively short
Garlic is an excellent component of any diversified vegetable farm because it stores well and provides multiple commodities for CSAs and farmers’ markets - marketable scapes in the spring and bulbs in the fall (Sideman, 2016). In New England, garlic is planted in the fall (mid-October – mid-November), because it requires a vernalization period of 40°F for approximately two months to induce bulbing (Grubinger, 2005). Garlic cloves planted in the fall begin to grow roots but often do not sprout until the following spring, when warmer temperatures and longer periods of daylight initiate bulb formation (Grubinger, 2005; Sideman, 2016). Hardneck garlic varieties, preferred by growers in northern climates, produce false flower stalks called scapes in the early summer. Scape removal promotes larger bulb size and provides an additional early season commodity to diversified vegetable farmers (Grubinger, 2005; UMass Amherst New England Vegetable Guide, 2020).

A heavy feeder, garlic requires relatively high fertilizer and labor inputs: 134 – 140 kg / ha N (120 - 125 lb / ac); 56 – 112 kg / ha P (50 – 100 lb / ac), and 56 – 112 kg / ha K (50 – 100 lb / ac) (Bachmann, 2008; Ford, 2014). Currently, it is recommended that growers seed garlic into raised beds with high organic matter content and cover the soil with straw mulch to maintain appropriate soil conditions for garlic production (Sideman, 2016; UMass Amherst New England Vegetable Guide, 2020). Mulching can inhibit soil temperature fluctuations and frost heaving during the winter months, help conserve soil moisture, and reduce weed pressure when garlic sprouts in the spring (Sideman, 2016; UMass Amherst New England Vegetable Guide, 2020). However, in addition to being labor intensive and expensive, straw mulch can also exacerbate the
effects of soil-borne disease in wet years (Grubinger, 2005). Moreover, straw mulch can harbor a large amount of weed seed that can emerge during the cropping season.

The adoption of sustainable agronomic practices by farmers is dependent on both immediate economic incentives and risk factors associated with transitioning to new methods of production (Lu et al., 2003). As the demand for local, organic produce increases and the devastating impacts of conventional agricultural practices on soil and the environment become more apparent, a growing number of vegetable producers are beginning to experiment with sustainable methods of production (Shennan, 1992; Thakur & Sharma, 2005). Incorporating annual, fall-planted cover crops and arbuscular mycorrhizal (AM) fungi into traditional garlic production can reduce off-farm inputs, improve the yield and quality of garlic, and provide cover crop ecosystem services without sacrificing production acreage (Bachmann, 2008).

1.5 Applied Research Knowledge Gaps and Project Objectives

Many growers in the northeast are exploring alternative methods of garlic production to increase the sustainability and resiliency of their farming operations. Applied research focused on developing alternative, regionally specific methods of production is necessary to evaluate yield response, assess management risks, and ensure that the economic security of farmers is not sacrificed during transition to sustainable, ecologically sound production systems (Plaza-Bonilla, 2016). This research project will examine the potential benefits of an alternative garlic production system designed to enhance soil health, improve N use efficiency, reduce off-farm inputs, and increase the yield and quality of garlic in New England field conditions. The proposed method
incorporates two major elements of agricultural sustainability, cover crops and arbuscular mycorrhizal (AM) fungi, into garlic production. An overview of the primary objectives and research activities associated with this project are presented in Figure 1.2.

Figure 1.3. Primary objectives, research activities, and outcomes associated with this dissertation project
1.6 Chapter References


29


https://doi.org/10.21273/HORTSCI.27.7.749

extension.unh.edu/resource/growing-garlic-new-hampshire-fact-sheet.


https://doi.org/10.2136/sssaj2017.05.0139


https://doi.org/10.1007/978-1-4612-4790-6_3


https://doi.org/10.1300/J064v26n01_10


https://doi.org/10.1007/s11356-017-8371-4


CHAPTER 2

ASSESSING THE ROOT AND SHOOT COMPOSITION, C CONTRIBUTION, DECOMPOSITION AND N MINERALIZATION TRENDS OF SINGLE SPECIES AND MIXED COVER CROPS

2.1 Abstract

Annual, fall planted cover crops provide a wealth of ecosystem of services to cropping systems, including reduced risk of erosion, improved nutrient retention, and enhanced soil health and quality in the long-term. This study quantified the fall biomass, N and C accumulation of the roots and aerial parts of three commonly planted cover crop species, oat (Avena sativa), field pea (Pisum sativum) (FP), tillage radish (Raphanus sativus) (TR) and their mixture (MX), as well as dry matter (DM) decomposition, N and C release, and residue C:N changes after frost termination the following spring. Additionally, this study characterized the relationship of initial root and aerial litter quality to decomposition rate \((k)\), N release \((k_n)\) and C release \((k_c)\) over the experimental period. MX had the highest fall biomass (2514.1 kg ha\(^{-1}\)), N yield (65.1 kg ha\(^{-1}\)) and C yield (1190 kg ha\(^{-1}\)) of all treatments. The highest rate of DM decomposition, C and N release was observed in DR root and aerial residues, with less than 20% the initial residues remaining in the field at the end of the sampling period. OT accumulated the lowest amount of N (30 kg ha\(^{-1}\)) of all cover crop treatments, and N released from OT residues during the experimental period was significantly less (17.95 kg ha\(^{-1}\)) than that of other treatments (48.7 – 51.1 kg ha\(^{-1}\)). Different litter quality parameters were associated with the rate of DM decomposition, N and C release of root and aerial residues, with
cellulose content being the best predictor of decay rate in root residues and moisture content the most reliable predictor of DM decay in aerial residues left on the soil surface.

2.2 Introduction

Cover crops are the backbone of general soil health and necessary for enhancing the sustainability and resiliency of farming systems. (Dabney et al., 2001). Although the benefits of cover crops are well established, managing cover crops to include both short-term benefits to crop production, such as increased nitrogen (N) supply to cash crops, and long-term ecosystem services, such as C sequestration and improved soil food web structure, can be difficult and requires a thorough understanding of litter decomposition dynamics and nutrient release (Blanco-Canqui et al., 2013; Dabney et al., 2001; X. Zhang et al., 2017). Differences in cover crop residue quality and management can impact the fate of N mineralization and C stabilization in the soil, impacting the yields of successor crops and rate of SOM formation. The timing of residue decomposition and N release relative to successor crop uptake determines the magnitude of the benefits provided by cover crops and must be considered to ensure appropriate nutrient management (Dabney et al., 2001; Ghimire et al., 2017; Romdhane et al., 2019; Shennan, 1992; Thapa et al., 2022; Tonitto et al., 2006; Trinsoutrot et al., 2000). The root biomass of cover crops ranges from 100 – 3850 kg ha⁻¹, depending on the species and stage of growth (Dean & Weil, 2009; Jani et al., 2016; Kuo et al., 1997a; Ruis et al., 2020; Sainju et al., 2005). However, the biomass, structural composition, and decomposition of cover crop roots are often overlooked in cover crop studies (Christensen, 1986; Douglas et al., 1980; Lupwayi & Soon, 2009; Poffenbarger et al., 2015; Ruis et al., 2019).
Fall planted cover crops can accumulate substantial amounts of N by taking up residual soil N before frost termination and reduce nitrate leaching by up to 70% compared to bare fallow systems [9,10,]. In the spring, decomposing cover crop residues can release stored nutrients to subsequently planted cash crops, impacting yield and quality (G. Chen & Weil, 2011; Dinnes et al., 2002; Fageria et al., 2005; Wyland, 1996). Therefore, incorporating annual, fall planted cover crops into production systems can improve on-farm nutrient cycling while simultaneously reducing environmental pollution and decreasing reliance on external inputs (Dabney et al., 2001; Dean & Weil, 2009; Delgado et al., 2007; Tonitto et al., 2006; Wang et al., 2008; Wyland, 1996). Daikon radish (Raphanus sativus), oat (Avena sativa), and field pea (Pisum sativum) are annual cover crops commonly planted in fall that differ in quality and growth habit. Daikon radish produces a large, fleshy taproot that extends deep into the soil, rendering it an exceptionally efficient cover crop for nutrient cycling. Additionally, its fast growing, broad leaves provide substantial weed suppression capacity (Lawley et al., 2012; Wang et al., 2008). Legume species, such as field pea, can supply successor crops with significant amounts of biologically fixed atmospheric N (Dabney et al., 2001; M. S. Smith et al., 1987). The extensive fibrous root systems of cereal crops, such as oat, are efficient in scavenging the soil for residual nitrate, which would otherwise be lost to the environment during fall-spring (Dabney et al., 2001; Dinnes et al., 2002; Fageria et al., 2005; Tonitto et al., 2006). The decomposition trends of cover crop residues are variable and highly dependent on litter quality and management. In general, cereal crops tend towards slower decomposition and may cause net N immobilization in the soil during decomposition (Aulakh et al., 1991;
Dabney et al., 2001; Doran, 1991). By contrast, annual legume cover crops have low C:N and decompose more quickly than cereal cover crops. The rapid decomposition of legume residues may result in poor synchrony with the active growth and N uptake of successor crops, thus increasing N losses to the environment (Plaza-Bonilla et al., 2017; Sievers & Cook, 2018). Using legume and cereal cover crop mixtures can help ‘balance’ the C:N of residues, modifying the rate of N release and providing a combination of benefits to cropping systems (Dabney et al., 2001; Fageria et al., 2005; Lawson et al., 2015; Tribouillois et al., 2016).

Termination strategy and tillage management impact the rate of residue decomposition in agricultural systems. Residues in no-till systems decompose more slowly than residues incorporated into the soil, impacting the rate of nutrient release and longevity of residue surface cover (Christensen, 1986; Douglas et al., 1980; Etemadi et al., 2018; Jahanzad et al., 2016; Poffenbarger et al., 2015; Thapa et al., 2022). However, studies evaluating differences in the biomass and decomposition dynamics of both cover crop roots and aerial parts under no-till conditions are lacking. A greater understanding of root and shoot decomposition dynamics in no-till systems is needed to increase the efficacy of residue management and nutrient release in farming systems. Moreover, because the chemical composition of aerial residues differs from that of root residues, it is necessary to evaluate the decomposition of winterkilled cover crop root and aerial residues separately.

Cover crop residues are a major source of carbon (C) in agroecosystems, essential for enhancing soil organic C (SOC) levels [17–19]. SOC can help to buffer the negative effects of climate change in agriculture and enhance agricultural productivity by
improving soil structure, reducing the risk of soil compaction, increasing water retention capacity, and increasing N retention in soils (Blanco-Canqui et al., 2013; Tonitto et al., 2006). Changes in SOC are not readily observed in the short-term; it can take over three years to observe minor increases in SOC after incorporating cover crops into a management system (Duval et al., 2016). Moreover, cover crop roots retain a greater proportion of C following termination than cover crop shoots, suggesting greater long-term contributions to SOC formation (Puget & Drinkwater, 2001). Thus, quantifying the C inputs fall-planted cover crop roots and shoots is a necessary step toward quantifying the long-term benefits of annual cover crops in agricultural systems, which are often overlooked in short-term agricultural studies.

A negative exponential decay model is frequently used to model litter decomposition in agricultural and forest systems (Berg & Ågren, 1984; Makkonen et al., 2012). Residues with a higher decay constant $k$ are generally associated with rapid N mineralization, while lower values are associated with C stabilization in agricultural soils (Ntonta et al., 2022; Thapa et al., 2022). Factors influencing litter decay rate include weather, soil conditions, and litter quality characteristics such as C:N, lignin content, and lignin:nitrogen ratio (L:N) (B. H. Janssen, 1996; Craine et al., 2007; Douglas & Rickman, 1992; Melillo et al., 1982; Talbot & Treseder, 2012; Taylor et al., 1989; D. Zhang et al., 2008). Studies linking root litter quality parameters with root decay constants are currently lacking (Silver & Miya, 2001; D. Zhang et al., 2008). During the decomposition process, the chemical composition of crop residue changes as various components of litter biomass are assimilated by soil microbes (Berg & Ågren, 1984). It is well known that the initial C:N of organic inputs can be used to predict net mineralization or
immobilization of N in the soil (Justes et al., 2009; Sylvia, 2005; Trinsoutrot et al., 2000). However, fluctuations in cover crop residue C:N during the decay period have not been documented. Characterizing changes in C and N during residue decomposition is essential to building a better understanding of complex decomposition dynamics in annual cropping systems.

The aims of this study were to 1) quantify the dry matter (DM), N and C accumulation of the roots and aerial parts of three common fall-planted annual cover crops (daikon radish, oat, and field pea) and their mixture; 2) characterize the DM decomposition, N and C release trends of the monocultures and mixture cover crop the spring following winterkill; 3) evaluate the relationships between initial litter quality characteristics and DM, N and C decay constants $k$, $k_n$ and $k_c$ of cover crop roots and aerial parts in annual cropping systems, and 4) quantify C:N changes of cover crop roots and aerial parts during spring decomposition.

2.3 Materials and Methods

2.3.1 Experimental Site and Weather Conditions

A three-year field experiment (2020-2022) was conducted at the University of Massachusetts Crop, Animal, Research and Education Center, located in South Deerfield, MA (42° N, 73° W). The soil at this location is characterized as a non-acid, mesic Typic Udifluvent, Hadley fine sandy loam. Relevant weather conditions for the experimental period, including annual precipitation and temperature data, are presented in Table 2.1 alongside twenty-year averages (2000-2019).
2.3.2 Experiment Layout

The spring residue decomposition of the roots and aerial parts of three annual, fall-planted cover crop species and their mixture were evaluated in this experiment. Experimental plots (3.8 m²) were laid out in a randomized, complete block design consisting of four replications. Three cover crop species, oat (OT) (*Avena sativa*, var: “Saddle Oat”), daikon radish (DR) (*Raphanus sativus*, var: generic), field pea (FP) (*Pisum sativum*, var: 4010), and their mixture (MX) were seeded on 04/9 and 25/8 (2020 and 2021, respectively), using a 7-row cone seeder with 17.7 cm row spacing set at 73.5 cone speed. Monoculture plots were planted with the following rates: 126 kg ha⁻¹ (OT), 13.5 kg ha⁻¹ (DR), and 90 kg ha⁻¹ (FP). The seeding rates of cover crop species were adjusted to 45 kg ha⁻¹ (OT), 5 kg ha⁻¹ (DR), and 90 kg ha⁻¹ (FP) for the MX plots.

2.3.3 Preparation of Litter Bags and Data Collection

The biomass of cover crop roots and aerial parts was determined in November of each year after three consecutive days of frost temperatures. Samples were collected on 11/12 and 11/5 in 2020 and 2021, respectively. Total cover crop biomass was harvested from 1.5 m in each plot. Following harvest, the aerial parts and roots of each sample were separated and roots carefully washed. Biomass samples from mixed cover crop plantings were sorted by species and weighed to obtain the fresh weight ratio of each species before drying. Samples were dried in a forced air oven at a temperature of 109°C until constant weight and dry matter (DM) of cover crops roots and shoots were obtained.

To evaluate the decomposition trends of each cover crop treatment, litter bags were assembled and placed into the experimental plots at winterkill and collected bi-
weekly the following spring. The separated root and aerial residues were placed into 2 mm mesh screen bags (23 x 23 cm). 25 g of fresh weight (FW) plant matter was placed into each single-species aerial litter bag and 5 g FW of roots were used for FP and OT root litter bags. 50 g of R roots were used to assemble the DR root litter bags to account for the disproportionately large size of the taproot. To assemble mixed cover crop litter bags, the ratios determined by the species separation during biomass collection were used. As a result, the mixed cover crop treatment aerial litter bags contained 15 g FW of OT residue, 10 g FW of DR residue, and 25 g FW of FP residue. The mixed root litter bags contained of 4 g OT, 4 g DR, and 4 g FP root residue. Initial dry weight, nitrogen (N) concentration, and carbon (C) of litter bags was obtained by placing four replications of each litter bag treatment into a forced air oven at a temperature of 109° C, drying until constant weight, and processing for analysis.

12 aerial litter bags and 12 root litter bags per experimental plot were placed in the field on 11/16 in 2020 and 11/6 in 2021. Bags containing shoots were placed on the soil surface while the root litter bags were buried at a depth of 12 cm to simulate no-till conditions. Litter bags were collected from the field bi-weekly from April – June on the following dates: 4/1, 4/14, 4/28, 5/12, 5/26 and 6/10. Two aerial and two root litter bags per plot were retrieved at each sampling date and combined for analysis.

2.3.4 Sample Processing and Laboratory Analysis

At each harvest, litter bags were collected and the contents placed into a forced air oven and dried at 109° C until constant weight. Dried samples were carefully brushed with a coarse artist’s brush to remove any residual soil debris, weighed to obtain DW
biomass, and ground to 1 mm particle size (Foss Cyclotec 1093 Sample Mill; Foss Electric A/S, Hillerød, Denmark). Processed samples were sent to a commercial laboratory, where C concentration (% DW) and N concentration (% DW) were determined using a CHN analyzer. Initial N and C yield were calculated by multiplying the initial residue N and C concentrations by cover crop biomass and adjusted to kg ha\(^{-1}\). The same calculation was used to determine the percent mineralization of N and C during the experimental period.

In the second year, dried cover crop biomass samples (roots and aerial parts) were ground to 1 mm particle and sent to a commercial laboratory to determine Acid Detergent Fiber (ADF), Neutral Detergent Fiber (NDF) and lignin content. Cellulose and hemicellulose content were calculated using the following formulas: % cellulose = ADF - % lignin, and % hemicellulose = NDF – ADF.

**2.3.5 Calculation of \(k\), \(k_n\), and \(k_c\)**

The annual decomposition rate constants (k year\(^{-1}\)) for a first order exponential decay model were calculated for biomass (k), N (\(k_n\)) and C (\(k_c\)) using the following formula:

\[
k = - \frac{\ln \left( \frac{M_t}{M_0} \right)}{t}
\]

where \(M_t\) = mass of residues at 10/6 (final mass), \(M_0\) = initial mass, and \(t\) = time in field (year\(^{-1}\)). N and C mass were calculated by multiplying their measured concentrations at each time point by the DW of the associated residues.
2.3.6 Statistical Analysis

Statistical analyses were performed using the proc GLM and proc CORR procedures in SAS, version 9.4 (SAS Institute, 2016). Means of effects that were significant at the p<0.05 level were separated using Tukey’s HSD in the case of fixed variables or fit to regression curves where variables were continuous.

2.4 Results

2.4.1 Weather at the Experimental Site

The average monthly precipitation and the maximum, minimum, and average monthly temperatures (°C) for the experimental period are presented in Table 2.1, alongside regional twenty-year (2000-2019) averages. In general, temperature and precipitation did not deviate strongly from the norm for the region, although the fall temperatures during both experimental years were slightly warmer than the twenty-year regional average. September of the first experimental year (2020) had decreased precipitation (~4.7 cm) compared to the norm, which coincided with cover crop planting and establishment. Lower precipitation than is normal for the region was observed in March – June of both experimental years, with temperatures close to the regional norm.
Table 2.1 Regional weather conditions at the experimental site and deviation from the regional norm.

<table>
<thead>
<tr>
<th></th>
<th>Avg. temperature (C)</th>
<th>Dev. from regional avg.</th>
<th>Min Temp (C)</th>
<th>Dev. from regional min.</th>
<th>Max Temp (C)</th>
<th>Dev. from regional max.</th>
<th>Precipitation (cm)</th>
<th>Dev. From regional avg. precipitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2020</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>September</td>
<td>16.6</td>
<td>0.0</td>
<td>9.8</td>
<td>- 0.8</td>
<td>23.2</td>
<td>+ 0.4</td>
<td>5.2</td>
<td>- 4.7</td>
</tr>
<tr>
<td>October</td>
<td>10.3</td>
<td>+ 0.5</td>
<td>5.0</td>
<td>+ 0.8</td>
<td>15.6</td>
<td>+ 0.1</td>
<td>11.9</td>
<td>0.2</td>
</tr>
<tr>
<td>November</td>
<td>5.6</td>
<td>+ 1.8</td>
<td>- 0.4</td>
<td>+ 1.0</td>
<td>12.1</td>
<td>+ 3.0</td>
<td>9.7</td>
<td>1.8</td>
</tr>
<tr>
<td>December</td>
<td>- 0.3</td>
<td>+ 1.2</td>
<td>- 5.3</td>
<td>+ 1.0</td>
<td>4.1</td>
<td>+ 1.0</td>
<td>10.8</td>
<td>1.3</td>
</tr>
<tr>
<td>2021</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>January</td>
<td>- 3.1</td>
<td>+ 1.8</td>
<td>- 6.8</td>
<td>+ 3.35</td>
<td>0.72</td>
<td>+ 0.8</td>
<td>5.4</td>
<td>- 0.9</td>
</tr>
<tr>
<td>February</td>
<td>- 4.0</td>
<td>- 0.4</td>
<td>- 8.6</td>
<td>+ 0.9</td>
<td>0.56</td>
<td>- 1.1</td>
<td>4.7</td>
<td>- 2.3</td>
</tr>
<tr>
<td>March</td>
<td>2.9</td>
<td>+ 1.9</td>
<td>- 3.4</td>
<td>+ 1.1</td>
<td>9.17</td>
<td>+ 2.8</td>
<td>4.1</td>
<td>- 3.63</td>
</tr>
<tr>
<td>April</td>
<td>8.6</td>
<td>+ 0.8</td>
<td>2.2</td>
<td>+ 0.7</td>
<td>15.0</td>
<td>+ 1.0</td>
<td>10.8</td>
<td>+ 2.1</td>
</tr>
<tr>
<td>May</td>
<td>14.3</td>
<td>+ 0.2</td>
<td>7.7</td>
<td>0.0</td>
<td>20.8</td>
<td>+ 0.5</td>
<td>10.2</td>
<td>+ 2.1</td>
</tr>
<tr>
<td>June</td>
<td>21.3</td>
<td>+ 2.6</td>
<td>14.8</td>
<td>+ 2.0</td>
<td>27.8</td>
<td>+ 3.3</td>
<td>5.1</td>
<td>- 6.64</td>
</tr>
<tr>
<td>July</td>
<td>21.0</td>
<td>- 0.7</td>
<td>16.3</td>
<td>+ 0.6</td>
<td>25.7</td>
<td>- 1.9</td>
<td>31.3</td>
<td>+ 22.1</td>
</tr>
<tr>
<td>August</td>
<td>23.0</td>
<td>+ 2.24</td>
<td>18.1</td>
<td>+ 3.1</td>
<td>27.8</td>
<td>+ 1.0</td>
<td>7.3</td>
<td>- 2.36</td>
</tr>
<tr>
<td>September</td>
<td>17.5</td>
<td>+ 0.9</td>
<td>12.1</td>
<td>+ 1.5</td>
<td>23.0</td>
<td>+ 0.6</td>
<td>11.8</td>
<td>+ 1.89</td>
</tr>
<tr>
<td>October</td>
<td>13.1</td>
<td>+ 3.3</td>
<td>8.1</td>
<td>+ 3.9</td>
<td>18.0</td>
<td>+ 2.5</td>
<td>13.3</td>
<td>+ 1.59</td>
</tr>
<tr>
<td>November</td>
<td>3.4</td>
<td>- 0.3</td>
<td>- 2.3</td>
<td>- 0.9</td>
<td>9.3</td>
<td>+ 0.2</td>
<td>6.4</td>
<td>- 1.45</td>
</tr>
<tr>
<td>December</td>
<td>1.0</td>
<td>+ 2.5</td>
<td>- 3.1</td>
<td>+ 3.2</td>
<td>5.1</td>
<td>+ 2.1</td>
<td>7.9</td>
<td>- 1.6</td>
</tr>
<tr>
<td>2022</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>January</td>
<td>- 6.8</td>
<td>+ 2.1</td>
<td>- 12.5</td>
<td>- 2.4</td>
<td>- 1.0</td>
<td>+ 1.1</td>
<td>3.25</td>
<td>- 3.0</td>
</tr>
<tr>
<td>February</td>
<td>- 2.3</td>
<td>+ 1.2</td>
<td>- 8.0</td>
<td>+ 1.3</td>
<td>3.3</td>
<td>+ 1.6</td>
<td>10.8</td>
<td>+ 3.88</td>
</tr>
<tr>
<td>March</td>
<td>2.4</td>
<td>+ 1.2</td>
<td>- 3.3</td>
<td>+ 1.0</td>
<td>8.2</td>
<td>+ 1.6</td>
<td>7.1</td>
<td>- 0.7</td>
</tr>
<tr>
<td>April</td>
<td>7.8</td>
<td>0.0</td>
<td>1.8</td>
<td>+ 0.4</td>
<td>13.8</td>
<td>- 0.1</td>
<td>8.4</td>
<td>- 0.3</td>
</tr>
<tr>
<td>May</td>
<td>15.9</td>
<td>+ 1.8</td>
<td>9.3</td>
<td>+ 1.6</td>
<td>22.4</td>
<td>+ 2.0</td>
<td>6.3</td>
<td>- 1.7</td>
</tr>
<tr>
<td>June</td>
<td>18.8</td>
<td>+ 0.1</td>
<td>12.4</td>
<td>- 0.4</td>
<td>25.3</td>
<td>+ 0.7</td>
<td>4.8</td>
<td>- 6.9</td>
</tr>
</tbody>
</table>

Regional averages were compiled based on monthly averages data obtained from the Orange Municipal Airport weather station in Orange, MA, USA, 22 miles from the experimental site.

2.4.2 Dry Weight Biomass Yield, N and C Accumulation, and C:N of Cover Crop Roots and Aerial Parts

Total biomass, determined by combining the dry weight biomass of the aerial and root parts of each cover crop treatment, did not vary significantly by cover crop treatment in this experiment. However, the proportion of biomass attributed to roots and aerial parts differed significantly among cover crops (Table 2.2). DR produced significantly higher root biomass (1080.2 kg ha\(^{-1}\)) than all other cover crop treatments, and the lowest aerial biomass (1334.5 kg ha\(^{-1}\)), while MX produced the highest aerial biomass (2159.2 kg ha\(^{-1}\)).
Unsurprisingly, the N concentration of FP roots and aerial parts were significantly higher than that of other treatments (Table 2.3). The N concentrations of DR and MX were intermediate, and the initial %N of OT residues was significantly smaller than that of other treatments. However, due to differences in aerial and root biomass, the total N (kg ha\(^{-1}\)) was not significantly different among any treatments other than OT, which produced significantly less N (30 ha\(^{-1}\) total) at winterkill than the other cover crops. There were few differences in the initial C concentration and C yield (kg ha\(^{-1}\)) among cover crop treatments. Despite having the highest root C concentration (41.3%), FP roots had the lowest C yield. Despite having a low C concentration in the roots compared to other treatments, MX had the highest overall C concentration and C yield (1190.4 kg ha\(^{-1}\)). FP had the lowest C yield at 767.5 kg ha\(^{-1}\), while DR and OT produced intermediate amounts of C (1044.4 kg ha\(^{-1}\) and 872.2 kg ha\(^{-1}\), respectively).

### Table 2.2 Dry Matter Biomass, N and C Yield Cover Crops at Time of Winterkill

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DM biomass (kg ha(^{-1}))</th>
<th>N kg ha(^{-1})</th>
<th>C kg ha(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>FP</td>
<td>root: 44.6 c 1740.1 ab 1784.7</td>
<td>root: 1.3 b 63.7 a 65.0 a 20.7 c</td>
<td>766.8 ab 787.5 b</td>
</tr>
<tr>
<td>DR</td>
<td>root: 1080.2 a 1334.5 c 2414.6</td>
<td>root: 17.6 a 41.6 ab 59.2 a 444.5 a</td>
<td>599.86 b 1044.4 ab</td>
</tr>
<tr>
<td>OT</td>
<td>root: 431.8 b 1536.9 bc 1968.6</td>
<td>root: 2.55 b 27.5 b 30.0 b 177.3 b</td>
<td>694.8 b 872.2 ab</td>
</tr>
<tr>
<td>MX</td>
<td>root: 354.9 bc 2159.2 a 2514.1</td>
<td>root: 6.61 b 58.5 b 65.1 a 143.2 bc</td>
<td>1047.18 a 1190.4 a</td>
</tr>
<tr>
<td>Pr&gt;F</td>
<td>&lt;.0001 0.0145 .0676 .0003 .0033 .0055 &lt;.0001 0.0074 0.0438</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FP = field pea, DR = daikon radish, OT = oat, MX = mixed cover crop treatment, DM = dry matter. Values presented are the combined averages of the 2021 and 2022 experimental years. Values presented in the same column with the same letter are not significantly different from each other.
The initial C:N of cover crop roots and aerial parts were significantly impacted by cover crop treatment and differed in the two experimental years, with root C:N being significantly higher overall in the second year than in the first (Figure 2.1). The initial C:N of OT roots and aerial parts were significantly higher than those of other cover crops (Figure 2.1). Among shoots, FP had the lowest aerial C:N, and DR and MX had intermediate C:N. There were few differences initial C:N differences among the roots of the other cover crops treatments.
Figure 2.1. Initial carbon: nitrogen (C:N) ratios of the cover crop aerial and root residues at time of winterkill. FP = field pea; DR = daikon radish; MX = mixed cover crop treatment; OT = oat. The red horizontal line represents the immobilization / mineralization threshold level C:N (24:1) established by Trinsoutrot et al. (2000); C:N values above this line encourage net soil N immobilization while values below this line tend towards net soil N mineralization. *, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively; ns = non-significant. Letters across columns signify significant statistical differences in C:N among cover crop treatments. Cover crops were harvested on November 12, 2020 and November 5, 2021 for analysis.

2.4.3 Moisture, Lignin, Cellulose, and Hemicellulose Content of Cover Crop Roots and Aerial Parts

Lignin, cellulose, and hemicellulose content (% DM) of the cover crop roots and aerial parts were quantified in the second year only.

A greater percentage of cellulose was found in the aerial parts of cover crops rather than the roots of all treatments, other than FP, in which a greater percentage was found in the roots (Figure 2.2). The percent of hemicellulose in the roots of OT and MX was significantly lower than in the aerial parts, but higher than in the aerial parts of FP. DR had similar hemicellulose composition in the roots and aerial parts. Lignin concentration was significantly higher in FP roots than aerial parts, and significantly
lower in MX roots compared to the aerial portions. Lignin concentration was not significantly different in the roots and aerial parts of DR and OT.

**Figure 2.2.** Percent initial hemicellulose, cellulose, and lignin composition of root and aerial residues. Values presented reflect averages of the second year only. MX = cover crop mixture; FP = field pea; DR = daikon radish; OT = oat. Letters across columns show treatment differences in lignin, hemicellulose, and cellulose content; letters that are different from each other signify statistically significant differences. Capital letters to the right of the columns show differences in lignin concentration. *** = statistically significant at the .001 probability level. Cover crops were harvested November 5, 2021 for analysis.

Differences in aerial and root lignin, cellulose, and hemicellulose content were highly significant among cover crop treatments (Figure 2.2). The aerial OT and MX residues had significantly higher cellulose content (25.7 and 25.7, respectively) than FP
(21.0%) and DR (14.4%), which had the lowest. FP had the highest root cellulose content (26.7%), OT had an intermediate percentage at 20.6%, and DR and MX had the lowest root cellulose content (10.2% and 11.9, respectively). The strongest compositional differences among cover crop treatments were evident in the hemicellulose content of both the roots and aerial parts. OT had the highest root and aerial hemicellulose content (22.5% and 24.1%, respectively), while DR had the lowest hemicellulose concentration (4.6% in leaves and 3.8% in roots) of all treatments. The root lignin concentration of FP was significantly higher than that of the other residues at 12.7%, while the lignin concentration of DR roots was significantly lower than all other treatments at 1.4%. The aerial lignin concentration of DR (1.5%) was also lower than the lignin concentration of other cover crop treatments. MX and FP had the highest lignin concentrations at 6.6% and 5.1%, respectively.

The lignin:nitrogen ratio (L:N) of oat and MX residues was significantly higher (Pr > F = <.0001) than that of DR, but not significantly different from the L:N of FP (data not shown). The aerial FP and DR L:N were not significantly different from each other. OT and FP L:N were not significantly different from each other but were significantly higher than the L:N of DR and MX root residues, which were not significantly different from each other.

The initial moisture content of cover crop aerial parts in the second year was significantly different (Pr > F = <.0001) among all cover crop treatments, with DR having the highest water content (90.8%) and OT having the lowest at 80.8% (data not shown). Similarly, DR root had a significantly higher moisture content at 90.3% than all
other treatments except FP (84% moisture). MX residues had the lowest root moisture content at 83.4% moisture.

2.4.4 Spring Dry Matter Decomposition, N and C Release from Cover Crop Residues

% DM, N and C remaining in residues were calculated for each sample date by comparing with the initial biomass, N and C yield of cover crop treatments at time of litter bag placement.

The %DM, N and C yield of FP roots and aerial parts decreased linearly throughout the spring sampling period; trends did not differ significantly between the two experimental years (Figures 2.3 – 2.5). However, the low $r^2$ value associated with the linear model suggests that the percentage of the initial N remaining in roots during spring decomposition is highly variable (Figure 2.4). The percentage of total DM, N and C lost from FP roots was similar in both experimental years, as well as C lost from aerial residues. However, a greater percentage of initial DM and N was lost from FP aerial residues in the second year than in the first.

DM decomposition and % C and N loss from DR root and aerial residues followed linear trends in both experimental years (Figures 2.3-2.5). There were no significant differences among DR DM and C trends between years; however, the trend of N loss was significantly different between the two experimental years. Residue C concentration data was missing for sample date 5 and 6 of the first year (27/5 and 10/6), thus, values were estimated using the linear regression equation with the best fit across the first four sample dates ($y = -10.8x + 64.5$). A greater portion of initial DR DM, N and
aerial C were lost before April 1 in the second year. The percentage of DR root C released over the winter was similar both years.

Spring C loss and DM decomposition of OT root residues were linear in both 2021 and 2022 (Figures 2.3 and 2.5) and did not differ significantly between years. The percent of initial N lost from oat roots during the spring decomposition period was not significant in either year (Figure 2.4). Aerial residue C and N release followed a linear trend in the spring of the first year, but neither C nor N release were significant in the spring of the second experimental year. Aerial biomass decomposition was significantly impacted by the interaction of year and sampling time. In the second year, OT aerial biomass decomposition followed a linear trend, while in the first year the trend was quintic. However, it is likely that the non-linear trend observed in the first year can primarily be explained by sample variation, as by far greatest sum of squares is associated with a linear relationship. OT aerial residues lost a greater percentage of initial DM, N and C before April 1 in the second experimental year than the first. However, this trend was reversed in OT root residues, which lost a greater proportion of their initial DM, N and C in the first year than the second.

The interaction of sampling time and year was significant for the DM and the percentage of initial N and C remaining in the MX aerial residues (Figures 2.3-2.5). In the first year, aerial DM, and percent N and C remaining in aerial residues decreased linearly during the spring sampling period. However, in the second experimental year, neither aerial DM nor percent N or C remaining in aerial residues was significantly different across spring sampling times. The root biomass and root N decomposition of MX residues followed linear trends both years. The amount of residue C released by the
MX roots in the spring of the second year was not significantly different among sampling times. The percentage of MX DM remaining at the start of the spring sampling period was similar both years. While there was more C lost from the roots and aerial parts of MX residues during the winter period of the second year, the differences were not drastic (59% and 51% of initial root C remaining April 1, and 73% and 61% of initial aerial C remaining April 1 in 2021 and 2022, respectively). A greater amount of N was lost from MX residues during the winter of the second experimental year than the first.

Both years, OT aerial residues released a significantly smaller proportion of total aerial N than all other treatments before April 1, and the lowest proportion of total aerial N throughout the experimental period. There were no differences in the proportion of total N or total C released from cover crop roots before April 1 in the first experimental year. However, in the second experimental year, OT roots released a significantly smaller portion (23.4%) of total root N than the MX and FP root residues (70.5% and 62.5%, respectively) and a significantly smaller portion of total root C (26.2%) than FP and FR (58.9% and 72.4%, respectively) before April 1. The percentage of total root C released by MX residues (49.5%) before April 1 of the second experimental year was not significantly different from the percentage released by OT, FP, and DR. DR roots released 40.3% of the total N found in the roots before April 1 of the second year, which was not significantly different than the proportion of N released by the other treatments.

During the spring sampling period (April 1 – June 10), there were no significant difference in % of total aerial N, % of total aerial C, or % of total root C released among the various treatments. However, in spring of the second experimental year, DR released a significantly greater portion of the total N found in the aerial residues than any other
cover crop treatment (45.3% of total DR aerial N, compared to <10% of total aerial N in other treatments), and a greater % of total root N (67.1% of total root N compared to <25% total root N in other treatments).

The total % DM, N and C lost from residues during the experimental period (November – June) varied by cover crop treatment and year (Figures 2.3-2.5). In the first year, DR and MX aerial residues released 79.7% and 68.7% of their total N before June 10, 2021. This was significantly higher than the proportion of total N released from the residues of OT (34.7%) but not significantly different from the total percentage of N released by FP (65.0%) which released an intermediate amount that was not significantly different from any of the other cover crop treatments. In the second year, FP released 82.3% of the total N contained in the aerial residues by June 10, DR released 91.1%, MX aerial residues released 82.2%, and OT aerial residues released 64.5% of their total N by the end of the experimental period. The proportion of N released by DR aerial residues was significantly higher than the proportion released by OT; the other treatments were not significantly different from each other.

The percentage of total aerial C released by DR in the first year (72.1%) was significantly higher than the percentage released by aerial OT residues (45.0%). FP and MX released intermediate amounts of the total C found in their aerial residues (57.9% and 64.5%, respectively) in the first year, proportions that did not differ significantly from the other treatments. In the second year, DR released a significantly greater proportion of total aerial residue C by the end of the experimental period (92.7%) than the aerial residues of all other treatments (<56%), which did not differ significantly from each other.
FP and DR released 81.8% and 79.7% of their total root N by June 10 in the first experimental year, significantly higher than the amount released by OT and MX root residues, 62.6% and 69.1%, respectively. OT roots released a significantly smaller proportion of total root N (26.7%) than all other cover crop root residues in the second year (>80%). The percentage of total root C released by the residues by the end of the experimental period was significantly different in the second year, but not in the first. In the second experimental year, DR roots released significantly greater proportions of total residue C (93.3%) than all other treatments June 10, while OT released a significantly smaller proportion of total root C (48.8%) than the other treatments. FP and MX root residues released intermediate amounts of their total root C before June 10 of the second experimental year (71.5% and 79.8%, respectively), values that were significantly different from DR and OT but not from each other.
Figure 2.3. Dry Matter (DM) remaining in cover crop aerial and root residues during spring decomposition. FP = field pea; DR = daikon radish; OT = oat; MX = mixed cover crops. *, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively; ns = non-significant. Litter bags were placed in the field on November 15, 2020 and November 6, 2021 and collected every two weeks from April 1 – June 10 the following spring.
Figure 2.4. Percent N remaining in cover crop aerial and root residues during spring decomposition. FP = field pea; DR = daikon radish; OT = oat; MX = mixed cover crops. *, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively; ns = non-significant. Litter bags were placed in the field on November 15, 2020 and November 6, 2021 and collected every two weeks from April 1 – June 10 the following spring.
Figure 2.5. Percent C remaining in cover crop aerial and root residues during spring decomposition. FP = field pea; DR = daikon radish; OT = oat; MX = mixed cover crops. *, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively; ns = non-significant. Litter bags were placed in the field on November 15, 2020 and November 6, 2021 and collected every two weeks from April 1 – June 10 the following spring.
Total N (kg ha\(^{-1}\)) and C (kg ha\(^{-1}\)) released from cover crop residues was calculated by adjusting the initial N and C yield (kg ha\(^{-1}\)) of residues to the percentage of cover crop residues remaining at the end of the spring sampling period, averaged between two the two experimental years.

OT residues released significantly less N (18 kg ha\(^{-1}\)) during the experimental period than the other cover crop residues, whose N release ranged from 48 kg ha\(^{-1}\)- 50 kg ha\(^{-1}\) (Figure 2.6). DR and MX released significantly more C than OT and FP during the experimental period, although the majority of DR C was released from root residues, while the majority of MX C was released from the aerial residues.

![Figure 2.6 Total nitrogen (N) and carbon (C) released from cover crop residues during the experimental period.](image)

**Figure 2.6 Total nitrogen (N) and carbon (C) released from cover crop residues during the experimental period.** Left: Total N (kg ha\(^{-1}\)) released from cover crop residues, averaged across the two experimental years. Right: Total C (kg ha\(^{-1}\)) released from cover crop residues, averaged across the 2021 and 2022 experimental years. Letters that are different from each other signify statistically significant differences between the means of the treatments. The level of significance is presented over each set of columns; *, **, and *** are significant at the 0.05, 0.01, and 0.001 probability levels, respectively; ns = non-significant. OT = oat; DR = daikon radish; FP = field pea; MX = cover crop mixture.
2.4.5 Differences among rate constants $k$, $k_c$, and $k_n$

Table 2.4. Influence of cover crop treatment on biomass, nitrogen and carbon exponential decay constants $k$, $k_c$, and $k_n$

<table>
<thead>
<tr>
<th></th>
<th>$k_a$</th>
<th>$k_r$</th>
<th>$k_n$</th>
<th>$k_a$</th>
<th>$k_r$</th>
<th>$k_c$</th>
<th>$k_c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>FP</td>
<td>1.0 b</td>
<td>2.2 b</td>
<td>2.3 b</td>
<td>2.9 a</td>
<td>1.5 b</td>
<td>2.5 ab</td>
<td></td>
</tr>
<tr>
<td>DR</td>
<td>2.8 a</td>
<td>3.5 a</td>
<td>3.6 a</td>
<td>3.3 a</td>
<td>3.5 a</td>
<td>3.8 a</td>
<td></td>
</tr>
<tr>
<td>OT</td>
<td>0.8 b</td>
<td>2.6 a</td>
<td>1.2 c</td>
<td>0.9 b</td>
<td>0.9 b</td>
<td>1.6 b</td>
<td></td>
</tr>
<tr>
<td>MX</td>
<td>1.4 b</td>
<td>1.7 b</td>
<td>2.5 b</td>
<td>2.6 a</td>
<td>1.6 b</td>
<td>2.7 ab</td>
<td></td>
</tr>
<tr>
<td>Pr&gt;F</td>
<td>&lt;.0001</td>
<td>.0081</td>
<td>.0003</td>
<td>.0003</td>
<td>&lt;.0001</td>
<td>.0048</td>
<td></td>
</tr>
</tbody>
</table>

FP = field pea, DR = daikon radish, OT = oat, MX = mixed cover crop treatment, $k$ = biomass exponential decay rate constant; $k_n$ = nitrogen exponential decay rate constant; $k_c$ = carbon exponential decay rate constant. Values presented are the combined averages of the two experimental years. Values presented in the same column with the same letter are not significantly different from each other.

The DR aerial DM decomposition rate constant $k$ was significantly higher than the $k$ values of all other treatments (Table 2.4). The year by treatment interaction was significant for $k$, but no meaningful differences were observed; thus, the results were averaged between the two years. DR aerial $k_n$ (3.6) and $k_c$ (3.5) values were significantly higher than those of other treatments, with the $k_c$ values of other residues not differing significantly from each other. FP (2.3) and MX (2.5) had intermediate aerial $k_n$ values, while OT had the lowest $k_n$ value at 1.2.

The rate constant $k$ for DR roots (3.5) was significantly higher than that of FP (2.2) and MX (2.5) $k$ values, but not significantly different than the OT (2.6) $k$ value. The year by treatment interaction was significant for $k_n$ (Pr = 0.0261). In the first year, there were few differences $k_n$ among cover crops; most notably, the $k_n$ of FP roots (3.07) was significantly higher than that of the MX root residues (2.18). DR and OT had intermediate $k_n$ values of 2.69 and 1.35, respectively, that did not differ significantly from
the other treatments. In the second year, OT roots had a significantly lower \( k_n \) (0.39) than all other treatments, while DR roots had the highest \( k_n \) at 3.81. The differences in \( k_c \) among root residues, although significant, were less pronounced. Most notably, DR had a significantly higher \( k_c \) value than OT (3.8 and 1.6, respectively).

2.4.6 Relationships between decomposition rate constants, proportion of litter decomposed, and litter quality parameters

A correlation analysis was conducted to quantify the relationships between rate constants \( k, k_c, \) and \( k_n \), and litter quality parameters (Table 2.5).

Table 2.5. Pearson correlation coefficients (r) between decay parameters \( k, k_n, k_c \) and residue quality parameters (n=16)

<table>
<thead>
<tr>
<th>Root</th>
<th>C:N</th>
<th>% lignin</th>
<th>% hemicellulose</th>
<th>% cellulose</th>
<th>L:N</th>
<th>% moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k )</td>
<td>-0.37 ns</td>
<td>-0.24 ns</td>
<td>-0.58 *</td>
<td>-0.69 **</td>
<td>-0.59 *</td>
<td>0.21 ns</td>
</tr>
<tr>
<td>( k_n )</td>
<td>-0.76 ***</td>
<td>0.07 ns</td>
<td>-0.43 ns</td>
<td>-0.80 ***</td>
<td>-0.60 *</td>
<td>0.18 ns</td>
</tr>
<tr>
<td>( k_c )</td>
<td>-0.44 ns</td>
<td>-0.23 ns</td>
<td>-0.60 *</td>
<td>-0.75 ***</td>
<td>-0.64 *</td>
<td>0.18 ns</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Aerial</th>
<th>C:N</th>
<th>% lignin</th>
<th>% hemicellulose</th>
<th>% cellulose</th>
<th>L:N</th>
<th>% moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k )</td>
<td>-0.54 *</td>
<td>-0.52 *</td>
<td>-0.73 **</td>
<td>-0.68 **</td>
<td>-0.59 *</td>
<td>0.79 ***</td>
</tr>
<tr>
<td>( k_n )</td>
<td>-0.67 **</td>
<td>-0.36 ns</td>
<td>-0.66 **</td>
<td>-0.73 **</td>
<td>-0.54 *</td>
<td>0.74 ***</td>
</tr>
<tr>
<td>( k_c )</td>
<td>-0.69 **</td>
<td>-0.58 *</td>
<td>-0.87 ***</td>
<td>-0.75 ***</td>
<td>-0.69 **</td>
<td>0.86 ***</td>
</tr>
</tbody>
</table>

\( k \) = biomass exponential decay rate constant; \( k_n \) = nitrogen exponential decay rate constant; \( k_c \) = carbon exponential decay rate constant; L:N = lignin:nitrogen ratio. *, **, *** Significant at the 0.05, 0.01, and 0.001 probability level, respectively; ns = non-significant.

The initial hemicellulose, cellulose and moisture content of aerial residues were significantly correlated with all rate constants \( (k, k_c, \) and \( k_n) \). However, C:N was more strongly correlated with rate constants \( k_n \) and \( k_c \) (\( r = -0.67 \) and \( r = -0.69 \), respectively) than with \( k \) (\( r = -0.54 \)). Similarly, aerial cellulose concentration showed a significant strong negative correlation (\( r < -0.70 \)) with \( k_c \) and \( k_n \). There was a significant strong correlation (\( r > 0.70 \)) between aerial moisture content and all three decay rate constants. Aerial hemicellulose showed strong negative correlations (\( r < -0.79 \)) to aerial \( k \) and \( k_c \) and
a weaker, but significant relationship to $k_n$ ($r = -0.66$). Although there were some
significant relationships between both lignin content and L:N and rate decay constants in
aerial residues, these relationships were generally weaker ($r > -0.60$), except for the
correlation between L:N and $k_c$ ($r = -0.69$).

There were fewer significant relationships between litter quality parameters and
the decay rate constants of root residues aerial residues. C:N was strongly and negatively
correlated to $k_n$ but to no other rate decay constants. The correlation between cellulose
and $k$ ($r = -0.69$) was the strongest relationship between $k$ and any litter quality parameter.
Cellulose content showed strong correlation with $k_n$ ($r = -0.80$) and $k_c$ ($r = -0.75$).
Hemicellulose showed significant correlations with $k$ and $k_c$, ($r = -0.58$ and $r = -0.60$,
respectively).

### 2.4.7 Changes in C:N of Cover Crop Root and Aerial Residues During Spring Decomposition

There were no significant changes in the C:N of the aerial cover crop residues
during spring decomposition. However, the C:N of cover crop root residues were
significantly impacted by sampling time. The C:N of OT roots decreased linearly both
years (Figure 2.7). The sampling date by year interaction was significant for the C:N of
FP, DR, and MX root residues. In the first year, there were no significant changes in the
C:N of DR root residues during the decomposition period, and in the second year the
changes in C:N followed a non-linear trend. Although there were significant differences
in C:N during the in-season decomposition of MX root residues both years, the trends
were not consistent from year to year. Spring changes in FP root residue C:N followed a
cubic trend both years.
Figure 2.7. Changes in C:N of cover crop root residues during spring decomposition. FP = field pea; DR = daikon radish; OT = oat; MX = mixed cover crop. *, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively; ns = non-significant. The red horizontal line represents the immobilization / mineralization threshold level C:N (24:1) established by Trinsoutrot et al. (2000); C:N values above this line encourage net soil N immobilization while values below this line tend towards net soil N mineralization. Litter bags were placed in the field on November 15, 2020 and November 6, 2021 and collected every two weeks from April 1 – June 10 the following spring.

2.5 Discussion

This study quantified the DM decomposition and nutrient release of three commonly planted cover crops and their mixture in the spring following winterkill. Winter cover crops have the potential to produce both short-term benefits to cropping systems by reducing soil erosion, preventing N leaching, and recycling nutrients to
successor crops, and long-term benefits by providing substantial C inputs to the soil profile, contributing to C sequestration and soil organic matter (SOM) formation.

### 2.5.1 Cover crop biomass, N and C yield, and residue quality

Overall, cover crop biomass, N yield and C:N were similar among all treatments except for oat, which accumulated significantly less N than other cover crops during the fall growing period and, consistent with other studies, had a significantly higher C:N than legume and brassica cover crops (Brennan et al., 2009; Clark et al., 1994; Duval et al., 2016; Kuo et al., 1997b; Ranells & Wagger, 1996; Tadiello et al., 2022). Grasses contain a higher proportion of carbon-rich structural components than other crop types and are less efficient at acquiring N from the environment; as a result, grass species accumulate less N than legume and brassica species and tend towards high C:N (Clark et al., 1994; Sainju et al., 2000, 2007). Despite producing the least amount of biomass, FP had a higher N concentration than other cover crops, resulting in a comparable N yield to DR and the mixed cover crop treatment. Legumes generally produce lower biomass but higher N concentrations (resulting in low C:N) than other cover crops planted in monoculture (Clark et al., 1994; Sainju et al., 2000, 2007; Snapp et al., 2005; Tadiello et al., 2022).

The results of this study showed no significant yield advantages to planting mixed cover crops as compared to well-performing monocultures, supporting previous findings that planting cover crop species in mixture rather than in monoculture does not necessarily lead to advantages in total biomass yield (Finney et al., 2016; Florence & McGuire, 2020; Garnier et al., 1997; Koudahe et al., 2022; Liebig et al., 2015; R. G.
Smith et al., 2014; Wortman et al., 2012). Consistent with other studies, the cover crop mixture had an ‘intermediate’ C:N as compared to legume or grass monocultures (Finney et al., 2016; Ranells & Wagger, 1996; Schipanski et al., 2014). However, the mixed cover crop residues did not release a greater amount of spring N than other cover crop treatments nor was the decomposition rate significantly different from that of the other treatments. Smith et al. (2014) demonstrated that the short-term ecosystem benefits, including total biomass production, of a five-species cover crop mixture did not exceed those provided by the species grown individually in monoculture. Furthermore, Finney et al. (2017) found that while cover crop mixtures generally tend towards greater multifunctionality than monocultures, oat and tillage radish monocultures can provide similar multifunctionality to cover crop mixtures, and pea monoculture can provide greater multifunctionality than cover crop mixtures. Therefore, planting a multi-species mixture in place of well performing monocultures may not be economically advantageous for farmers primarily interested in short-term ecological services from cover crops. However, it is important to consider the limitations in quantifying short-term nutrient transformations in sustainably managed agronomic systems and emphasize a necessary shift towards examining the influence of sustainable management on long-term nutrient pools and soil quality.

Trinsoutrot et al. (2000) reported the decomposition of residues with a C:N of 24:1 or lower reliably resulted in net mineralization after 168 days, while residues with a higher C:N led to net N immobilization. However, it is important to note that mineralization and immobilization dynamics are based not only on residue quality, but also on soil conditions and field history (Hu et al., 2018; Mary & Recous, 1996; Recous
et al., 1995). Increased cover crop biomass is positively correlated to N accumulation in plant tissues (Finney et al., 2016, 2017), which can help mitigate winter N losses in cropping systems (De Notaris et al., 2018; Thapa et al., 2018). However, high cover crop biomass and associated N uptake can result in decreased soil inorganic N availability the following spring, particularly in residues with high C:N (Finney et al., 2016; Garba et al., 2022; Liebig et al., 2015). Soil response to cover crop decomposition was not measured in this study, thus, the fate of N released from the residues, including the amount mineralized, amount retained in soil vs. leached, and amount available for spring planted crops is ultimately unknown. Based on the decomposition trends observed in this study and previous observations made in the literature linking net soil mineralization and immobilization trends to initial residue C:N (Justes et al., 2009; Sylvia, 2005; Trinsoutrot et al., 2000), we can predict that winterkilled oat residues led to net soil immobilization, while DR and FP residues caused net N mineralization in the soil. However, Gieske et al. (2016) reported lower soil nitrate levels and reduced yields of spring planted oat following fall planted brassica cover crops, although initial residue C:N was not measured in their study. The interannual variation in the initial C:N of the cover crop mixture, and thus the potential influence on soil N, can likely be associated with planting date and crop maturity at maturation. It is important to consider that net mineralization trends do not guarantee successor crop uptake or positive yield response, as N mineralized from residues during the winter period is susceptible to loss through leaching before the planting of a successor crop. In particular, crops with low C:N and high initial N concentrations have been linked to increase winter N losses via leaching (Christensen,
1986; Finney et al., 2016), although sowing date, climate, and soil quality characteristics are also important factors (Teixeira et al., 2016).

One of the major ecological benefits provided by cover crops is the contribution of large amounts of residue C to agricultural systems, essential for maintaining and building soil organic carbon (SOC) (Blanco-Canqui et al., 2013; Y. Chen et al., 2015; Franzluebbers, 2005). SOC, the main component of SOM, promotes the macroaggregation of soil particles, providing microbial habitat, increasing water retention, and alleviating the risks of soil compaction, thus enhancing resiliency to extreme climate events such as flooding and drought (Blanco-Canqui et al., 2013). C cycling in soils is impacted by the quantity and quality of residue inputs, which influence the rate and magnitude of C stabilized in soils over time (Duval et al., 2016; Ghimire et al., 2017; Ntonta et al., 2022). Lignin, resistant to microbial degradation, limits access to the more easily degradable holocellulose components in plant cell walls (Berg & McClaugherty, 2014). Residues with increased lignin content are associated with decreased rates of decomposition, promoting C stabilization in soils (Ntonta et al., 2022; Talbot & Treseder, 2012). In forest systems, a reduction in the rate of litter decomposition is observed after 70-80% of the mass has been lost, with the remaining mass facilitating humus formation (Prescott, 2010). Ghimire et. al (2017) found that residue quantity was the major factor influencing labile SOC mineralization over an 8-week period despite differences in biomass decomposition rate among species. Mixed species residues have been shown to increase both microbial biomass and functional diversity during decomposition as compared to single species decomposition, which can
enhance soil ecosystem functions such as disease suppression and nutrient cycling (Chapman & Newman, 2010; Drost et al., 2020; Shu et al., 2022).

In this study, cover crops accumulated between 787.7 – 1190.4 kg ha C in the period before winterkill. Consistent with the findings of Kuo et al. (1997), the total C concentration of cover crop roots varied significantly across treatments, but there were no significant differences in % aerial C among cover crop species. DR and mixed cover crop treatments had the greatest C yield, consistent with the amount of biomas produced. Despite similar amounts of C produced by DR and MX, differences in their composition may alter trajectory of C released from residues during decomposition. At the time of winterkill, DR roots, which contained the majority of C produced by DR, had a significantly lower lignin content than any of the other treatments. By contrast, the aerial portions of MX, which contained the majority of C produced by the mixture, had the highest lignin content of any of the cover crop treatments. Moreover, only 36 – 47% of initial MX aerial residue C was remaining at the end of the experimental period, compared to only 10 – 15% of initial residue C of DR roots. Thus, there is greater potential of MX to provide ecosystem benefits via soil C stabilization than DR residues.

2.5.2 Cover crop root and aerial residue decomposition

By the end of the experimental period, 60 – 80% DM of OT aerial residues remained undecomposed, compared to 30 – 50% of root residues. These results indicate slower decomposition compared to other studies that evaluated the decomposition of gramineous species. For example, Roth et al. (2023) found that only 33% of cereal rye residues remained after 120 days, while Christensen et al. (1986) reported 50% biomass
loss from barley straw residues by late spring, although litter bags were placed in the field in September rather than November in the latter study. Both studies concluded that little to no plant available N was available to successor crops following cover crops. Furthermore, Malpassi et al. predicted that less than 55% of N contained in spring killed oat roots would be available to the successor crops. Thus, it is likely that fall-planted oat cover crops lead to short-term N immobilization and provide little supplemental N to spring planted crops.

By contrast, a majority of DR DM was decomposed, and N and C released, during the decomposition period. The roots and aerial residues of DR decomposed significantly faster than those of the other residues and released more both cumulatively and during the spring sampling season. Residue N decomposition was consistent with that described by Jahanzad et al. (2016) who reported <20% DM and N remaining from initial DR root residue after 12 weeks. However, contrary to the findings of Hu et al. (2018), who found that DR aerial residues decomposed more quickly than root residues, a similar amount of root and aerial residues were remaining at the end of the experimental period in this study. Additionally, while their study reported only 58% C mineralization after 180 days in both roots and aerial parts, our results suggest between 60-80% mineralization of aerial C and around 80% root C mineralization in the first 180 days since litter bag placement. Despite the rapid mineralization rate of DR residues, DR has been found to significantly reduce winter N losses via leaching compared to fallow controls (Norberg & Aronsson, 2020).

Our results suggest more complete decomposition by roots than aerial parts of FP during the winter and spring period following winterkill, which can likely be explained
by their high N concentration and proximity to soil microbes responsible for
decomposition. By the end of the experimental period, less than 35% initial N remained
in FP root and aerial residues, compared to 50-65% aerial DM and 30% root DM. Less
than 30% of initial root C remained after June 10, compared to 42% of initial aerial C.
The percentage of N released by aerial residues in this study was greater than that
observed by Jahanzad et al. (2016), despite a greater portion of DM decomposed.
Lupwayi and Soon (2000) reported significantly lower amounts of N mineralization from
FP residues, 19 – 24% over a one-year period. Both studies reported increased levels of
soil nitrate under FP decomposition, suggesting net mineralization.

Decomposition of cover crop mixtures that contain leguminous and non-
leguminous species have the potential to result in net soil N mineralization if the
proportion of legume species is kept above 40% (Kuo et al., 1997b). In this study, MX
was composed of 64% FP, 32% oat, and 4% DR, suggesting a tendency towards
mineralization. However, despite the composition of the mixture, the C:N of the aerial
mixture at the time of winterkill in the first year was relatively high (36.8), predicting net
immobilization. In the second year, the aerial C:N of residues was substantially lower,
indicating likely net soil N mineralization following residue decomposition. Moreover,
the decomposition of other cover crop treatments was consistent from year to year, with
almost no significant differences in DM decomposition, N or C release trends between
years (DR root biomass being the only exception); however, the decomposition trends of
the MX residues varied significantly from year to year. These results indicate greater
variability in the decomposition patterns of mixed residues as compared to monoculture
residues. Future research should focus on the sources of variability associated with mixed
residue decomposition to better understand how the factors influencing their decomposition can be manipulated to enhance on farm sustainability and meet nutrient management goals.

DM decomposition was closely associated to C release in both the aerial and root residues of the cover crop mixture during the experimental period. 58-70% DM and 57-66% C were released from aerial residues, while 71-80% DM and 75-77% C were released from root residues. Aerial and root residues released the same amount of N both years (81% and 80%, respectively, in the first year, and 69% in the second year), despite differences in DM decomposition and C release. Overall, the decomposition of mixed residues was not remarkably different than the decomposition of FP and DR, and the total amount of N (kg ha⁻¹) released during the experimental period was not significantly different.

2.5.3 Differences in residue decay rate, N and C release

Despite differences in initial litter composition, there were few significant differences among the rate of DM residue decay or C release among cover crop treatments during the experimental period, with the greatest $k$ and $k_c$ observed for DR in both roots and aerial parts. Previous studies have observed that litter residue decomposition is dominated by two stages: an initial phase of rapid, labile mass loss that occurs within the first four weeks of termination and a second, longer phase categorized by slower decay during which refractory litter fractions are decomposed and fewer differences in decomposition rate between residues of different quality are observed (Berg & Ågren, 1984; Douglas & Rickman, 1992; Jensen et al., 2005; Stott et al., 1986;
Trinsoutrot et al., 2000). In the present study, the $k$-values represent an average decay rate during the entire experimental period after cover crop termination (205 days in the first year and 216 days in the second year); thus, differences in decay rate during initial phase of decomposition are not reported and there are few overall differences among cover crop decay rate as residues have already entered the second phase of decomposition.

2.5.4 Relationships between litter quality and decay rate constants

It is well established that litter C:N, lignin content, and L:N are consistent predictors of litter decay rate and N release (B. H. Janssen, 1996; Melillo et al., 1982; Talbot & Treseder, 2012; Taylor et al., 1989; D. Zhang et al., 2008), although climate, soil characteristics, and litter placement are also important factors influencing rate of decay (Craine et al., 2007; Jahanzad et al., 2016; Thapa et al., 2022). In this study, we found that the litter quality of aerial surface residues is significantly associated with residue DM, N and C decay rate constants $k$, $k_n$ and $k_c$; however, the parameters of litter quality associated with aerial decay rate constants were not consistent with those associated with root decay rate constants. Initial C:N of residues were significantly, but moderately, associated with $k_c$ and $k_n$ in aerial residues, and showed a significant but weaker correlation to aerial $k$. Initial C:N was strongly and significantly correlated to $k_n$ in root residues, indicating that initial C:N is a stronger predictor of residue N and C mineralization than of overall decay rate, and that root C:N in particular is a strong predictor of root $k_n$. L:N values were moderately associated with $k$, $k_c$, and $k_n$ values in both roots and aerial parts, suggesting that L:N is a consistent, if not strong, predictor of residue decay and nutrient release.
Our results support the findings of Jensen et al. (2005), who concluded that holocellulose content best explained the variability in C release during residue decomposition and that lignin was not as valuable in predicting decay rate. Although initial lignin content was significantly correlated with aerial $k$ and $k_c$ in our study, the r values were relatively low compared to relationships with other litter quality parameters and lignin content was not significantly correlated to any of the root decay rate constants. Residue cellulose concentration had the strongest relationship to $k$, $k_a$ and $k_c$ in roots, and a strong negative correlation with $k_a$ and $k_c$ in aerial parts. Hemicellulose was only moderately, but significantly associated with $k_a$ and $k_c$ in roots, but had a stronger inverse relationship to $k$ and $k_c$ of aerial residues than cellulose.

Residue moisture content showed the strongest relationship to decay rate in aerial residues; however, this relationship was not significant in root decomposition. This is likely due to increased moisture loss from surface residues via evaporation due to increased exposure to high temperatures and wind. The $k$ and $k_c$ values of root residues were higher than those of aerial residues, which can likely be attributed to a lower percentage of carbon rich structural components such as lignin, cellulose, and hemicellulose (for all treatments except FP, in which the inverse relationship was observed), conditions favoring moisture retention, which is positively correlated with decomposition rate (Schomberg et al., 1994; Stott et al., 1986), and residue placement (buried vs. soil surface) (Douglas et al., 1980; Etemadi et al., 2018; Schomberg et al., 1994), resulting in closer proximity to soil microorganisms responsible for decomposition. Additionally, increased variability in weather conditions on the soil surface, including inconsistencies in moisture levels resulting in the drying and rewetting
of residues have shown to negatively impact decomposition rate of surface residues, as surface residues dry more quickly following precipitation events than do buried residues (Cabrera et al., 2005; Manstretta & Rossi, 2015; Schomberg et al., 1994).

Our results suggest that the while structural composition can serve as a valuable predictor of decay patterns in winterkilled cover crop residues, the parameters of litter quality by which decay rate is predicted is different in surface residues than in roots. According to our results, cellulose content is the best predictor of root decay ($k$, $k_n$ and $k_c$), and root C:N is a strong predictor of the rate of root N release. These findings are consistent with those of Silver and Miya (2001), who found that lignin, N content, and L:N were not strong indicators of root decomposition across peer-reviewed studies published globally, despite being reliable predictors of leaf litter decomposition. Overall, greater proportion of litter quality characteristics was associated with shoot $k$, $k_n$ and $k_c$, suggesting that litter quality is a better predictor of decay rate of aerial residues than root residues. It is important to consider that although individual litter quality characteristics can be helpful predictors for mass loss, it is necessary to take edaphic and climate conditions into account to accurately predict litter decay rate (Prescott, 2010; Silver & Miya, 2001; Thapa et al., 2022).

2.5.5 Changes in root residue C:N during decomposition

During the decomposition process, the chemical composition of residues changes, leading to shifts in microbial communities and potential changes in soil mineral N status (Berg & Ågren, 1984; Nevins et al., 2018). In this study, we found that despite significant changes observed during spring decomposition, the root C:N of FP and DR remained
lower than the residue C:N immobilization threshold (24:1) described by Trinsoutrot et al. (2000). The greatest change was observed in oat root residues, the C:N of which decreased steadily throughout the spring but remained high, suggesting that oat roots contribute to N immobilization dynamics in soils during decomposition, despite changes in chemical composition. Changes in mixed residue root C:N during spring decomposition were variable, dropping below the 24:1 C:N threshold within the first four weeks of decomposition in the first year but remaining higher in the second year. These results emphasize the variability of cover crop mixture decomposition and suggest that greater research is necessary to better understand the complexities of this variation and how it relates to management and climate fluctuations.

2.6. Conclusions

Overall, this study found that fall planted, annual cover crops can provide a significant amount of C to agroecosystems and release substantial amount of N during the winter and spring after frost termination. DR is a promising candidate to supply N to spring planted cash crops because of its high N content and quick decomposition rate. Although there may be few economic advantages to planting cover crop mixtures in the short term, cover crop mixtures provide C inputs that are substantially greater than FP and oat monocultures, which can influence the degree of C stabilization in the soil and build SOM in the long-term, increasing N retention and providing greater ecological services. Additionally, we found that the DM decomposition rate, as well as the rate of N and C release, are associated with different litter quality parameters in root and aerial residues. Our results indicate that initial cellulose content of root residues is the strongest predictor of $k$, $k_n$ and $k_c$, while moisture content is the strongest predictor of $k$ in aerial
residues. Consistent with other studies, we found that L:N and C:N are reliable predictors of decay rate, although C:N is primarily associated with N and C release rates than DM decomposition. Lastly, we observed significant changes in root residue C:N over the course of spring decomposition.
2.7 Chapter References

https://doi.org/10.2136/sssaj1991.0361599500550040022x


https://doi.org/10.2489/jswc.68.5.129A

https://doi.org/10.2134/agronj2008.0194x


CHAPTER 3

EVALUATING THE IMPACTS OF ANNUAL, FALL PLANTED COVER CROPS AND ARBUSCULAR MYCORRHIZAL (AM) FUNGI ON GARLIC YIELD, BULB NUTRIENT ACCUMULATION, AND NITROGEN USE EFFICIENCY IN A RELAY CROP SYSTEM

3.1 Abstract

Agricultural intensification in the 21st century must prioritize soil health, environmental sustainability, and the reduction of synthetic fertilizer use to support a growing global population while mitigating the deleterious effects of agriculture on the environment. Management strategies that support sustainable intensification include reduced tillage, continuous cropping, and facilitating the development of arbuscular mycorrhizal (AM) fungi associations in cropping systems. This study aimed to improve the sustainability of hardneck garlic production by incorporating AM fungi and cover crops (CC) into a garlic relay cropping (RC) system. Additionally, this study quantified the biomass and N accumulation of garlic roots, bulbs and aerial parts under optimum N nutrition in the spring. Hardneck garlic (*Allium sativum*) was relay planted into three species of commonly planted annual CC – oat (*Avena sativa*) (OT), daikon radish (*Raphanus sativus*) (DR), field pea (*Pisum sativum*) (FP), and their mixture (MIX) and amended with commercially available AM inoculant at time of planting. A no cover crop (NCC) control was amended with straw mulch to mimic traditional garlic production practices. Garlic bulb yield response to RC was dependent on cover crop fall biomass accumulation; CC accumulated 2300.9 – 3696 kg ha\(^{-1}\) dry weight (DW) in 2021, compared to >1500 kg ha\(^{-1}\) in 2020. In the second experimental year, garlic yield was
reduced by 21 - 31 % in RC treatments compared to the NCC control. In the first year, garlic yield was only reduced in the OT treatment, which was likely a result of N immobilization rather than competition. Agronomic efficiency (AE) was not improved under the relay cropping system. Garlic bulb N, P, K and Mn concentrations were reduced in CC treatments as compared to NCC in the first year, but were normalized in the second cropping season. The soil iron (Fe) concentration was below optimum for garlic production in this study. Garlic amended with AM had significantly higher bulb Fe concentration than unamended garlic both years (0.040 and 0.38, respectively) and significantly lower potassium (K) concentration in the second year. Despite the OT treatment producing the lowest garlic bulb yield in the first year, it performed best among the treatments containing cover crops in the second year and had the best nitrogen use efficiency (NUE\textsubscript{crop}) both years. Overall, the impacts of cover crops and AM were cumulative across the experimental period, emphasizing the need for long-term research to accurately quantify the benefits of sustainable management systems.

3.2 Introduction

To keep up with calorie demands of a rapidly growing global population and mitigate the negative environmental impacts exacerbated by industrial agriculture, agricultural intensification in the 21st century must prioritize environmental health, sustainable resource use and resilience to climate change (Hunter et al., 2019; Pimentel & Burgess, 2013). Current estimates predict the world population will reach 9.7 billion by 2050, reflecting unprecedented population expansion over the last 100 years that will require food production to increase dramatically to maintain (UN 2015) (Hunter et al.,
Since the development of the Haber-Bosch process in the early 20th century, the application of synthetic nitrogen (N) fertilizer to cropping systems has resulted in a 30–50% increase in crop yield worldwide and a heavy reliance on synthetic N for world food production. Consequently, environmental pollution and atmospheric emissions have increased at an alarming rate while agricultural nitrogen use efficiency (NUE) has dropped (Verzeaux, Nivelle, et al., 2017). Moreover, agricultural intensification has degraded soil quality and significantly accelerated topsoil erosion, contributing to significant economic declines and major reductions in soil fertility (Lal et al., 1991; Telles et al., 2011). Moving forward, management practices that reduce inputs and enhance soil health should be adapted to various production systems and scales to ensure their viability and encourage their adoption. Such practices include reduced tillage, incorporation of cover crops into cropping systems, encouraging conditions for mycorrhizal development, and minimizing reliance on external inputs.

Relay cropping, the practice of planting a second, or ‘relay’ crop into an already established ‘primary’ crop, can provide a sustainable management option for agricultural intensification (Gesch et al., 2023). Some of the benefits of relay cropping systems include increased land use efficiency, reduced nitrate leaching, and the spatial and temporal diversification of agricultural systems, which helps to mitigate pest and disease pressure while enhancing nutrient cycling and supporting soil and environmental health (Gesch et al., 2023; Lamichhane et al., 2023; Tanveer et al., 2017). The feasibility of incorporating relay cropping as a management practice is increasing due to lengthened growing seasons in traditionally colder climates because of global warming, making it possible to grow two crops in one year where the growing season is not long enough to
grow two crops sequentially (Lamichhane et al., 2023). Although the practice of relay cropping has gained popularity in soybean and wheat production in the United States over the last few decades, it is still poorly adopted worldwide, and most of the scientific literature is confined to optimizing relay cropping for these systems (Amossé et al., 2014; Cecchin et al., 2021; Gesch et al., 2023; Lamichhane et al., 2023; Martens et al., 2001; Ott et al., 2019). Due to the complex management associated with relay cropping systems, crop yield and soil response are system dependent and must be evaluated thoroughly to ensure the economic viability of individual cropping systems (Cecchin et al., 2021; Lamichhane et al., 2023). Thus, there is a need to evaluate the feasibility of using relay crop systems in specialty crop production before these practices can be recommended for farmer adoption.

Garlic (*Allium sativum*) is an economically important crop worldwide, with global production increasing annually to meet market demand (Food and Agriculture Organization of the United Nations, 2017). Hardneck garlic can contribute to the diversification of specialty crop systems by providing multiple marketable commodities: garlic scapes spring and bulbs in the summer. Bolting, or ‘hardneck’ garlic cultivars, typically selected for cultivation in northern climates, require a vernalization period of 30 – 60 days, depending on temperature (Khokhar, 2023). To meet this vernalization requirement, garlic bulbs are planted in late October, complete vegetative growth in the spring and are harvested mid-July. The excessively long cultivation period of garlic crops prevents growers from planting winter cover crops, which are typically established in the early fall in regions limited by short growing seasons. Omitting winter cover crops from rotations can disrupt efforts to build soil health in northern cropping systems, as growers
must prioritize cash crop production during the spring and summer. Relay planting garlic into fall planted annual cover crops can improve the soil health and environmental sustainability of garlic cropping systems without taking land out of production. Annual cover crops naturally senesce before the critical nutrient accumulation period of garlic begins in the spring, eliminating the need for mechanical termination and alleviating risk of competition during garlic bulb development. Moreover, the proposed relay cropping system has the potential to enhance garlic quality and increase bulb yield by recycling nutrients to growing garlic via decomposing cover crop residues in the spring.

Generally, crops selected for relay cropping should have a slow growth habit during the establishment, or ‘competition’, phase to reduce the risk of yield loss (Lamichhane et al., 2023). In this regard, garlic is an excellent candidate for relay cropping systems, as only root production begins in the fall, while most of the active growth and biomass accumulation occurs in the spring months after cover crops have been frost killed. The residues of frost terminated cover crops can protect the soil from erosion until their decomposition in the spring, which in turn can release nutrients to actively growing garlic, improving the synchrony of N uptake and enhancing NUE.

Improving NUE on farms is a critical step towards enhancing both the environmental and economic sustainability of agricultural production worldwide (Sharma & Bali, 2017). Synthetic N fertilizers are the primary method used by farmers to meet the nutritional demands of cropping systems, despite a disproportionately large amount of applied N being lost from intensive production systems annually (Hirel et al., 2014). In addition to the deleterious environmental effects caused by agricultural N pollution, the inefficiency of fertilizer use has economic consequences as the cost of synthetic
fertilizers continues to increase annually (Sharma & Bali, 2017). Although many factors can influence the NUE of cropping systems, including crop type, field history, and fertilizer application rate, placement, and timing, the greatest contributor to decreased agricultural NUE is a lack of synchrony between the timing of residue mineralization and N uptake of cash crops (Alghamdi & Cihacek, 2022; Cassman et al., 2002; Hirel et al., 2014; Sharma & Bali, 2017). Thus, evaluating management practices that can improve synchrony of N uptake on farms, such as the recycling of cover crop residue N to successor crops, is critical to improve NUE. In particular, legumes can supply 40 – 60% of the total N accumulated in their biomass to successor crops, reduce leaching from agricultural systems, and enhance cash crop colonization by AM fungi, thus improving system NUE (Hirel et al., 2014). However, due to their low carbon/nitrogen ratio (C:N), legume residues often mineralize too quickly to provide sufficient N to successor crops (Alghamdi & Cihacek, 2022). Daikon radish, a fast growing, low C:N cover crop, can accumulate substantial amounts of biomass before winterkill when planted in the fall, potentially supplying large amounts of N to spring cash crops (Lawley et al., 2012; Wang et al., 2008). Gramineous cover crop species, such as oat, decompose more slowly than legumes and brassicas but may immobilize soil N in the short term due to their high C:N (Aulakh et al., 1991; Dabney et al., 2001; Doran, 1991). Multi-species cover crop mixtures can maximize the synchrony of residue N release with successor crop uptake by ‘balancing’ the C:N of residues (Dabney et al., 2001; Fageria et al., 2005; Lawson et al., 2015; Tribouillois et al., 2016). Management practices that contribute to the retention of soil organic matter (SOM) and arbuscular mycorrhizal (AM) fungi development in
agricultural soils, such as continuous cropping and reduced tillage, can lead to improved NUE in the long term (Cassman et al., 2002).

The benefits of AM fungi to cropping systems, including increased cash crop yield, optimized nutrient uptake, enhanced nitrogen use efficiency and improved aggregate stability have been well documented (Asghari & Cavagnaro, 2011; Boutasknit et al., 2020; Hirel et al., 2014; Pellegrino et al., 2015; Verzeaux, Nivelle, et al., 2017). Accordingly, AM fungi has been identified as a critical component of sustainable agricultural intensification (Murrell et al., 2019). As AM fungi are particularly sensitive to disturbance and are dependent on the presence of host roots for their development, reduced tillage, continuous cropping and the retention of crop residues in agricultural fields favor AM fungi development and fungal spore density in agricultural soils (Verzeaux et al., 2016; Verzeaux, Nivelle, et al., 2017). Fall planted cover crops can increase mycorrhizal colonization of subsequently planted crops by facilitating AM overwintering, which is favored by attachment or proximity to living roots (Z. Kabir et al., 1997; Z. Kabir & Koide, 2002.; Verzeaux, Nivelle, et al., 2017). Extraradical hyphae, one of the principal sources of inoculum in the soil, continue to proliferate in the soil even after the death of their host plant (Z. Kabir et al., 1997). Thus, annual cover crops can contribute to AM overwintering in northern climates, despite being frost terminated. We hypothesized that in a garlic – cover crop relay crop system, inoculating garlic with AM fungi at time of planting could increase the nutrient concentration of garlic bulbs in the spring, as the relay cropping system provides favorable conditions for AM winter survival via the presence of cover crop and garlic roots, and nutrients released by cover crop residues in the spring could be more efficiently recovered by AM associated garlic.
For this study, we selected a commercially available, granular source of inoculum that is likely to be applied by farmers transitioning to no-till, continuous cropping systems to evaluate the benefits of incorporating an easily accessible AM inoculant into sustainable cropping systems.

This study aimed to evaluate the feasibility of a reduced-tillage relay cropping system in which garlic is planted into three commonly planted annual cover crops, oat (Avena sativa), daikon radish (Raphanus sativus), field pea (Pisum sativum), and their mixture, and amended with commercially available AM fungi inoculant at the time of planting. Although brassica species, such as daikon radish, do not form associations with AM fungi, fall planted forage radish does not inhibit mycorrhizal colonization of subsequently planted crops (Murrell et al., 2019; White & Weil, 2010). Our goal was to quantify the impacts of this system on garlic yield, nutrient uptake, and NUE. We hypothesized that 1) all cover crop treatments would increase garlic yield compared to a no cover crop treatment, with the highest yields obtained from garlic planted into mixed cover crops due to more synchronous release from their residues; 2) the nutrient concentration of garlic bulbs planted into cover crop residues and inoculated with AM fungi would be higher than that of uninoculated garlic planted into a fallow system; and 3) NUE would be highest in mixed cover crop treatments inoculated with AM fungi and lowest in uninoculated garlic planted into bare soil.
3.3 Materials and Methods

3.3.1 Experimental Site and Weather Conditions

A three-year field experiment (2020-2022) was conducted at the University of Massachusetts Crop and Animal Research and Education Center in South Deerfield, MA (42° N, 73° W). The soil at this location is characterized as a non-acid, mesic Typic Udifluent, Hadley fine sandy loam. Weather conditions, including annual precipitation and temperature data, for the experimental period, are presented in Table 3.1 alongside twenty-year averages (2000-2019).

3.3.2 Experiment Layout and Management

This experiment evaluated the impacts of four fall planted cover crop treatments and the in-field application of a commercially available mycorrhizal inoculant on the yield, nitrogen use and utilization efficiency, and nutrient concentrations of field planted garlic in a relay-cropped system.

Experimental plots (3.8 m²) were laid out in a randomized, complete block design consisting of four replications. Plots were planted in the same location both years to evaluate the cumulative effects of cover crops and mycorrhizae in a two-year system. Baseline soil samples were taken on 8/25 in 2020 and 2021. Cover crop treatments included monocultures of oat (*Avena sativa*, var: “Saddle Oat”) (OT), daikon radish (*Raphanus sativus*, var: generic) (DR), field pea (*Pisum sativum*, var: 4010) (FP), a mixture of the three species (MX), and a no cover crop treatment (NCC). Cover crops were seeded on 9/04 and 8/25 (2020 and 2021, respectively), using a 7-row cone seeder with 17.7 cm row spacing set at 73.5 cone speed and allowed to grow until frost.
terminated. Monoculture plots were planted following a summer buckwheat cover crop using the following seeding rates: 126 kg ha\(^{-1}\) (OT), 13.5 kg ha\(^{-1}\) (DR), and 90 kg ha\(^{-1}\) (FP); the seeding rates of each species were adjusted to 45 kg ha\(^{-1}\) (OT), 5 kg ha\(^{-1}\) (DR), and 90 kg ha\(^{-1}\) (FP) for the MX plots. NCC treatments were left fallow.

Garlic (\textit{Allium sativum}, var: German Extra Hardy) was relay-planted by hand into rows of standing cover crops on 10/18 and 10/15 in 2020 and 2021, respectively, 15 cm apart at a depth of 5 cm. Each plot contained 60 garlic plants. NCC plots were mulched with straw to mimic traditional garlic cultivation practices. At the time of planting, half of the garlic plots were inoculated with a commercially available, granular mycorrhizae blend composed of 4 species (\textit{Rhizophagus irregularus, Funneliformus mosseae, Rhizophagus aggregatum, Claroideoglomus etunicatum}) (+M), each present in the blend at a rate of 33 propagules gram\(^{-1}\), at the recommended rate for restoration (22.5 kg ha\(^{-1}\)), while the other half received no mycorrhizal inoculant (-M). Granular inoculant was placed into the furrows beside garlic cloves at the time of planting.

Garlic emerged by the third week in March in the spring after planting in both years and was drip irrigated throughout the experimental period. In the first experimental year, plots were weeded regularly during the spring growth period, while in the second year, straw mulch was applied to cover crop plots in April to eliminate weed competition. Garlic seedstalks (scapes) were removed on 6/10 and 6/14 in 2021 and 2022, respectively, to mimic traditional garlic production practices. Garlic was harvested on 7/6 in 2021, and 7/8 in 2022.

Nitrogen (N) fertilizer was applied in the form of 32\% Urea Ammonium Nitrate (UAN) at four levels: 0 kg ha\(^{-1}\), 44.4 kg ha\(^{-1}\) (33\% of the optimal recommended rate for
garlic production), 88.7 kg ha\(^{-1}\) (66% of the recommended rate) and 134.5 (100% of the recommended rate). Nitrogen was applied in a split application, with half of the N in each treatment applied when the garlic was 15 cm tall (4/9 and 4/13 in 2021 and 2022, respectively), and the other half two weeks later.

3.3.3 Sample and Data Collection

Cover crop biomass was determined by harvesting 1.5 m of cover crop biomass (roots and aerial parts) per plot in November of each year following three consecutive days of frost temperatures. Sample collection occurred on 11/12 and 11/5 in 2020 and 2021, respectively. Roots were carefully washed, and all samples were placed in a forced air oven at a temperature of 109\(^\circ\) C until constant weight to obtain dry matter (DM) yield, which was adjusted to kg ha\(^{-1}\). Cover crop DM samples were then ground to 1 mm particle size (Foss Cyclotec 1093 Sample Mill; Foss Electric A/S, Hillerød, Denmark). Processed samples were sent to a commercial laboratory, where N concentration (g kg\(^{-1}\)) was determined using a CHN analyzer.

The garlic growth curve was developed with garlic grown in the plots receiving the (134.5 kg ha\(^{-1}\)) x (-M) x (NCC) treatment combination, as it reflected traditional garlic production practices in which garlic is mulched in the fall and receives sufficient mineral N. Each year, 2 plants plot\(^{-1}\) were harvested every two weeks starting April 1 until harvest in early July. To obtain DM biomass, garlic roots, aerial parts, and bulbs were separated and dried in a forced air oven at 109\(^\circ\) C until constant weight, and their DM weights (g) divided by two to determine the average weight of each component per plant. Roots, aerial parts, and bulbs were then ground in a stainless-steel container using a
Vitamix 5200 high-power blender to pass through a 20-mesh sieve (Dual Manufacturing Company, Inc., Franklin Park, IL) and homogenized to prepare for laboratory analysis.

Garlic leaf chlorophyll concentrations were measured throughout the spring growing period using a SPAD-502 chlorophyll meter (Konica-Minolta, Japan). Measurements were taken from the second fully developed leaf as counted from the top of the plant and were collected from each experimental plot every two weeks, starting from April 14 until garlic harvest.

Soil nitrate concentration was measured every two weeks during the spring growing season in all plots receiving 0 kg ha\(^{-1}\) N. Twelve subsamples were collected from each plot at a depth of 15 cm using a soil auger (2 cm diameter). Samples were air dried, homogenized, and analyzed for nitrate using Quick-Chem Methods (Lachat Quick-Chem 8000 FIA; Zellweger Analytical, Milwaukee, WI, USA).

To obtain garlic yield, ten plants from each plot were harvested, bundled, and allowed to cure completely for three weeks by hanging in a well-ventilated tobacco barn. Following the curing process, roots and aerial parts were removed and bulbs were weighed to obtain the fresh weight (FW) yield, which was adjusted to kg ha\(^{-1}\).

At the time of garlic harvest, an additional two plants plot\(^{-1}\) were harvested, cleaned, and measured to obtain diameter (cm\(^2\)). Bulbs were finely chopped and dried in a forced air oven at a temperature of 109\(^\circ\) C until constant weight. Dried bulbs were ground to pass through a 20-mesh sieve (Dual Manufacturing Company, Inc., Franklin Park, IL) using a Vitamix 5200 high-power blender and retained for laboratory analysis.
### 3.3.4 Laboratory Analysis

A dry ashing procedure was used to determine the nutrient concentrations of garlic bulbs. After homogenization, 0.2 g of sample from each plot were weighed into porcelain crucibles, placed into a combustion oven at a temperature of 500 °C for 6 hours, and allowed to cool to room temperature. An amount of 15 ml 10% HCl was then added to each crucible, mixed, and filtered through Whatman #2 filter paper. Finally, the phosphorus (P), potassium (K), magnesium (Mg), manganese (Mn), copper (Cu), and iron (Fe) concentrations of each sample were quantified using microwave plasma-atomic emission spectroscopy (4210 MP-AES, Agilent Technologies, Santa Clara, CA).

The N concentration of garlic roots, aerial parts, and bulbs was quantified using the Kjeldahl method (Standard Method 4500-N(Org) C. Semi-Micro-Kjeldahl), followed by analysis with a Lachat 8500 FIA spectrophotometer, Lachat Total Kjeldahl Nitrogen (TKN) Method Number 13-107-06-2-D (Zellweger Analytical, Milwaukee, WI, USA).

### 3.3.5 Determination of Garlic Bulb NUE and NUtE

Agronomic Efficiency (AE) and Crop Nitrogen Use Efficiency (NUEcrop) of garlic were calculated using the following formulas (Martinez-Feria et al., 2018):

\[
AE = \frac{\text{kg garlic bulb yield}}{\text{kg fertilizer N applied}};
\]

and

\[
\text{NUEcrop} = \frac{\text{kg garlic bulb N yield}}{\text{kg fertilizer N applied + kg N released from cover crops}}.
\]
N released from each cover crop treatment was calculated by multiplying the initial N yield of each cover crop treatment by the percentage of N released from each treatment during spring residue decomposition (Chapter 1).

3.3.6 Statistical Analysis

Statistical analyses were performed using the proc GLM procedure in SAS, version 9.4 (SAS Institute, 2016). Means of effects that were significant at the p<0.05 level were separated using Tukey’s HSD in the case of fixed variables or fit to regression curves where variables were continuous.

3.4 Results

3.4.1 Weather Conditions at the Experimental Site

The average monthly precipitation and the minimum, maximum, and average monthly temperatures (°C) for the experimental period are presented in Table 3.1, alongside regional twenty-year (2000-2019) averages. Overall, weather conditions during the experimental period did not deviate notably from the regional norm, although fall temperatures were slightly warmer than the twenty-year regional average during both experimental years. However, in the first experimental year (2020), September had lower precipitation (~4.7 cm) as compared to the norm, which coincided with cover crop planting and establishment. In 2021, cover crops were planted at the end of August, which was warmer than both the regional average (+2.2 °C) and August 2020. October of the second experimental year was warmer and wetter than both 2020 and the twenty-year average, facilitating better conditions for cover crop growth.
During the spring growing season (April – July) of the first experimental year (2021), precipitation was lower than the regional norm in May (-6.7 cm) but showed a drastic increase in June (22.2 cm above average). Conditions during the spring growing period of the second year (2022) were exceptionally dry, with much lower precipitation than normal but temperatures close to the regional norm.

Table 3.1. Regional weather conditions at the experimental site and deviation from the regional norm.

<table>
<thead>
<tr>
<th></th>
<th>Avg. temperature (°C)</th>
<th>Dev. from regional avg.</th>
<th>Min Temp (°C)</th>
<th>Dev. from regional min.</th>
<th>Max Temp (°C)</th>
<th>Dev. from regional max.</th>
<th>Precipitation (cm)</th>
<th>Dev. from regional avg. precipitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2020</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>September</td>
<td>16.6</td>
<td>0.0</td>
<td>9.8</td>
<td>-0.8</td>
<td>23.2</td>
<td>+0.4</td>
<td>5.2</td>
<td>-4.7</td>
</tr>
<tr>
<td>October</td>
<td>10.3</td>
<td>+0.5</td>
<td>5.0</td>
<td>+0.8</td>
<td>15.6</td>
<td>+0.1</td>
<td>11.9</td>
<td>0.2</td>
</tr>
<tr>
<td>November</td>
<td>5.6</td>
<td>+1.8</td>
<td>-0.4</td>
<td>+1.0</td>
<td>12.1</td>
<td>+3.0</td>
<td>9.7</td>
<td>1.8</td>
</tr>
<tr>
<td>December</td>
<td>-0.3</td>
<td>+1.2</td>
<td>-5.3</td>
<td>+1.0</td>
<td>4.1</td>
<td>+1.0</td>
<td>10.8</td>
<td>1.3</td>
</tr>
<tr>
<td>2021</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>January</td>
<td>-3.1</td>
<td>+1.8</td>
<td>-6.8</td>
<td>+3.35</td>
<td>0.72</td>
<td>+0.8</td>
<td>5.4</td>
<td>-0.9</td>
</tr>
<tr>
<td>February</td>
<td>-4.0</td>
<td>-0.4</td>
<td>-8.6</td>
<td>+0.9</td>
<td>0.56</td>
<td>-1.1</td>
<td>4.7</td>
<td>-2.3</td>
</tr>
<tr>
<td>March</td>
<td>2.9</td>
<td>+1.9</td>
<td>-3.4</td>
<td>+1.1</td>
<td>9.17</td>
<td>+2.8</td>
<td>4.1</td>
<td>-3.63</td>
</tr>
<tr>
<td>April</td>
<td>8.6</td>
<td>+0.8</td>
<td>2.2</td>
<td>+0.7</td>
<td>15.0</td>
<td>+1.0</td>
<td>10.8</td>
<td>+2.1</td>
</tr>
<tr>
<td>May</td>
<td>14.3</td>
<td>+0.2</td>
<td>7.7</td>
<td>0.0</td>
<td>20.8</td>
<td>+0.5</td>
<td>10.2</td>
<td>+2.1</td>
</tr>
<tr>
<td>June</td>
<td>21.3</td>
<td>+2.6</td>
<td>14.8</td>
<td>+2.0</td>
<td>27.8</td>
<td>+3.3</td>
<td>5.1</td>
<td>-6.64</td>
</tr>
<tr>
<td>July</td>
<td>21.0</td>
<td>-0.7</td>
<td>16.3</td>
<td>+0.6</td>
<td>25.7</td>
<td>-1.9</td>
<td>31.3</td>
<td>+22.1</td>
</tr>
<tr>
<td>August</td>
<td>23.0</td>
<td>+2.24</td>
<td>18.1</td>
<td>+3.1</td>
<td>27.8</td>
<td>+1.0</td>
<td>7.3</td>
<td>-2.36</td>
</tr>
<tr>
<td>September</td>
<td>17.5</td>
<td>+0.9</td>
<td>12.1</td>
<td>+1.5</td>
<td>23.0</td>
<td>+0.6</td>
<td>11.8</td>
<td>+1.89</td>
</tr>
<tr>
<td>October</td>
<td>13.1</td>
<td>+3.3</td>
<td>8.1</td>
<td>+3.9</td>
<td>18.0</td>
<td>+2.5</td>
<td>13.3</td>
<td>+1.59</td>
</tr>
<tr>
<td>November</td>
<td>3.4</td>
<td>-0.3</td>
<td>-2.3</td>
<td>-0.9</td>
<td>9.3</td>
<td>+0.2</td>
<td>6.4</td>
<td>-1.45</td>
</tr>
<tr>
<td>December</td>
<td>1.0</td>
<td>+2.5</td>
<td>-3.1</td>
<td>+3.2</td>
<td>5.1</td>
<td>+2.1</td>
<td>7.9</td>
<td>-1.6</td>
</tr>
<tr>
<td>2022</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>January</td>
<td>-6.8</td>
<td>-2.1</td>
<td>-12.5</td>
<td>-2.4</td>
<td>-1.0</td>
<td>-1.1</td>
<td>3.25</td>
<td>-3.0</td>
</tr>
<tr>
<td>February</td>
<td>-2.3</td>
<td>+1.2</td>
<td>-8.0</td>
<td>+1.3</td>
<td>3.3</td>
<td>+1.6</td>
<td>10.8</td>
<td>+3.88</td>
</tr>
<tr>
<td>March</td>
<td>2.4</td>
<td>+1.2</td>
<td>-3.3</td>
<td>+1.0</td>
<td>8.2</td>
<td>+1.6</td>
<td>7.1</td>
<td>-0.7</td>
</tr>
<tr>
<td>April</td>
<td>7.8</td>
<td>0.0</td>
<td>1.8</td>
<td>+0.4</td>
<td>13.8</td>
<td>-0.1</td>
<td>8.4</td>
<td>-0.3</td>
</tr>
<tr>
<td>May</td>
<td>15.9</td>
<td>+1.8</td>
<td>9.3</td>
<td>+1.6</td>
<td>22.4</td>
<td>+2.0</td>
<td>6.3</td>
<td>-1.7</td>
</tr>
<tr>
<td>June</td>
<td>18.8</td>
<td>+0.1</td>
<td>12.4</td>
<td>-0.4</td>
<td>25.3</td>
<td>+0.7</td>
<td>4.8</td>
<td>-6.9</td>
</tr>
<tr>
<td>July</td>
<td>22.5</td>
<td>+0.7</td>
<td>15.8</td>
<td>-0.1</td>
<td>29.2</td>
<td>+1.5</td>
<td>9.9</td>
<td>+1.0</td>
</tr>
</tbody>
</table>

Regional averages were compiled based on monthly averages data obtained from the Orange Municipal Airport weather station in Orange, MA, USA, 22 miles from the experimental site.
3.4.2 Cover Crop Biomass and N Yield

The year by cover crop treatment interaction was significant for both cover crop biomass and N yield (kg ha\(^{-1}\)); therefore, results were separated, and treatments compared within each year (Figure 1). Overall, cover crop biomass and N yield (kg ha\(^{-1}\)) were significantly higher in the second experimental year than in the first.

Cover crop biomass was not significantly different among cover crops within the first experimental year (Figure 1). In the second year, MX cover crops produced significantly greater biomass yield than FP (3696.8 kg ha\(^{-1}\) and 2300.9 kg ha\(^{-1}\), respectively), while OT and DR produced intermediate amounts of biomass that were not significantly different from the other treatments.

In 2021, the total N yield of cover crop treatments was ordered FP>MX>DR>OT, with OT having a significantly lower N yield than FP, and MX and DR having intermediate N yields that did not differ significantly from the other cover crop treatments. In the second experimental year, the N yields of the cover crop treatments were similar, except for OT, which accumulated significantly less N than the other cover crops.
Figure 3.1. Total dry matter (DM) biomass and N yield (kg ha\(^{-1}\)) accumulated by cover crop treatments before winterkill in the two experimental years. *Top:* Total dry matter (DM) biomass yield (kg ha\(^{-1}\)) accumulated by cover crop treatments before winterkill in the two experimental years. *Bottom:* Total N (kg ha\(^{-1}\)) accumulated by cover crop treatments before winterkill in the two experimental years. Letters that are different from each other signify statistically significant differences between the means of the treatments. The level of significance is presented to the right of each set of columns; *, **, and *** are significant at the 0.05, 0.01, and 0.001 probability levels, respectively; ns = non-significant. OT = oat; DR = daikon radish; FP = field pea; MX = cover crop mixture.
3.3 Garlic Bulb Yield and Diameter

Garlic bulb yield was significantly impacted by the interaction of year and cover crop; thus, results were separated, and the means of cover crop treatments presented within year (Figure 3.2). In both experimental years, bulb yields were highest in the NCC treatment (Figure 3.2). However, there were fewer statistically significant differences in the first year, in which the only the bulb yields of the NCC and OT treatment were significantly different from each other. In the second year, NCC garlic yield was significantly higher than all other cover crop treatments, the yields of which were not significantly different from each other (Figure 3.2).

Figure 3.2 Fresh weight (FW) garlic bulb yield (kg ha\(^{-1}\)) as affected by cover crop treatments during the two experimental years. Garlic was harvested on July 7, 2021 and July 8, 2022. Letters that are different from each other signify statistically significant differences between the means of the treatments. The level of significance is presented over each set of columns; *, **, and *** are significant at the 0.05, 0.01, and 0.001 probability levels, respectively; ns = non-significant. OT = oat; DR = daikon radish; FP = field pea; MX = cover crop mixture; NCC = no cover crop treatment.
Garlic bulb diameter (cm) was significantly impacted by cover crop treatment, nitrogen fertilization application, and year. The diameter of garlic bulbs, as affected by cover crop treatment, was ordered NCC>FR>MX>OT>FP, with the mean diameter of bulbs grown in FP and OT (4.4 cm) being significantly smaller than the average diameter of bulbs grown in NCC plots (4.7 cm). FR and MX bulb diameters (4.5 cm) were not significantly different from those of other cover crop treatments.

Bulb yield and diameter increased linearly ($r^2 = .91$ and $r^2 = .90$, respectively) with increased N fertilizer application rate (Figure 3.3). Overall, garlic bulb yield and diameter were higher at time of harvest in 2021 than in 2022. Neither bulb yield nor diameter were significantly impacted by the application of mycorrhizal inoculant at the time of planting.
Figure 3.3 Effects of nitrogen (N) fertilizer on garlic growth. *Top*: Effects of N fertilizer application rate on garlic yield (kg ha$^{-1}$) and diameter (cm). *Bottom*: Effects of N fertilizer application rate on garlic root, aerial and bulb dry weight (DW) at time of harvest (g plant$^{-1}$). Values presented are averaged over the 2021 and 2022 experimental years. *, **, and *** are significant at the 0.05, 0.01, and 0.001 probability levels, respectively; ns = non-significant.
3.4.3 Garlic Bulb, Aerial, and Root Dry Weight at Harvest

There were no significant differences in garlic root, aerial, and bulb DW plant\(^{-1}\) among cover crop treatments in the first experimental year (Table 3.2). In the second experimental year, garlic in the NCC plots produced significantly higher total biomass plant\(^{-1}\) (24.3 g) than in all other treatments except OT, which produced an intermediate amount of DW biomass plant\(^{-1}\) (21.1 g) (Table 3.2). NCC bulb DW plant\(^{-1}\) (17.5 g) was higher than other treatments’, whose bulb DW plant\(^{-1}\) ranged from 13.9 - 14.8 g. Notably, the garlic root biomass plant\(^{-1}\) was highest in DR, which may be a product of reduced compaction in DR plots resulting from the decomposition of the cover crop’s large, fleshy taproots. On average, garlic plants in the DR and FP plots produced significantly less aerial biomass than in the NCC and OT plots, with garlic grown in the MX treatment producing an intermediate amount of aerial biomass.

Table 3.2. Influence of cover crop treatments on the dry weight (g) of garlic shoots, bulbs, and roots at time of harvest

<table>
<thead>
<tr>
<th></th>
<th>2021 bulb</th>
<th>2021 shoot</th>
<th>2021 root</th>
<th>2021 total</th>
<th>2022 bulb</th>
<th>2022 shoot</th>
<th>2022 root</th>
<th>2022 total</th>
</tr>
</thead>
<tbody>
<tr>
<td>FP</td>
<td>17.7</td>
<td>7.7</td>
<td>0.9</td>
<td>26.2</td>
<td>14.3 b</td>
<td>5.1 b</td>
<td>0.5 b</td>
<td>19.8 b</td>
</tr>
<tr>
<td>DR</td>
<td>17.3</td>
<td>8.5</td>
<td>0.8</td>
<td>26.7</td>
<td>13.9 b</td>
<td>5.9 b</td>
<td>0.7 a</td>
<td>20.4 b</td>
</tr>
<tr>
<td>OT</td>
<td>16.9</td>
<td>8.2</td>
<td>0.9</td>
<td>25.7</td>
<td>14.8 b</td>
<td>5.8 a</td>
<td>0.6 ab</td>
<td>21.1 ab</td>
</tr>
<tr>
<td>MX</td>
<td>18.3</td>
<td>9.4</td>
<td>0.9</td>
<td>27.4</td>
<td>14.4 b</td>
<td>5.5 ab</td>
<td>0.5 b</td>
<td>19.9 b</td>
</tr>
<tr>
<td>None</td>
<td>17.7</td>
<td>8.0</td>
<td>1.0</td>
<td>28.0</td>
<td>17.5 a</td>
<td>6.1 a</td>
<td>0.6 ab</td>
<td>24.3 a</td>
</tr>
<tr>
<td>Pr&gt;F</td>
<td>.3203</td>
<td>.0053</td>
<td>.7517</td>
<td>.1800</td>
<td>.0013</td>
<td>.0056</td>
<td>.0256</td>
<td>.0086</td>
</tr>
</tbody>
</table>

 FP = field pea, DR = daikon radish, OT = oat, MX = mixed cover crop treatment. Values presented in the same column with the same letter are not significantly different from each other.

Bulb DW increased linearly with increased rate of N fertilizer application (Figure 3.3). However, aerial and root DW at time of harvest were not significantly impacted by the N fertilization rate. The addition of mycorrhizal inoculant did not significantly impact the dry weight of garlic plants in this study.
3.4.4 Spring Biomass Accumulation and N Concentration of Garlic Bulbs, Aerial Parts, and Roots

The growth patterns of garlic bulbs, roots and aerial parts were evaluated by quantifying the biomass accumulation and N concentration of each component during the period of active growth in the spring.

Biomass accumulation in garlic bulbs, aerial parts, and roots followed non-linear growth trends during the spring and early summer (Figure 3.4). Although the aerial parts and roots of garlic accumulated biomass more gradually, the bulbs showed little biomass accumulation in the first 6 weeks of sampling, followed by rapid biomass accumulation from mid-May until harvest (Figure 3.4). A dip in aerial biomass can be seen mid-June, corresponding with the removal of garlic scapes.

As expected, the N concentration (g kg⁻¹) of garlic bulbs and aerial parts decreased with sampling time as garlic accrued biomass (Figure 3.4). A slight increase in aerial concentration can be observed in early May, which corresponds with the initiation...
of garlic scape formation (Figure 3.4). There were no significant differences in the N concentration of garlic roots during the spring and early summer growing period.

### 3.4.5 Changes in Garlic Leaf Chlorophyll Concentrations During the Growing Period

Garlic leaf chlorophyll concentrations were measured from all plots every two weeks in the spring and early summer using a SPAD-502 chlorophyll meter (Konica-Minolta, Japan). Overall, SPAD values increased linearly with increased N application rate (Figure 3.5). The addition of mycorrhizal inoculant did not impact the chlorophyll concentration of garlic during growth.

![Figure 3.5. SPAD measurements of garlic shoots as affected by cover crop treatment, sample date, and N fertilizer application. Values presented are averaged over the 2021 and 2022 experimental years. SPAD measurements were taken from the 2nd fully expanded leaf from the top of the plant at each sampling date. Left: SPAD measurements of garlic shoots as affected by cover crop treatment and sample date during garlic growth from April 14 – July 7 (garlic harvest). OT = oat; DR = daikon radish; FP = field pea; MX = cover crop mixture. Trends are hexic and R^2 = 1 for all treatments. Right: SPAD measurements of garlic shoots as affected by N fertilizer application. *, **, and *** are significant at the 0.05, 0.01, and 0.001 probability levels, respectively; ns = non-significant.](image)

Changes in chlorophyll concentration as affected by the interaction of cover crop treatment and sampling date are presented in Figure 3.5. The increase in chlorophyll concentration and subsequent peak observed at the end of May corresponds with garlic
scape development, at which time a greater amount of N is allocated to garlic aerial parts. A pronounced decrease in chlorophyll concentration is observed in the sampling dates following scape formation and removal.

3.4.6 Garlic AE and NUE\textsubscript{crop}

NUE\textsubscript{crop} was significantly impacted by both N fertilizer application rate and cover crop treatment in this study.

Both years, the NUE\textsubscript{crop} values of NCC garlic were significantly higher than those of all treatments containing cover crops, and no significant differences were observed among the FP, DR, OT, and MX treatments (Figure 3.6). In the second year, all cover crop treatments had NUE\textsubscript{crop} values <1, besides OT, which had a NUE\textsubscript{crop} value of approximately 1 in both experimental years.

AE was not significantly impacted by any of the treatments in this study.
Figure 3.6. Nitrogen Use Efficiency (NUE<sub>crop</sub>) as affected by cover crop treatment and nitrogen (N) fertilization. Left: Nitrogen Use Efficiency (NUE<sub>crop</sub>) of garlic as affected by cover crop treatments during the 2021 and 2022 experimental years. Letters that are different from each other signify statistically significant differences between the means of the treatments. The level of significance is presented over each set of columns; *, **, and *** are significant at the 0.05, 0.01, and 0.001 probability levels, respectively; ns = non-significant. OT = oat; DR = daikon radish; FP = field pea; MX = cover crop mixture. NUE<sub>crop</sub> = kg garlic bulb yield / (kg fertilizer N applied + kg N released from cover crop residues). A dotted line placed at NUE<sub>crop</sub> = 1 represents the “ideal” system as described by Martinez-Feria et al (2018), where the amount of N supplied to the system is equivalent to the amount of N removed. Values >1 suggest net N removal from the system, and values <1 suggest surplus N. Right: Nitrogen Use Efficiency (NUE<sub>crop</sub>) of garlic as affected by rate of nitrogen fertilizer application.

3.4.7 Fluctuations in Spring Soil Nitrate Status

Soil nitrate was significantly impacted by the interaction of sampling date and cover crop treatment.

As expected, soil nitrate levels were low in all unfertilized plots. Notably, there was no significant change in soil nitrate status during the spring sampling period in the NCC plots. By contrast, soil nitrate levels were significantly different among sampling times in all treatments containing cover crop residues (Figure 3.7). At the beginning of the sampling period on April 1, the soil nitrate status of all treatments was similar; however, soil nitrate in April was much lower in all plots containing cover crop residues than in NCC plots (Figure 3.7). By the beginning of May, the nitrate levels had normalized once more and were similar among treatments.
Figure 3.7. Soil nitrate concentration (mg g⁻¹ soil) as affected by cover crop treatment and sampling date during the garlic growth and residue decomposition period in the spring. Plots sampled received no N fertilizer. Values presented reflect an average of the 2021 and 2022 experimental years. *, **, and *** are significant at the 0.05, 0.01, and 0.001 probability levels, respectively; ns = non-significant. OT = oat; DR = daikon radish; FP = field pea; MX = cover crop mixture; NCC = no cover crop treatment. Trendline $R^2$ values are as follows: FP $R^2 = .99$; DR $R^2 = 94$; OT $R^2 = 1$; MX $R^2 = .92$; NCC $R^2 = 1$.

### 3.4.8 Nutrient Concentration of Garlic Bulbs at Time of Harvest

The nutrient concentrations of garlic bulbs after harvest as affected by cover crop treatments are presented in Table 3.3. There were significant differences in N, P, K, and Mn concentrations among cover crop treatments, although the patterns of nutrient accumulation among cover crops were not consistent from year to year. For example, in the first year, the level of N concentration in bulbs was ordered NCC>MX>DR>FP>OT, with significantly higher garlic bulb N concentrations in the NCC vs OT treatment. In the second year, garlic bulb N concentrations decreased in the following order:
FP>NCC>MX>OT>DR, with significantly lower N concentrations in the DR treatment than in FP.

Table 3.3. Influence of cover crop treatments on the nutrient concentration (g kg⁻¹) of garlic bulbs at time of harvest

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>Mg</th>
<th>Ca</th>
<th>Mn</th>
<th>Cu</th>
<th>Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2021</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FP</td>
<td>14.9</td>
<td>4.2</td>
<td>9.8</td>
<td>0.6</td>
<td>1.2</td>
<td>0.005</td>
<td>0.003</td>
<td>0.043</td>
</tr>
<tr>
<td>DR</td>
<td>15.1</td>
<td>3.7</td>
<td>9.4</td>
<td>0.6</td>
<td>1.3</td>
<td>0.005</td>
<td>0.002</td>
<td>0.045</td>
</tr>
<tr>
<td>OT</td>
<td>14.0</td>
<td>3.8</td>
<td>9.3</td>
<td>0.6</td>
<td>1.1</td>
<td>0.007</td>
<td>0.002</td>
<td>0.043</td>
</tr>
<tr>
<td>MX</td>
<td>15.3</td>
<td>3.9</td>
<td>9.4</td>
<td>0.6</td>
<td>1.2</td>
<td>0.008</td>
<td>0.003</td>
<td>0.046</td>
</tr>
<tr>
<td>None</td>
<td>16.0</td>
<td>4.0</td>
<td>9.9</td>
<td>0.6</td>
<td>1.4</td>
<td>0.009</td>
<td>0.003</td>
<td>0.045</td>
</tr>
<tr>
<td><strong>Pr&gt;F</strong></td>
<td>&lt;.0001</td>
<td>.0006</td>
<td>.0102</td>
<td>.0542</td>
<td>.0640</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

|          |       |       |       |       |       |      |      |      |
| **2022** |       |       |       |       |       |      |      |      |
| FP       | 18.6  | 4.2   | 10.5  | 0.7   | 1.4   | 0.008| 0.004| 0.030|
| DR       | 16.7  | 4.2   | 10.5  | 0.7   | 1.5   | 0.009| 0.004| 0.034|
| OT       | 17.0  | 4.5   | 10.3  | 0.7   | 1.5   | 0.009| 0.004| 0.032|
| MX       | 17.6  | 4.3   | 10.1  | 0.7   | 1.4   | 0.008| 0.004| 0.033|
| None     | 18.3  | 4.3   | 10.8  | 0.7   | 1.4   | 0.009| 0.004| 0.036|
| **Pr>F** | .0318 | .0262 | .4002 | .2126 | .0789 | .001 | .1142| .2677|

FP = field pea, DR = daikon radish, OT = oat, MX = mixed cover crop treatment. Values presented in the same column with the same letter are not significantly different from each other.

Garlic bulb P, Mn, and K concentrations also varied from year to year. In 2021, the P concentrations of NCC and FP garlic bulbs were significantly higher than the concentrations in DR and OT treatments, with the MX treatment having intermediate concentrations. In the following year, OT had the highest P concentration in the bulb, significantly higher than FP and DR, which had the lowest P concentration in both years. OT treatments had the lowest Mn concentration in garlic bulbs both years. However, in the first year, NCC bulbs had significantly higher Mn concentrations than all other treatments, while in the second, DR had the highest Mn concentrations, and the differences among treatments were less pronounced. In the first year, K accumulation in garlic bulbs decreased in the order of NCC> FP> DR>MX>OT, with concentrations significantly higher in NCC plots than in OT. In the second experimental year, there were no significant differences in K concentration among treatments.
Figure 3.8. Nutrient concentrations (g kg⁻¹) of garlic bulbs at time of harvest, as affected by rate of nitrogen (N) fertilizer application. Values presented are averaged over the 2021 and 2022 experimental years. Left: N concentration (g kg⁻¹) of garlic bulbs at time of harvest as affected by rate of N fertilizer application; Middle: Phosphorus (P) concentration (g kg⁻¹) of garlic bulbs at time of harvest as affected by rate of N fertilizer application; Right: Magnesium (Mg) concentration (g kg⁻¹) of garlic bulbs at time of harvest as affected by rate of N fertilizer application. *, **, and *** are significant at the 0.05, 0.01, and 0.001 probability levels, respectively; ns = non-significant.

Nitrogen fertilization, year, and the addition of mycorrhizal inoculant also impacted the nutrient concentrations of garlic bulbs. Ca and Cu concentrations were significantly higher in the second year than in the first, and Mg, P and N concentrations increased linearly with the rate of N fertilizer application (Figure 3.8). The Fe concentration of garlic bulbs that received mycorrhizal inoculant at the time of planting was significantly higher (Pr>F = .0393) than the Fe concentration of bulbs that were not inoculated (0.040 and 0.38, respectively). By contrast, the K concentration of bulbs that received the mycorrhizal inoculum (10.3 g kg⁻¹) was significantly lower (Pr>F = .0347) than the concentration of bulbs that were planted without mycorrhizal addition (10.6 g kg⁻¹) in the second year. There were no differences in K concentration between the inoculated and uninoculated bulbs in the first year. Bulb Ca concentration was significantly impacted by the interaction of cover crop treatment and mycorrhizal application (Pr>F = 0.0435). There were no differences in bulb Ca concentration among cover crop treatments that did not receive the mycorrhizal inoculant; however, among the plots to which inoculant was added at time of planting, NCC plots had significantly
higher (Pr>F = .0435) Ca concentrations than MX (1.40 g kg\(^{-1}\) and 1.26 g kg\(^{-1}\), respectively), while the other treatments ranged in concentration from 1.28 – 1.3 g kg\(^{-1}\) and were not significantly different from each other.

### 3.5. Discussion

As cover crops are planted for their ecological benefits rather than for harvest, they are typically sown in otherwise fallow periods when environmental and field conditions are not ideal for crop growth. For this reason, cover crop biomass and nutrient accumulation can be highly variable from year to year and are especially sensitive to sowing date and weather fluctuations (Gabriel et al., 2016; Lal et al., 1991; Pavinato et al., 2017). Earlier sowing of winter cover crops can significantly increase cover crop biomass and N accumulation (Akbari et al., 2020; Hashemi et al., 2013; Lawson et al., 2015; Murrell et al., 2017; Odhiambo & Bomke, 2001; Toom et al., 2021). In the second experimental year, cover crops were sown ten days earlier than in the first, resulting in significantly higher biomass and N yield. In addition to sowing date, the weather during cover crop establishment may have contributed to yield differences between the two years. September of the first experimental year (2020) was substantially drier than the regional norm, which may have affected cover crop establishment and early growth. Warmer temperatures and increased rainfall were observed in August 2021, the cover crop sowing period in the second year.

Despite the numerous documented benefits of relay cropping systems, there are many management challenges that must be addressed to ensure the economic viability of their adoption (Cecchin et al., 2021; Lamichhane et al., 2023). Relay cropping systems
are designed to stagger the critical growth phase of their component crops, limiting interspecies resource competition during establishment (Teasdale, 1996). However, due to the complexity of management associated with these systems, particularly the intercropping phase (the period of time where the crops overlap in the field), relay crops often experience yield reduction compared to crops grown in monoculture because of competition for light, moisture, and nutrients (Gesch et al., 2023; Hoerning et al., 2020; Lamichhane et al., 2023; Ott et al., 2019; Tanveer et al., 2017). ‘Relay crops’, or the crops planted into the already established ‘primary crops’, are particularly susceptible to yield reduction due to early-stage competition during their critical establishment period (Tanveer et al., 2017). Factors influencing the crop yields and environmental outcomes of relay cropping include crop species and cultivar selection, time and density of planting, weather conditions during the establishment of the relay crop, and the length of the intercropping phase (Gesch et al., 2023; Lamichhane et al., 2023; Tanveer et al., 2017). Generally, the planting density of the primary crop should be reduced and the sowing date should take into account the competition dynamics of the two crops, allowing the relay crop a chance for robust establishment despite potential reductions in soil water and nutrient content by the primary crop (Lamichhane et al., 2023).

The reported impacts of relay cropping systems on cash crop yield and soil N status are variable and system dependent. For instance, the literature reports significant soybean yield decrease in relay cropping systems (Cecchin et al., 2021; Hoerning et al., 2020; Ott et al., 2019). By contrast, the incorporation of legumes into winter cereal systems via intercropping did not negatively impact yields in published studies (Amossé et al., 2014; Cicek et al., 2014; Martens et al., 2001). Moreover, planting legumes in a
relay cropping system has been reported to increase soil available N, whereas including
gramineous crops reduced total nitrate in the soil (Tanveer et al., 2017). Malone et al.
(2022) found that while winter hardy cover crop species decreased corn yield in a relay
cropping system, corn yield was not significantly impacted when frost sensitive cover
crop species were used. In this study, garlic yield under a relay crop management system
was dependent on the establishment and biomass of cover crops in the fall. In the first
year, when cover crop biomass was low, garlic yield was only reduced by the OT
treatment compared to the control. By contrast, garlic yield was significantly reduced by
relay cropping, regardless of cover crop type, in the second year when cover crop fall
biomass was high.

Although the majority of garlic biomass accumulation occurs in the spring
following fall planting, garlic root production is initiated soon after a mature clove is
planted into the soil and continues at a prolific rate until the beginning of bulb swelling in
the spring (Bertoni et al., 1992; Mann, Louis K., 1952). Intercropping practices can
compromise crop root growth and development, contributing to yield decline (Gill et al.,
2009). Garlic is especially sensitive to competition, even at early stages of growth, with
weed pressure reported as one of the major contributors to garlic yield reduction
(Rahman et al., 2012). Moreover, garlic bulb diameter is severely reduced by interspecies
and intraspecies competition (Castellanos et al., 2004; Rahman et al., 2012). Rahman et
al. (2012) found that decreasing the frequency of weed removal from 15 to 30-day
intervals resulted in significant yield reduction of garlic. When garlic is relay planted into
standing cover crops mid-October, the intercropping phase of garlic and cover crops lasts
between 30 – 40 days, after which time cover crops are frost terminated and no longer
competing for resources. Thus, it is likely that interspecies competition during the early establishment period of garlic contributed to the significant yield decline of relay cropped garlic in this study.

In systems where cover crop residues are frost terminated and residues remain in the field, the quality and quantity of residues is an important factor influencing nutrient cycling and N availability to actively growing relay crops. The growth habit, biomass accumulation, and nutrient uptake of cover crops as well as the quality of their residues can influence N availability to crop growth in the spring. Increased cover crop biomass is positively correlated with N accumulation in plant tissues, which can help mitigate winter N losses in cropping systems (De Notaris et al., 2018; Finney et al., 2016, 2017; Thapa et al., 2018). However, high cover crop biomass and associated N uptake in the fall can result in decreased soil inorganic N availability the following spring, particularly in residues with high C:N (Finney et al., 2016; Garba et al., 2022; Liebig et al., 2015). Thus, it is important to consider that in addition to resource competition during establishment, insufficient N supply due immobilization can contribute to reduced yields of relay crops. Gramineous species, such as oat, are less efficient at acquiring N from the environment and contain a high proportion of carbon rich structural components; as a result, they accumulate less N than legume and brassica species, have high C:N and are often associated with net N immobilization in agricultural soils during decomposition (A. J. Clark et al., 1994; Sainju et al., 2000, 2007). Leguminous species, such as field pea, tend to produce less biomass than other crop types but often have higher N concentrations, resulting in low C:N and more rapid N mineralization associated with residue decay (A. J. Clark et al., 1994; Sainju et al., 2000, 2007; Snapp et al., 2005; Tadiello et al., 2022).
In this study, despite producing lower biomass than other crops in the second year, field pea monoculture had comparable N yields to the daikon radish monoculture and the cover crop mixture, likely due to the high N concentration of its tissue.

The decrease in soil nitrate during garlic vegetative growth in treatments containing cover crop residues compared to NCC plots suggests resource competition between plants and microbial communities, pointing to N immobilization dynamics in April. These results are consistent with other studies that reported reduced soil N levels in the spring following annual, fall planted cover crops (Gieske et al., 2016; Rutan & Steinke, 2019). However, because the nitrate status was only quantified in unfertilized plots in this study, it was undetermined whether N fertilization offset immobilization caused by residue decomposition in any of the cover crop treatments.

Interestingly, of the cover crop treatments, bulb yield was highest in the OT treatment in the second year, but lowest in the first year. Furthermore, the N concentration of bulbs in OT plots was significantly lower than other treatments in the first year but was not significantly different from the bulbs with the highest N concentration in the second year. As OT fall biomass was lower than that of other cover crops, these findings suggest that soil N immobilization rather than competition in the OT treatment played a contributing role in the reduction of garlic yield. Improved yields and increased bulb N concentration in the second year indicate that soil available N in OT systems was balanced following two years of cover cropping and continued residue decomposition. By contrast, the N concentration of garlic in DR plots was lowest in the second year despite DR accumulating substantial amounts of N before winterkill, indicating poor synchrony between N release from DR residues and uptake by growing
garlic. Since DR residues decompose quickly, it is likely that the multi-year effects of DR on N dynamics are less pronounced. Based on the significant interannual differences in both cover crop biomass and cover crop yield response to relay cropping, it is likely that interspecies competition was the major contributor towards garlic bulb yield reduction in this study, as soil nitrate levels were not significantly different among the two experimental years.

Characterizing crop nitrogen response and biomass synthesis during active growth is a critical first step towards managing N effectively in agricultural systems. In this study, the yield, dry weight, and diameter of garlic bulbs increased linearly with increased N application rate. A possible mechanism for increased yield associated with N fertilization is an increase in garlic shoot growth, which can improve photosynthetic potential and in turn promote carbohydrate synthesis in the bulb (Bertoni et al., 1992). In this study, the garlic foliar growth began in the spring and continued to accelerate until garlic seed stalk (scape) removal in the second week of June, with aerial N concentration decreasing gradually throughout the growing period. The removal of garlic scapes, a common management practice, can increase both bulb size and yield by up to 15% (Rosen & Tongn, 2001). Kim et al. (2013) demonstrated that SPAD chlorophyll meter readings can serve as reasonable indicators of leaf N status and photosynthetic capacity in hardneck garlic. A spike in the SPAD readings in May demonstrated an increase in photosynthetic capacity during scape development, suggesting greater resource allocation to the aerial portion of the plant.

Scape development coincided with the initiation of bulb enlargement in this study (early May), which was unsurprising as both mark the termination of the vegetative
growth stage in garlic. Bulb growth and clove differentiation are favored by increased day lengths and high temperatures and occur rapidly from late April – early June (Khokhar, 2023; Mann, Louis K., 1952). The availability of high levels of N during bulb enlargement can reduce bulb size due to increased N allocation to garlic leaves (Bachmann, 2008). Thus, a majority of N supplied to garlic via both fertilization and mineralization from residue decomposition should be made available in the early spring before the initiation of the reproductive stage at the end of April. At bulb maturity, garlic leaves and roots begin to senesce, root absorption is decreased, and foliar and root N is gradually redistributed to the garlic bulb (Bertoni et al., 1992). In this study, an increase in both bulb and aerial N concentration can be seen at the end of the growing period, likely due to due the reallocation of N to the bulb from aerial parts and the senescence of the lower leaves, resulting in an increase in N concentration in the upper aerial portion.

One of the goals of incorporating cover crops into cropping systems is the improvement of agricultural nitrogen use efficiency (NUE), which is critical to reducing synthetic fertilizer inputs and improving environmental sustainability (Hirel et al., 2014). Currently, an estimated 50-70% of N applied to intensified agricultural cropping systems is lost to the environment, with crop recovery estimated to average around 35% (Anas et al., 2020; Hirel et al., 2014; Verzeaux, Hirel, et al., 2017). Increased N fertilization in agricultural systems is associated with decreased NUE, as increased mineral N availability does necessarily coincide with improved crop biomass production and usually leads to an excess of soil N, increasing both production costs and the risk of environmental pollution (Anas et al., 2020; Gabriel et al., 2016; Hausherr Lüder et al., 2020; Hirel et al., 2014). Careful management is crucial to creating a balanced soil N
pool, which would ideally be maintained at a minimal size, providing just enough N to meet crop requirements and maintain adequate yield while avoiding the accumulation of surplus N that could result in leaching (Cassman et al., 2002; Martinez-Feria et al., 2018).

A variety of metrics are used to evaluate NUE; Martinez-Feria et al. (2018) have categorized them as primarily agronomic, regional, or budget-based in scope (Congreves et al., 2021; Martinez-Feria et al., 2018). In this study, NUE was evaluated via agricultural efficiency (AE), which gauged garlic response to N fertilizer under various cover crop and mycorrhizal treatments and NUE$_{\text{crop}}$, used to assess the performance and sustainability of the relay cropping system as affected by various treatments (Martinez-Feria et al., 2018). Following the framework outlined by Martinez-Feria et al. (2018), NUE$_{\text{crop}} > 1$ is indicative of a cropping system with net N removal, whereas NUE$_{\text{crop}} < 1$ suggests a net surplus of N. By this interpretation, the ‘ideal’ system should have a NUE$_{\text{crop}}$ value of 1, where the N supply and N removal are near neutral (Martinez-Feria et al., 2018).

The AE of garlic was not significantly impacted by treatment in this study, which is consistent with the results of other short-term studies in which cover crops did not significantly impact cash crop response to fertilizer (Gabriel et al., 2016; Gaudin et al., 2015; Mancinelli et al., 2019). By contrast, NUE$_{\text{crop}}$ was significantly different among treatments. Both years, NUE$_{\text{crop}}$ of the NCC cover crop treatments was > 1.5, significantly higher than the relay crop systems, indicating net removal of N from the cropping system. All cover crop treatments in the relay cropping system, except for FP, were close to 1 in the first year. In the second year, the NUE$_{\text{crop}}$ of all treatments except for OT were <1, suggesting N surplus. These results suggest that the higher biomass
production of cover crops in the fall likely leads to an excess of available soil N, increasing the risk of N loss to the environment. However, it would be reasonable to assume that N available for cover crop uptake in the fall could be lost to the environment via leaching if not assimilated into cover crop biomass, thus adjusting NUE\textsubscript{crop} values but not necessarily reducing N loss to the environment. Additionally, when calculating ‘available N’ in this study, we considered the rate of mineral fertilizer applied as well as the amount of N released from cover crop residues during the experimental period. However, it is important to note that the fate of residue N (mineralization or immobilization) in the soil is dependent on residue quality, and as such the N released from residues may not be immediately available for uptake by successor crops (Christensen, 1986; Finney et al., 2016; Malpassi et al., 2000; Trinsoutrot et al., 2000). If N immobilization occurs in soils containing cover crop residues, as previously discussed, residue N would be unavailable for crop uptake in the spring, resulting in decreased NUE\textsubscript{crop} values that do not correspond with a surplus of leachable N. By this logic, we concluded that the OT relay cropping system was more sustainable in regard to N cycling than all other treatments evaluated in this study, as the NUE\textsubscript{crop} of the OT treatment was approximately 1 both years, indicating not only consistency regardless of biomass production but also a balanced N system in which N supply and demand are nearly equal. As predicted, NUE\textsubscript{crop} decreased with increased rate of N fertilizer application.

Some criticisms of NUE metrics include the prioritization of short-term yield response to N availability with a limited focus on N cycling during the crop growing period, which confounds the residual effects of long-term N management in agricultural systems (Congreves et al., 2021; Martinez-Feria et al., 2018). Management practices that
encourage SOM formation can increase the long-term NUE of cropping systems by increasing soil N sequestration and retention as indigenous soil N (Cassman et al., 2002; Gabriel et al., 2016). Gabriel et al. (2016) observed an increased soil capacity to supply N after seven consecutive years of incorporating cover crops into crop rotations. These results emphasize the necessity for long-term agricultural studies that consider the cumulative effect of sustainable management practices on both crop response and the environment. For example, in addition to recycling nutrients, cover crops can contribute to increased cash crop yields over time by decreasing soil compaction and improving soil health, facilitating a favorable environment for establishment and growth (Blanco-Canqui et al., 2012).

The cumulative effects of incorporating cover crops into crop rotations are elucidated by the differences in nutrient concentrations among cover crop treatments between the two years. In the first year, garlic relay cropped with cover crops had lower N and K bulb concentrations than garlic grown in the NCC plots. However, in the second year, these trends were normalized, with no significant differences in K concentration among treatments and fewer differences in bulb N concentration, with the only significant difference among treatments occurring between DR and FP. Similarly, garlic bulb Mn concentrations in the relay cropped treatments were significantly lower than the NCC treatment in the first year; however, in the second year, the Mn concentrations were normalized, with only the Mn concentrations of bulbs in the OT treatment significantly lower than the NCC treatment. Bulb P concentration was highest in OT treatments in the second year, despite being significantly lower than all treatments besides DR in the first year. It is likely that these interannual differences reflect the patterns of cover crop
nutrient uptake and subsequent mineralization in the soil; the nutrients assimilated by cover crops in the first year were likely unavailable for garlic uptake as they were confined to residue biomass. By the second garlic cropping season, more complete decomposition of first year residues likely resulted in greater nutrient availability to garlic.

Garlic bulb N, P and Mg concentrations of garlic bulbs increased linearly with increased rates of mineral N fertilizer application. As P uptake is highly dependent on root density, the higher bulb P concentrations observed under increasing fertilizer regimes are likely a result of improved root growth associated with increased N nutrition (Marschner, Petra, 2012). In agricultural systems, a majority of plant available P is found in the top 30 cm of soil where nitrate concentrations are highest; as irrigated systems encourage shallow root development, increased root density in these systems optimizes crop access to available soil P (Marschner, Petra, 2012).

Although mycorrhizal colonization is an important component of sustainable cropping systems that has the capacity to improve both crop yields and NUE, the application of a commercially available inoculant did not significantly impact either parameter in this study. However, the mycorrhizal treatment did have significant impacts on the nutrient concentration of garlic bulbs, most notably on bulb Fe concentrations. The recommended soil Fe range for optimum growth is 2.7 – 9.4 ppm. According to soil test results, soil Fe was limited in this study at 1.4 ppm. Notably, garlic bulbs in the plots that received the mycorrhizal inoculant had a higher Fe concentration than those that were planted without inoculant. These results are consistent with those of previous studies, which reported increased Fe uptake in AM fungi amended crops in soils with low levels
of micronutrients (A. H. Kabir et al., 2020; Liu et al., 2000). AM inoculation of sunflower using the same four species combination as used in this study (*Rhizophagus irregularus, Funneliformus mosseae, Rhizophagus aggregatum, Claroideoglomus etunicatum*) increased sunflower root and shoot Fe and Zn concentrations under nutrient limiting conditions (A. H. Kabir et al., 2020).

A limited amount of scientific studies have investigated the role of mycorrhiza on the uptake of macronutrients and reports of the shoot macronutrient concentrations as affected by AM associations are inconsistent in the literature (R. B. Clark & Zeto, 2000; Marschner & Dell, 1994). Overall, it has been established that macronutrient acquisition by mycorrhizal plants depends on crop type, AM isolate, and soil pH, which can account for the variability in results among studies (R. B. Clark & Zeto, 2000). Generally, mycorrhizae – crop relationships in acidic soils with low nutrient availability enhance K, Ca, and Mg acquisition in crops, whereas mycorrhizal associated plants in neutral to alkaline soils or in soils with high nutrient (particularly P) availability have been reported to have similar or decreased nutrient concentrations than non-mycorrhizal plants grown in similar conditions (R. B. Clark & Zeto, 2000; Liu et al., 2002; Raju et al., 1990). In this study, soil K was in the optimum range for garlic production, and P levels were above optimum. K concentration of garlic bulbs in the plots inoculated with AM fungi were lower than those uninoculated at time of planting in both years, although these differences were only statistically significant in the second year. Our results are consistent with those of other studies in which shoot K concentration was either unaffected or reduced in mycorrhizae associated maize grown in high P conditions (Kothari et al., 1990; Lambert et al., 1979; Liu et al., 2002; Marschner & Dell, 1994).
Similarly, Raju et al. (1990) found that while sorghum inoculated with mycorrhizal fungi increased macronutrient concentrations in low P conditions, macronutrient concentrations were similar or decreased under P fertilization. It is possible that the K acquisition response of mycorrhizal garlic would be different in more acidic soils or in limited P conditions, although further research is needed to better understand the dynamics of macronutrient uptake in mycorrhizal garlic.

3.6 Conclusions

Relay planting garlic into standing, annual cover crops can be an effective strategy to increase the sustainability of hardneck garlic production. However, proper cover crop management is crucial to the economic viability of this system, as garlic yield response is dependent on cover crop biomass accumulation in the fall. High cover crop biomass production significantly reduced garlic bulb yields in the second year, likely due to competition during the intercropping phase. Based on our results, the seeding rate of cover crops should be decreased to offset garlic yield reductions. Overall, oat was the best cover crop candidate for use in a multi-year garlic relay cropping system. Despite the bulb yield and nutrient concentrations of garlic relay planted into standing oat being the lowest of all treatments in the first year, nutrient concentrations were increased, and garlic yield was the highest among all cover crop treatments in the second year. Moreover, garlic relay planted into oat cover crops was the most sustainable in terms of NUE\textsubscript{crop} both years. In the first experimental year, bulb N, P and K concentrations were lower in the cover crop treatments; however, in the second year, bulb nutrient concentrations were normalized, suggesting that incorporating cover crops into crop
rotations over multiple years can balance nutrient availability in cropping systems. These differences highlight the importance of conducting long-term studies to better assess the cumulative impacts of sustainable intensification strategies on crop response and soil health. Although amending garlic with AM fungi at time of planting did not significantly impact the yield or NUE in garlic production, bulb Fe concentrations were significantly higher in AM amended plots, suggesting that AM fungi can improve micronutrient uptake in nutrient limited soils. Moreover, amending cropping systems with commercially available AM fungi is a feasible management strategy for sustainable intensification in reduced tillage, continuous cropping systems, although the benefits to cash crops are likely dependent on soil pH and nutrient status. Garlic bulb yield, diameter, and N, P and Mg concentrations increased linearly with N fertilizer application rate. The relay cropping system did not improve agronomic efficiency (AE) in this study, concluding that N fertilizer inputs cannot be offset by nutrients released via cover crop residue decomposition.
3.7 Chapter References


Hirel, B., Tetu, T., Lea, P., & Dubois, F. (2014). IMPROVING NITROGEN USE EFFICIENCY IN CROPS FOR SUSTAINABLE AGRICULTURE. In K. Etingoff,
https://doi.org/10.1201/b17368-20


Kabir, Z., & Koide, R. T. (2002). Effect of autumn and winter mycorrhizal cover crops on soil properties, nutrient uptake and yield of sweet corn in Pennsylvania, USA.


https://doi.org/10.1080/14620316.2022.2150326


https://doi.org/10.2136/sssaj1979.03615995004300050033x


https://doi.org/10.1016/j.fcr.2022.108795


https://doi.org/10.2134/agronj2011.0128


https://doi.org/10.1371/journal.pone.0129597


https://doi.org/10.2134/agronj15.0146


https://doi.org/10.2134/agronj2001.9351086x


https://doi.org/10.1017/S1742170519000061


https://doi.org/10.2134/agronj2001.932299x


https://doi.org/10.2134/agronj2018.04.0277

Pavinato, P. S., Rodrigues, M., Soltangheisi, A., Sartor, L. R., & Withers, P. J. A. (2017). Effects of Cover Crops and Phosphorus Sources on Maize Yield, Phosphorus Uptake, and


and is Correlated with Environmental Parameters in a Silty Loam Soil. *Agronomy*, 7(2), 38. https://doi.org/10.3390/agronomy7020038


CHAPTER 4

SHORT-TERM RESPONSE OF NEMATODE COMMUNITIES, PERMANGANATE OXIDIZABLE CARBON (POXC) LEVELS, AND BIOLOGICAL ACTIVITY TO COVER CROP RESIDUE DECOMPOSITION IN A GARLIC RELAY CROP SYSTEM

4.1 Abstract

Cover crops and arbuscular mycorrhizal (AM) fungi are key components of sustainable agriculture and can contribute to the sustainable intensification of annual production systems in the long-term. Decomposition of cover crop residues influences microbial community composition and can enhance soil food web services by increasing the supply of primary resources over time and facilitating soil conditions conducive to food web function. This study quantified short-term changes in permanganate oxidizable carbon (POXC), a fraction of labile carbon sensitive to management, biological activity, and soil food web status in response to the spring cover crop residue of three annual, frost terminated cover crop species and their mixture. The three cover crop species selected for this study were field pea (Pisum sativum), forage radish (Raphanus sativus), and oat (Avena sativa). Garlic was relay planted into standing cover crops in October and half of all plots were inoculated with commercially available AM fungi at time of planting. POXC and biological activity were not significantly influenced by cover crop or AM fungi treatment in this study; however, POXC levels were significantly higher in the second year (338.95 and 398.40 mg kg⁻¹, respectively), indicating soil health improvements. Nematode faunal analysis revealed significantly higher maturity index...
(MI) values in treatments containing cover crops compared to a no cover crop (NCC) control, and higher structure index (SI) values in the second year. Enrichment, calculated using the enrichment index (EI), was lower in cover crop treatments compared to NCC. AM fungi did not significantly influence POXC, biological activity, or soil food web response to cover crop decomposition in this study. Overall, this study demonstrated that incorporating cover crops into garlic production for multiple seasons can increase soil food web structure and improve ecosystem condition, thus contributing to soil health.

4.2 Introduction

Sustainable intensification has been proposed as a necessary method of meeting the increasing calorie demands of a growing global population while mitigating the negative impacts of agriculture on the environment (Garnett et al., 2013). Management practices associated with sustainable intensification prioritize reducing synthetic fertilizer use, increasing biodiversity and promoting soil health practices; reduced tillage, cover crops, and encouraging the development of arbuscular mycorrhizae (AM) in agricultural systems are key components of sustainable agriculture (Conservation Agriculture, 2022; Rillig et al., 2016). Relay cropping, the practice of planting a second crop into an already established ‘primary’ crop, is a sustainable option for production intensification as it can improve land use efficiency, promote biodiversity, and increase C sequestration in soils (Gesch et al., 2023; Tanveer et al., 2017). Relay cropping with annual, fall planted cover crops is an effective way to integrate cover crops into cropping systems without taking land out of cash crop production (Amossé et al., 2014; Martens et al., 2001; Teasdale, 1996).
As crop residues are a significant source of C input in annual cropping systems, residue decomposition is a primary channel for energy to enter soil food webs in agroecosystems. Soil microbial communities are impacted by input quality, and as such, soil C cycling is influenced not only by the quantity of C contributed by cover crop residues but also by organism selection driven by substrate specialization (Finney et al., 2017; Schimel & Schaeffer, 2012). Thus, the quantity and quality of residue additions can influence both the rate of decomposition and the fate of C in agricultural systems (Bardgett & Van Der Putten, 2014; Taylor et al., 1989; Wardle et al., 2004; D. Zhang et al., 2008). Moreover, multiple years of organic inputs into cropping systems can have cumulative effects on microbial biomass and community structure (Finney et al., 2017; Lundquist et al., 1999). Microbial respiration, a measure of biological activity, and permanganate oxidizable carbon (POXC), which represents a pool of labile, or ‘active’ C that can be readily used as a microbial food source, are indicators of soil health considered to be sensitive to management that provide insight into C cycling in agricultural soils (Bongiorno et al., 2019; Doran & Parkin, 1994; Parkin et al., 2015). In particular, POXC has emerged as a promising indicator of short-term soil health improvements as it is the labile C fraction most sensitive to management and is positively correlated with carbon pools, including total organic carbon (TOC) and microbial biomass carbon (MBC), and other indicators of soil health such as cation exchange capacity, aggregate stability, water holding capacity and bulk density (Bongiorno et al., 2019; Culman et al., 2012). Increased POXC levels have been observed following cover crop additions to annual cropping systems, even in short-term studies (Hu et al., 2023; Lucas & Weil, 2021; F. Wang et al., 2017).
Organisms within the soil food web play a critical role in maintaining soil health and fertility by modifying soil structure, decomposing organic matter, regulating pest populations, and sequestering C (Bongers & Ferris, 1999; Ferris et al., 2001). The primary origins of labile C, which provides readily available resources for soil food webs, are rhizodeposition, decomposition, and microbial turnover (Bongiorno et al., 2019; Margenot & Hodson, 2016). Energy flow through the soil food web is driven by trophic interchange, with the amount of C accumulated by each organism decreasing with each exchange (Ferris & Matute, 2003). As the size potential of soil food webs is limited by the amount of basal C input, a consistent supply of resources is necessary for the maintenance of structured food webs. In annual cropping systems, insufficient resources due to inconsistent pulses of enrichment often result in the dominance of short food webs that are unable to support soil organisms at higher trophic levels, facilitating low rates of microbial turnover and mineral immobilization (Dinnes et al., 2002).

Bacteria, fungi, and plant-parasitic nematodes are primary consumers in terrestrial ecosystems and constitute the basal resources from which channels in the soil food web are formed (Ruess, 2003). These channels determine the rate of nutrient turnover in soil and directly influence soil fertility via the mineralization and immobilization of important plant nutrients. Bacterially dominated decomposition channels are associated with high quality (low C:N) inputs and increased N mineralization rates, whereas fungal dominated channels are associated with low quality (high C:N) inputs, slow decomposition, and N immobilization in soils. Despite these apparent shortcomings, fungal dominated decomposition pathways can promote
higher C retention in ecosystems, greater trophic exchange, and more structured food webs, which are a critical component of nutrient cycling in systems with low disturbance, such as no-till (Bongers & Ferris, 1999).

Evaluating the condition of soil food webs in agroecosystems can provide valuable insight into soil health and ecosystem function, which can be particularly important in systems transitioning to low-disturbance, high residue management, such as reduced- or no-till. Because of the complexity of soil food webs and fluctuations in microbe populations, soil food web analysis can be difficult if not impossible to perform by examining the diversity and abundance of all organisms present. The use of nematodes as biological indicators of terrestrial ecosystems has been proposed as a way to evaluate food web structure, function and resilience (Ferris et al., 2001; Ferris & Matute, 2003). Because nematodes occupy key positions as primary and intermediate consumers in the soil food web, their abundance and community composition reflects the relative availability of their energy sources and can be linked to important ecological processes and changes in soil conditions (Bongers & Ferris, 1999; Ferris et al., 2001; Ferris & Matute, 2003; D. Neher, 2001). Furthermore, nematode faunal analysis can more reliable than microbial analysis when assessing food web structure because of nematodes’ longer generation time, higher occupancy in the food chain, and the generation of an indicator profile that is not inhibited by distribution or microenvironment effects in the soil (Howard Ferris & Tom Bongers, 2006; D. Neher, 2001). Evaluation of nematode community structure provides information about ecosystem condition and can serve as a tool to track environmental remediation or degradation (Bongers & Ferris, 1999).
The application of nematode faunal analysis to assess ecosystem function is based on the use of nematode taxa as representatives of functional guilds, which are used to calculate nematode based indices (NBIs) of food web condition (Howard Ferris & Tom Bongers, 2006). Nematodes can be categorized by their feeding habits, or trophic roles, which are readily inferred based on easily observable morphological features. Based on their life strategy characteristics, nematode families are placed along a colonizer – persister (c-p) scale (1-5), with low c-p values assigned to r strategists and high c-p values associated with k characteristics (Bongers & Bongers, 1998).

Nematodes are further classified into functional guilds, which contain nematodes with the same feeding habits and inferred function in the soil food web (Ferris et al., 2001). Due to the functional redundancy reflected in the vast taxonomic diversity of nematodes, the presence or absence of a guild is a reliable indicator of soil food web disturbance or recovery (Howard Ferris & Tom Bongers, 2006). The weighted abundance of observed nematode functional guilds are used to calculate the following NBIs, each of which provides unique insight into soil food web condition or function: maturity index (MI), basal index (BI), structure index (SI), enrichment index (EI), channel index (CI) and plant-parasitic index (PPI). While food web response to disturbance may take several weeks to detect using nematode analysis, this is considered a relatively short response time and their indices can provide useful information about nitrogen cycling, decomposition channels, and the ability of soil to suppress plant pathogens (Bongers & Bongers, 1998; DuPont et al., 2009; Ferris et al., 2001; Ferris & Matute, 2003).
Nematodes occur wherever decomposition takes place, and previous studies have reported that nematode indices are responsive to organic matter additions (Bongers & Bongers, 1998; DuPont et al., 2009; Ferris et al., 1996; Shi et al., 2023; K.-H. Wang et al., 2004a). In particular, adding organic amendments to agricultural soils can increase the populations of free-living nematodes while simultaneously decreasing plant parasitic populations (Freckman, 1988). Despite increased application of NBIs to evaluate soil food web condition in recent years, only 26% of published research papers focus on their response to cover crop amendments (Du Preez et al., 2022). Additionally, there is a lack of scientific literature documenting nematode community response to AM fungi. As such, further research is necessary to document and assess the complex relationships between these components of sustainable agriculture and soil food web dynamics.

In recent years, there has been a push to quantify relationships of NBIs to other measures of soil health with the goal of integrating nematode faunal analysis into soil health testing (Margenot & Hodson, 2016; Martin et al., 2022; Martin & Sprunger, 2023). However, it is unlikely that all soil health indicators will be sensitive to changes in nematode community structure. In this study, we aim to evaluate the relationships between selected NBIs (MI, SI, BI, EI, and PPI), POXC, and microbial respiration. As POXC represents the pool of labile C most sensitive to management, and microbial respiration is used as a measure of biological activity, we predict that meaningful relationships can be observed between these indicators and NBIs.

This study aims to evaluate the impacts of an alternative garlic production system in which garlic is relay cropped into fall planted, annual cover crops and inoculated with
commercially available AM fungi at the time of planting on POXC, biological activity, and NBIs of soil food web condition during residue decomposition. Relay cropping garlic into annual cover crops facilitates unique enrichment conditions in which C enters the food web both via cover crop decomposition and garlic root rhizodeposition. Cover crops selected for relay cropping, forage radish (*Raphanus sativus*), field pea (*Pisum sativum*), and oat (*Avena sativa*), were planted in monoculture and as a three-species mixture. We hypothesized that 1) microbial respiration and POXC would be highest in plots containing forage radish residues, as forage radish accumulates significant amounts of carbon before frost termination and has a relatively high decomposition rate, and lowest in plots containing oat residues, as oat has a high C:N and slow decomposition rate 2) POXC levels in cover crop plots would be higher in the second experimental year, reflecting cumulative increases in labile C following multiple years of cover crop additions; 3) decomposition channels, measured as CI, would be more fungal dominated in plots containing cover crop residues; 4) enrichment, determined by EI, would be highest in forage radish plots and lowest in plots containing oat residues; and 5) overall ecosystem condition (MI) and food web structure (SI) would be higher in plots containing cover crop residues compared to the no cover crop control.

**4.3 Materials and Methods**

**4.3.1 Experimental Site and Weather Conditions**

A three-year field experiment (2020-2022) was conducted at the University of Massachusetts Crop and Animal Research and Education Center in South Deerfield, MA (42° N, 73° W). The soil at this location is characterized as a non-acid, mesic Typic Udifluvent, Hadley fine sandy loam. Relevant weather conditions for the experimental
period, including annual precipitation and temperature data, are presented in Table 4.1 alongside twenty-year averages (2000-2019).

4.3.2 Experiment Layout and Management

This experiment evaluated the impacts of a relay-cropping system in which hardneck garlic was planted into three species of annual, fall planted cover crops and their mixture and inoculated with a commercially available mycorrhizal inoculant at time of planting, on soil POXC levels, biological activity, and nematode-based indicators (NBIs) of soil food web condition.

Experimental plots (3.8 m²) were planted in a randomized, complete block design consisting of four replications following a summer buckwheat cover crop. Plots were planted in the same location both years to evaluate the cumulative effects of cover crops and mycorrhizae in a two-year system. Cover crop treatments included monocultures of oat (*Avena sativa*, var: “Saddle Oat”) (OT), daikon radish (*Raphanus sativus*, var: generic) (DR), field pea (*Pisum sativum*, var: 4010) (FP), a mixture of the three species (MX), and a no cover crop treatment (NCC). Cover crops were seeded on 9/04 and 8/25 (2020 and 2021, respectively), using a 7-row cone seeder with 17.7 cm row spacing set at 73.5 cone speed. The following seeding rates were used for monoculture plots: 126 kg ha⁻¹ (OT), 13.5 kg ha⁻¹ (DR), and 90 kg ha⁻¹ (FP). For MX plots, seeding rates were adjusted to 45 kg ha⁻¹ (OT), 5 kg ha⁻¹ (DR), and 90 kg ha⁻¹ (FP).

Hardneck garlic (*Allium sativum*, var: German Extra Hardy) was relay planted between rows of standing cover crops on 10/18 and 10/15 in 2020 and 2021, respectively, 15 cm apart at a depth of 5 cm. NCC plots were mulched with straw to mimic traditional
garlic cultivation practices. Half of the garlic plots were inoculated with a commercially available, granular mycorrhizae blend composed of 4 species (*Rhizophagus irregularus*, *Funneliformus mosseae*, *Rhizophagus aggregatum*, *Claroideoglomus etunicatum*) (+M) at the recommended rate for ecosystem restoration (22.5 kg ha\(^{-1}\)), placed into furrows besides garlic cloves at time of planting, while the other half received no mycorrhizal inoculant (-M).

Cover crops were frost terminated in November of each experimental year, and their residues remained in the field throughout the experimental period. Drip irrigation was installed at the end of March to ensure sufficient moisture was supplied to the growing garlic crop. The experimental plots were hand-weeded regularly during the spring growth period of the first year; in the second experimental year straw mulch was applied over the cover crop residues in April to eliminate weed competition. Following garlic harvest in July, the field was disked, and a summer buckwheat crop was planted prior to establishment of cover crops for the second experimental season.

**4.3.3 Sample and Data Collection**

Cover crop dry matter (DM) biomass yield was quantified in November of each year following three consecutive days of frost temperatures by harvesting 1.5 m of cover crop biomass (roots and aerial parts), drying in a forced air oven at 109°C until constant weight and adjusting to kg ha\(^{-1}\). Sample collection occurred on 11/12 and 11/5 in 2020 and 2021, respectively. DM samples were ground to 1 mm particle size (Foss Cyclotec 1093 Sample Mill; Foss Electric A/S, Hillerød, Denmark) and sent to a
commercial laboratory to determine C and N concentration (g kg$^{-1}$) using a CHN elemental analyzer.

In the spring following winterkill, soil samples were collected every two weeks from April 1 – June 10. Twelve soil samples were collected from each plot at a depth of 15 cm using a soil auger (2 cm diameter). After homogenization, samples were separated for laboratory analysis. Soil samples for substrate-induced respiration (SIR) and nematode faunal analysis were stored at 4 °C until analysis, while soil for permanganate-oxidizable C were sieved and air dried.

4.3.4 Laboratory Analysis

Soil biological activity was determined via substrate-induced respiration (SIR) as described by Fierer and Schimel (2003). Soil slurries comprised of 4 g dry mass equivalent soil amended with an autolyzed yeast substrate were incubated at 20 C for 4 h, after which respiration was determined on an infrared gas analyzer (IRGA, Model LI-7000, Li-Cor Biosciences Lincoln, Nebraska, USA).

Permanganate oxidizable carbon (POXC) was quantified using methods adapted from Culman et al., (2012) and Stott et al. (2019). Air-dried soil (2.5 g) was weighed into a falcon tube to which 18 ml of deionized water and 2 ml of 0.2 M potassium permanganate (KMnO$_4$) solution was added. The tube was tightly capped and shaken for 2 minutes at 180 rpm, after which the tube was gently inverted, uncapped and placed on a lab bench to continue the oxidation reaction. After 10 minutes, the reaction was terminated by removing 0.2 ml the solution and diluting in 20 ml of deionized water.
Sample absorbance was determined at 550 nm (Abs) using a GENESYS 30 visible spectrophotometer, and POXC calculated according to Weil et al., (2003):

\[
POXC (mg \ kg^{-1}) = [0.02 \ mol \ L^{-1} - (a + b \ast Abs)] \ast (9000 \ mg \ C \ mol^{-1}) \ast \left( \frac{0.02 \ mol \ L^{-1}}{9000 \ mg \ C \ mol^{-1}} \right) \ast \left( \frac{9000 \ mg \ C \ mol^{-1}}{0.02 \ L \ solution} \right) \ast \left( \frac{0.02 \ L \ solution}{0.0025 \ kg \ soil^{-1}} \right);
\]

where 0.02 mol L\(^{-1}\) is the initial solution concentration, a is the intercept and b is the slope of the standard calibration curve, Abs is the absorbance, 9000 mg of C (0.75 mol) is assumed to be oxidized by 1 mol of MnO\(_4^\) changing from Mn\(^{7+}\) to Mn\(^{2+}\), 0.02 L is the volume of KMnO\(_4\) solution reacted, and 0.0025 is the weight of soil used.

Nematodes were extracted from 25 cubic centimeters (cc) of each homogenized composite soil sample using a modified Cobb’s sifting and gravity method followed by sugar floatation-centrifugation (Caveness FE & Jensen HJ, 1955; D. A. Neher et al., 1995, p. 199; Neher, Deborah, 1999). Using an inverted microscope, nematodes were classified by trophic group, identified to taxonomic family, and assigned to appropriate colonizer-persister (c-p) guilds (Bongers & Bongers, 1998; Yeates & Bongers, 1993). The weighted abundance of nematode guilds was used to calculate the following nematode-based indices (NBIs) of soil food web condition: maturity index (MI), structure index (SI), enrichment index (EI), channel index (CI) and plant-parasitic index (PPI) (Bongers, 1990; Ferris et al., 2001; D. Neher, 2001, p. 2). MI and PPI were calculated as \[MI \ or \ PPI = \Sigma(v_i \ast f_i)/n,\] where \(v_i\) is the c-p level of a family, \(f_i\) is the frequency of individuals in a sample, and \(n\) is the total number of nematodes present (Bongers, 1990; D. Neher et al., 2004). The MI is used as a gauge of general ecosystem condition, with higher values associated with greater soil food web maturity, while the PPI represents the plant parasitic nematode assemblages in a sample (Bongers, 1990; Du Preez et al., 2022). BI, calculated as \[BI = 100*[b/(e+s+b)],\] is indicative of the
level of soil perturbation (Du Preez et al., 2022; Ferris et al., 2001). SI, used to calculate trophic complexity by measuring predatory and omnivorous nematodes with higher c-p values (3-5), was calculated as $SI = 100^*[s/(s+b+)]$, with resulting values corresponding to low (0-30), moderate (30-60), or high (60-100) food web structure (Du Preez et al., 2022; Ferris et al., 2001). The EI was calculated as $EI = 100^*[e/(e+b)]$, and represents nematode response to resource availability as the weighted abundance of bacterial- and fungal-feeding enrichment opportunists (c-p 1 and 2) (Berkelmans et al., 2003a; Du Preez et al., 2022; Ferris et al., 2001). CI, calculated as $CI = 100^*[(0.8*Fu_2)/(3.2*Ba_1 + 0.8*Fu_2)]$, assesses the level of fungal decomposition occurring in soil, with higher values (>50) indicative of a primarily fungal decomposition channel, and lower values (<50) representing a primarily bacterial decomposition channel in soil. $E$, $b$ and $s$ are weighted sum-products calculated based on the frequency of families observed in a sample.

4.3.5 Statistical Analysis

Statistical analyses were performed using the proc GLM and proc corr procedures in SAS, version 9.4 (SAS Institute, 2016). Means of effects that were significant at the p<0.05 level were separated using Tukey’s HSD in the case of fixed variables or fit to regression curves where variables were continuous. Relationships between soil health indicators and NBIs were determined by correlation analysis.

4.4 Results

4.4.1 Weather conditions and cover crop biomass, composition, and C input
Relevant weather conditions for the duration of the experimental period are presented, alongside twenty-year averages, in Table 4.1. Overall, the weather conditions during the experimental period did not deviate significantly from the norm, although the period of cover crop establishment was drier than usual in the first experimental year.

Table 4.1 Regional weather conditions at the experimental site and deviation from the regional norm.

<table>
<thead>
<tr>
<th>Month</th>
<th>Avg. temperature (°C)</th>
<th>Dev. from regional avg.</th>
<th>Min Temp (°C)</th>
<th>Dev. from regional min.</th>
<th>Max Temp (°C)</th>
<th>Dev. from regional max.</th>
<th>Precipitation (cm)</th>
<th>Dev. from regional avg. precipitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2020</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>September</td>
<td>16.6</td>
<td>0.0</td>
<td>9.8</td>
<td>-0.8</td>
<td>23.2</td>
<td>+0.4</td>
<td>5.2</td>
<td>-4.7</td>
</tr>
<tr>
<td>October</td>
<td>10.3</td>
<td>+0.5</td>
<td>5.0</td>
<td>+0.8</td>
<td>15.6</td>
<td>+0.1</td>
<td>11.9</td>
<td>0.2</td>
</tr>
<tr>
<td>November</td>
<td>5.6</td>
<td>+1.8</td>
<td>-0.4</td>
<td>+1.0</td>
<td>12.1</td>
<td>+3.0</td>
<td>9.7</td>
<td>1.8</td>
</tr>
<tr>
<td>December</td>
<td>-0.3</td>
<td>+1.2</td>
<td>-5.3</td>
<td>+1.0</td>
<td>4.1</td>
<td>+1.0</td>
<td>10.8</td>
<td>1.3</td>
</tr>
<tr>
<td>2021</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>January</td>
<td>-3.1</td>
<td>+1.8</td>
<td>-6.8</td>
<td>+3.35</td>
<td>0.72</td>
<td>+0.8</td>
<td>5.4</td>
<td>-0.9</td>
</tr>
<tr>
<td>February</td>
<td>-4.0</td>
<td>-0.4</td>
<td>-8.6</td>
<td>-0.9</td>
<td>0.56</td>
<td>-1.1</td>
<td>4.7</td>
<td>-2.3</td>
</tr>
<tr>
<td>March</td>
<td>2.9</td>
<td>+1.9</td>
<td>-3.4</td>
<td>+1.1</td>
<td>9.17</td>
<td>+2.8</td>
<td>4.1</td>
<td>-3.63</td>
</tr>
<tr>
<td>April</td>
<td>8.6</td>
<td>+0.8</td>
<td>2.2</td>
<td>+0.7</td>
<td>15.0</td>
<td>+1.0</td>
<td>10.8</td>
<td>+2.1</td>
</tr>
<tr>
<td>May</td>
<td>14.3</td>
<td>+0.2</td>
<td>7.7</td>
<td>0.0</td>
<td>20.8</td>
<td>+0.5</td>
<td>10.2</td>
<td>+2.1</td>
</tr>
<tr>
<td>June</td>
<td>21.3</td>
<td>+2.6</td>
<td>14.8</td>
<td>+2.0</td>
<td>27.8</td>
<td>+3.3</td>
<td>5.1</td>
<td>-6.64</td>
</tr>
<tr>
<td>July</td>
<td>21.0</td>
<td>-0.7</td>
<td>16.3</td>
<td>+0.6</td>
<td>25.7</td>
<td>-1.9</td>
<td>31.3</td>
<td>+22.1</td>
</tr>
<tr>
<td>August</td>
<td>23.0</td>
<td>+2.24</td>
<td>18.1</td>
<td>+3.1</td>
<td>27.8</td>
<td>+1.0</td>
<td>7.3</td>
<td>-2.36</td>
</tr>
<tr>
<td>September</td>
<td>17.5</td>
<td>+0.9</td>
<td>12.1</td>
<td>+1.5</td>
<td>23.0</td>
<td>+0.6</td>
<td>11.8</td>
<td>+1.89</td>
</tr>
<tr>
<td>October</td>
<td>13.1</td>
<td>+3.3</td>
<td>8.1</td>
<td>+3.9</td>
<td>18.0</td>
<td>+2.5</td>
<td>13.3</td>
<td>+1.59</td>
</tr>
<tr>
<td>November</td>
<td>3.4</td>
<td>-0.3</td>
<td>-2.3</td>
<td>-0.9</td>
<td>9.3</td>
<td>+0.2</td>
<td>6.4</td>
<td>-1.45</td>
</tr>
<tr>
<td>December</td>
<td>1.0</td>
<td>+2.5</td>
<td>-3.1</td>
<td>+3.2</td>
<td>5.1</td>
<td>+2.1</td>
<td>7.9</td>
<td>-1.6</td>
</tr>
<tr>
<td>2022</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>January</td>
<td>-6.8</td>
<td>-2.1</td>
<td>-12.5</td>
<td>-2.4</td>
<td>-1.0</td>
<td>-1.1</td>
<td>3.25</td>
<td>-3.0</td>
</tr>
<tr>
<td>February</td>
<td>-2.3</td>
<td>+1.2</td>
<td>-8.0</td>
<td>+1.3</td>
<td>3.3</td>
<td>+1.6</td>
<td>10.8</td>
<td>+3.88</td>
</tr>
<tr>
<td>March</td>
<td>2.4</td>
<td>+1.2</td>
<td>-3.3</td>
<td>+1.0</td>
<td>8.2</td>
<td>+1.6</td>
<td>7.1</td>
<td>-0.7</td>
</tr>
<tr>
<td>April</td>
<td>7.8</td>
<td>0.0</td>
<td>1.8</td>
<td>+0.4</td>
<td>13.8</td>
<td>-0.1</td>
<td>8.4</td>
<td>-0.3</td>
</tr>
<tr>
<td>May</td>
<td>15.9</td>
<td>+1.8</td>
<td>9.3</td>
<td>+1.6</td>
<td>22.4</td>
<td>+2.0</td>
<td>6.3</td>
<td>-1.7</td>
</tr>
<tr>
<td>June</td>
<td>18.8</td>
<td>+0.1</td>
<td>12.4</td>
<td>-0.4</td>
<td>25.3</td>
<td>+0.7</td>
<td>4.8</td>
<td>-6.9</td>
</tr>
<tr>
<td>July</td>
<td>22.5</td>
<td>+0.7</td>
<td>15.8</td>
<td>-0.1</td>
<td>29.2</td>
<td>+1.5</td>
<td>9.9</td>
<td>+1.0</td>
</tr>
</tbody>
</table>

Regional averages were compiled based on monthly averages data obtained from the Orange Municipal Airport weather station in Orange, MA, USA, 22 miles from the experimental site.

In the second year, cover crops were planted ten days earlier than in the first year and accumulated significantly greater biomass before frost termination. The total dry weight (DW) biomass of the roots and aerial parts of cover crops in each year is presented in...
Table 4.2. Cover crop composition, C:N, and the amount of C released from the residues of each treatment during the experimental period are presented in Table 4.3.

### Table 4.2. Dry Matter Biomass of Cover Crops at Time of Winterkill

<table>
<thead>
<tr>
<th></th>
<th>aerial</th>
<th>root</th>
<th>aerial</th>
<th>root</th>
</tr>
</thead>
<tbody>
<tr>
<td>2021</td>
<td>1226.9</td>
<td>41.5 b</td>
<td>2253.3 ab</td>
<td>47.6 c</td>
</tr>
<tr>
<td>2022</td>
<td>981.1</td>
<td>337.3 a</td>
<td>1687.9 b</td>
<td>1823.1 a</td>
</tr>
<tr>
<td>FP</td>
<td>793.6</td>
<td>193.6 ab</td>
<td>2280.2 ab</td>
<td>669.9 b</td>
</tr>
<tr>
<td>OT</td>
<td>1205.4</td>
<td>126.0 b</td>
<td>3113. a</td>
<td>583.9 bc</td>
</tr>
<tr>
<td>MX</td>
<td>.1201</td>
<td>.0019</td>
<td>.0054</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Pr&gt;F</td>
<td>.0298</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

FP = field pea, DR = daikon radish, OT = oat, MX = mixed cover crop treatment. Values presented in the first two columns are the combined averages of the 2021 and 2022 experimental years. Values presented in the same column with the same letter are not significantly different from each other.

### Table 4.3. Cover crop residue composition, C:N, and C kg ha⁻¹ released during the experimental period

<table>
<thead>
<tr>
<th></th>
<th>C kg ha⁻¹</th>
<th>C:N</th>
<th>% lignin*</th>
<th>% hemicellulose*</th>
<th>% cellulose*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerial</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FP</td>
<td>375.0 ab</td>
<td>12.0</td>
<td>5.10 ab</td>
<td>21.00 b</td>
<td>7.75 c</td>
</tr>
<tr>
<td>DR</td>
<td>461.1 a</td>
<td>19.9 bc</td>
<td>1.47 c</td>
<td>14.43 c</td>
<td>4.60 d</td>
</tr>
<tr>
<td>OT</td>
<td>224.3 b</td>
<td>76.2 a</td>
<td>3.30 bc</td>
<td>25.76 a</td>
<td>24.10 a</td>
</tr>
<tr>
<td>MX</td>
<td>523.7 a</td>
<td>28.3 b</td>
<td>6.60 a</td>
<td>25.70 a</td>
<td>15.43 b</td>
</tr>
<tr>
<td>Pr&gt;F</td>
<td>.0298</td>
<td>&lt;.0001</td>
<td>.0025</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Root</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FP</td>
<td>14.0 b</td>
<td>8.0 c</td>
<td>12.67 a</td>
<td>26.67 a</td>
<td>14.70 b</td>
</tr>
<tr>
<td>DR</td>
<td>350.0 a</td>
<td>8.2 c</td>
<td>1.35 c</td>
<td>10.15 c</td>
<td>3.75 d</td>
</tr>
<tr>
<td>OT</td>
<td>80.4 b</td>
<td>20.3 a</td>
<td>2.53 b</td>
<td>20.63 b</td>
<td>22.50 a</td>
</tr>
<tr>
<td>MX</td>
<td>98.0 b</td>
<td>12.8 b</td>
<td>2.35 b</td>
<td>11.93 c</td>
<td>5.96 c</td>
</tr>
<tr>
<td>Pr&gt;F</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

FP = field pea, DR = daikon radish, OT = oat, MX = mixed cover crop treatment; C:N = carbon:nitrogen. Carbon (C) values represent the amount of C (kg ha⁻¹) released from residues during the experimental period. Values presented in the first two columns are the combined averages of the 2021 and 2022 experimental years; *values presented are the averages of the second year only. Values presented in the same column with the same letter are not significantly different from each other.

#### 4.4.2 Biological Activity

Biological activity, as measured by substrate-induced respiration, was significantly impacted by the year by sampling date interaction (Pr>F = .0481) in this study, but not by cover crop treatment or mycorrhizal amendment. In the first year, microbial respiration was significantly higher than in the second (2.78 and 0.70 µl CO₂ g⁻¹ soil h⁻¹,
respectively). Both years, microbial respiration fluctuated during the sampling period, following an oscillating pattern marked by peaks in respiration rate (Figure 4.1).

**Figure 4.1. Microbial respiration (µl CO$_2$ g$^{-1}$ soil h$^{-1}$) as affected by sampling date during the experimental period.** Values represent averages separated by year. *, **, and *** are significant at the 0.05, 0.01, and 0.001 probability levels, respectively; ns = non-significant.

**4.4.3 Permanganate Oxidizable C (POXC)**

The year by sampling date interaction significantly influenced permanganate-oxidizable C (POXC) levels in this study. POXC fluctuated significantly during the sampling period of the first experimental year (Figure 4.2). From the first sampling date (April 1) until early May, POXC levels decreased, followed by a sharp increase in POXC that remained relatively steady for the remainder of the experimental period. In the
second experimental year, POXC levels did not differ significantly throughout the sampling period and were overall significantly higher than in the first experimental year (338.95 and 398.40 mg kg\(^{-1}\), respectively) (Figure 4.2). POXC levels were not significantly impacted by management practices in either year.

![Figure 4.2](image)

**Figure 4.2.** Soil potassium permanganate (KMnO\(_4\)) oxidizable C (POX–C) (mg kg\(^{-1}\)) as affected by sampling date during the experimental period. Values represent averages separated by year. *, **, and *** are significant at the 0.05, 0.01, and 0.001 probability levels, respectively; ns = non-significant.

### 4.4.4 Nematode Faunal Analysis

The overall abundance of nematodes recovered per sample was significantly higher (Pr>F = .0058) in the second experimental year than in the first (78 and 193, respectively). Both years, nematodes of all trophic groups increased significantly over the sampling period, and the primary trophic groups represented were fungal feeders,
bacterial feeders, and plant parasites. As expected in agricultural systems, the number of higher trophic level nematodes (predators and omnivores) was relatively low. The dominant family of bacterial feeding nematodes represented in this study was *Rhabditae*; other bacterial feeding families recovered were *Cephalobidae, Panagrolaimidae, Plectidae, Diplogasteridae, and Alaimidae*. The families of fungal feeders represented in this study were limited to *Aphelenchidae, Aphelenchoididae, and Leptonchidae*, with *Aphelenchus avenae* (family *Aphelenchidae*) being the dominant nematode species present across all plots throughout the study. *Tylenchidae* and *Tylenchulidae* were the most highly represented families of plant parasitic nematodes, although nematodes from the *Pratylenchidae, Longidoridae, and Anguinidae* families were also recovered.

All NBIs were significantly higher in the second experimental year than the first, except for PPI, which was significantly lower in the second year (Table 4.2). The MI, SI, EI, and BI were all significantly impacted by the year by sampling date interaction (Figure 3). MI values were close to 2 throughout the experimental period in both years, indicating high levels of disturbance and low soil food web structure (Figure 3). In the second year, decreasing MI values in the second half of April (< 2) reflect increased nutrient availability. Low PPI values, indicating primary herbivorous activity by ectoparasitic nematodes of a small size, were present throughout the experimental period in both years. In the first year, there was a significant decrease in the BI during the experimental period, indicating increased food web structure and complexity by the early summer as compared to early spring (Figure 4.3). In the second year, initial BI values were similar to those observed by the end of the experimental period in the first year and remained similar throughout the spring.
Figure 4.3. Indices of food web condition as determined by nematode faunal analysis and affected by sampling date during the experimental period. *Top left:* Changes in Structure Index (SI) during the experimental period. Values presented reflect an average of the two experimental years. *Top right:* Changes in Channel Index (CI) during the experimental period. Values below 50 indicate a primary bacterial
decomposition channel, while values above 50 indicate a primary fungal decomposition channel. **Bottom left:** Changes in Enrichment Index (EI) during the experimental period, separated by year. **Bottom Right:** Changes in Plant Parasitic Index (PPI) during the experimental period. Values presented reflect an average of the two experimental years, separated by year. *, **, and *** are significant at the 0.05, 0.01, and 0.001 probability levels, respectively; ns = non-significant.

There was an increase in SI values between the first two sampling dates, followed by a decrease between April 14 – May 12, after which values increased until the end of the sampling period (Figure 4.3). Throughout the experiment, SI values were indicative of an intermediate level of food web trophic complexity. There were significant differences among sampling dates in both EI and CI in the first experimental year, but not in the second (Figure 4.3). In the first year, changes in EI followed the same trend as those observed in the SI, suggesting that deviations in trophic complexity throughout the spring were likely influenced by the fluctuations in resource availability. There were significant fluctuations in CI throughout the decomposition period in the first year, with a fungal dominated, slow cycle decomposition channel dominated for most of the experimental period. In the second year, a fast cycle, bacterially dominated primary decomposition channel was observed throughout the spring sampling period.

MI, CI, and EI were significantly affected by cover crop treatment (Table 4.4). The year by cover crop interaction was significant for SI, with significant differences only observed in the second year. The MI of all treatments containing cover crop residues was significantly higher than that of NCC. The SI of OT was significantly higher than that of NCC, which was the lowest of all treatments. The CI values were ordered 

FP>OT>FR>MIX>NCC, with the only significant differences observed between FP and NCC. The EI of NCC was significantly higher than that of all cover crop treatments except for FR, which did not differ significantly from any of the other treatments.
Table 4.4. Nematode-based Indices (NBIs) associated with cover crop spring residue decomposition

<table>
<thead>
<tr>
<th></th>
<th>MI</th>
<th>PPI</th>
<th>CI</th>
<th>BI</th>
<th>SI*</th>
<th>EI</th>
</tr>
</thead>
<tbody>
<tr>
<td>FP</td>
<td>2.18 a</td>
<td>1.3</td>
<td>43.4 a</td>
<td>26.1</td>
<td>55.0 ab</td>
<td>56.5 b</td>
</tr>
<tr>
<td>DR</td>
<td>2.17 a</td>
<td>1.2</td>
<td>39.5 ab</td>
<td>22.2</td>
<td>53.4 ab</td>
<td>62.8 ab</td>
</tr>
<tr>
<td>OT</td>
<td>2.21 a</td>
<td>1.4</td>
<td>40.6 ab</td>
<td>26.1</td>
<td>59.5 a</td>
<td>55.8 b</td>
</tr>
<tr>
<td>MX</td>
<td>2.18 a</td>
<td>1.2</td>
<td>38.7 ab</td>
<td>25.5</td>
<td>52.4 b</td>
<td>58.2 b</td>
</tr>
<tr>
<td>NCC</td>
<td>1.97 b</td>
<td>1.2</td>
<td>30.4 b</td>
<td>23.1</td>
<td>50.6 b</td>
<td>67.2 a</td>
</tr>
<tr>
<td>Pr&gt;F</td>
<td>.0019</td>
<td>.3838</td>
<td>.0405</td>
<td>.1488</td>
<td>.0120</td>
<td>.0035</td>
</tr>
</tbody>
</table>

FP = field pea, DR = daikon radish, OT = oat, MX = mixed cover crop treatment; MI = maturity index, PPI = plant-parasitic index, CI = channel index, BI = basal index; SI = structure index, EI = enrichment index. Values presented are the combined averages of the 2021 and 2022 experimental years; *SI was significant only in the second year, so values presented are the averages of the second year only. Values presented in the same column with the same letter are not significantly different from each other.

4.4.5 Relationships between soil health indicators and NBIs

Although POXC was significantly correlated to four out of the six NBIs selected, the demonstrated relationships were very weak across all NBIs (Figure 4.4). MI, BI, and CI were all negatively related to POXC, while EI had a positive relationship (Figure 4). All of the selected NBIs were significantly correlated to microbial respiration, although the majority of these relationships were weak (Figure 4.4). EI and PPI were negatively correlated with microbial respiration, while the relationships between respiration and the other measured NBIs were positive. Most notably, respiration was moderately related to CI, indicating higher respiration rates with increased dominance of a fungal decomposition channel.
Figure 4.4 Relationships between potassium permanganate (KMnO₄) oxidizable C (POXC) (mg kg⁻¹), microbial respiration (µl CO₂ g⁻¹ soil h⁻¹), and nematode-based indices of food web condition. Indices of food web condition include Maturity Index (MI), Structure Index (SI), Enrichment Index (EI) and Plant Parasitic Index (PPI). Values presented in figures (r) are Pearson correlation coefficients (n = 480). *, **, and *** are significant at the 0.05, 0.01, and 0.001 probability levels, respectively; ns = non-significant.
4.5 Discussion

4.5.1 Changes in Biological Activity and POXC levels

Microbial respiration during the spring experimental period followed an oscillating pattern, likely responding to periods of drying and rewetting due to irrigation and precipitation events. Rewetting soil after a period of drying causes a flush of organic substrate availability, resulting in rapid C assimilation by microorganisms and an associated increase in respiration (Clein, J.S & Schimel, JP, 1994; Davidson et al., 1998; Lundquist et al., 1999; Orchard & Cook, 1983; Schimel & Schaeffer, 2012). Clein and Schimel (1994), who observed similar patterns in respiration to those in this study in response to the drying and rewetting of a birch leaf substrate, found that shorter drying periods between rewetting result in more erratic fluctuations in respiration than longer periods of dry conditions. Orchard and Cook (1983) observed similar peaks in soil respiration following multiple rewetting events, even when soil remained moist between rewetting, with fluctuations lasting for 14 days before reaching equilibrium. Thus, it is likely that the fluctuations in respiration throughout the experimental period in this study were influenced by pulses of irrigation and precipitation.

Recent reports have found that the level of POXC response to cover crop additions is sensitive to management history, soil type, and tillage regime (Bongiorno et al., 2019; Decker et al., 2022; Lucas & Weil, 2021). For instance, POXC levels in fields with more frequent disturbance events, such as those in annual cropping systems or under conventional tillage regimes, are more responsive to organic matter additions than those under perennial systems or reduced tillage systems (Bongiorno et al., 2019; Lucas & Weil, 2021). Consistent with the results of other studies evaluating POXC response to
cover crops in the short-term, POXC levels were not significantly affected by cover crop treatment in this study (Ghimire et al., 2017; Pokhrel et al., 2021; X. Zhang et al., 2017). In a global meta-analysis, Hu et al. (2023) reported the effect size of cover crops with respect to POXC was significantly influenced by soil order, more so than cover crop type, tillage regime, or experiment duration. Similarly, Decker et al. (2022) reported improved POXC levels following cover crops compared to fallow in a loamy sand, but no significant differences among treatments in a silt loam. It is likely that the lack of response to cover crop amendments in this study was impacted to some degree by the soil type at the experiment location, which is categorized as a sandy loam. However, the significant increase in POXC levels across all plots in the second year suggests that supplementing garlic production with a summer buckwheat cover crop can increase soil health in the short-term, regardless of fall management.

4.5.2 Nematode faunal analysis and soil food web response to cover crop amendments

Nematode abundance was not significantly impacted by cover crop treatment in this study, suggesting microbial populations were similar across treatments. These results are corroborated by the lack of significant differences in microbial respiration across treatments, indicating similar levels of biological activity. The higher abundance of nematodes in the second year is reflected in increased levels of POXC, as POXC has been found to be positively correlated with microbial biomass carbon (Bongiorno et al., 2019). The presence of Rhabditae as the dominant bacterial-feeding family was expected, as it predominates in the early spring and summer (Bongers, 1990; Ferris et al., 1998).
The EI reflects soil food web response to available resources (e.g. labile C) by quantifying the magnitude of response by enrichment opportunists (Ba1 and Fu2). As such, it is particularly sensitive to management and can reflect changes in soil resource status following disturbance, the addition of organic amendments, or residue decomposition (Howard Ferris & Tom Bongers, 2006). Generally, the addition of crop residues increases the EI compared to fallow systems, particularly in systems where residues are incorporated into the soil profile rather than left on the surface (Berkelmans et al., 2003b; Du Preez et al., 2022; DuPont et al., 2009; Ferris & Matute, 2003; Howard Ferris & Tom Bongers, 2006). The decomposition and corresponding nutrient release is slower in surface residues, which was reflected in the EI values measured in this study (sources) (Christensen, 1986; Douglas & Rickman, 1992; Etemadi et al., 2018; Jahanzad et al., 2016; Thapa et al., 2018). Enrichment indices associated with the various cover crop treatments were proportional to the amount of C released from the residues during the experimental period, and all but DR, which released the greatest total amount of C, were significantly lower than the EI of the NCC treatment. The presence of actively growing garlic provided a continuous source of labile C enrichment to all plots in this experiment, contributing to the relatively high EI value of the NCC treatment. The lower EI values of cover crop treatments are indicative of a decreased abundance of bacterial enrichment opportunists in cover crop plots, possibly due to lower levels of available N or higher concentrations of recalcitrant C compounds in the litter, as both factors are negatively associated with bacterial decomposition (Ferris et al., 1996, 1997; Ruess, 2003).
Channels in the soil food web are influenced by the quality of inputs and associated enrichment response. Nematodes in the *Rhabditidae* family (Ba$_1$) respond rapidly to bacterial blooms and active plant growth, and colonize decomposing residues in the soil by migrating to sources of enrichment (Griffiths & Caul, 1993; Howard Ferris & Tom Bongers, 2006). Typically, the abundance of c-p 1 bacterial feeders is associated with low C:N amendments and to a lesser extent with more complex materials, as low C:N organic matter inputs favor a bacterially dominated decomposition channel (Du Preez et al., 2022; DuPont et al., 2009; Howard Ferris & Tom Bongers, 2006). By contrast, members of the *Aphelenchidae* family (Fu$_2$), the primary fungivorous nematode family represented in this study, increase following enrichment with medium to high C:N organic material, of which fungi are more likely to be the primary decomposers (Du Preez et al., 2022; Ettema & Bongers, 1993; Ferris et al., 1996; Ferris & Matute, 2003; Howard Ferris & Tom Bongers, 2006). The CI reflects the relative proportion of fungal to bacterial decomposition activity. Ecosystems in which bacterial decomposition channels predominate have low CI values (<50) and are described as having ‘fast cycle’ decomposition, classified by a rapid turnover rate and amplified rates of N mineralization by bacterial feeding nematodes and protozoa (Howard Ferris & Tom Bongers, 2006). Fungal dominated, or “slow cycle” decomposition channels have high CI values (>50), are marked by slow mineralization rates, and may result in immobilization of nutrients due to high levels of C (Howard Ferris & Tom Bongers, 2006). Crop residues left on the soil surface instead of incorporated surface decompose more slowly than those incorporated via tillage and are typically associated with higher fungal: bacterial decomposition (Holland & Coleman, 1987; Jahanzad et al., 2016; Neely et al., 1991).
this study, the decomposition channels in all treatments were predominantly bacterially-dominated, although soil food webs in the cover crop treatments had higher CIs than the NCC treatment, with the highest CI observed in FP. This can most likely be explained by the lignin concentration of FP roots, which was significantly higher than that of other cover crop residues. As lignin is a complex polymer resistant to bacterial degradation, a higher proportion of fungal decomposition is expected to coincide with the addition of lignin-rich materials to soil (Ferris & Matute, 2003; Howard Ferris & Tom Bongers, 2006; Neely et al., 1991; Strickland & Rousk, 2010).

In agricultural systems, food webs are generally driven by periodic resource pulses, resulting in food webs dominated by enrichment opportunists with low trophic interchange and a predominantly bacterial decomposition channel that can be beneficial for nutrient cycling (Ferris, 2010) (Ruess, 2003). Bacterial nematodes increase N availability in soil by excreting excess N assimilated for growth and respiration in mineral form (NH4+) (Ferris et al., 1998; Freckman, 1988; Ingham et al., 1985). However, bacterial decomposition channels tend to be ‘bottom heavy’, dominated by organisms with high metabolic requirements and respiration rates, limiting the amount of C that can be successfully transferred to higher trophic levels and resulting in high levels of C loss (Howard Ferris & Tom Bongers, 2006). By contrast, although mineralization rates are slower in fungal decomposition channels, metabolic rates are lower and a greater amount of C is transferred to higher trophic levels, contributing to food web structure (Howard Ferris & Tom Bongers, 2006). Increased hyphal networks in soil, and longer, more structured food webs can increase the abundance of higher trophic level burrowing organisms, which are necessary for the dispersal of nutrients in low disturbance systems,
such as no-till cropping systems (Howard Ferris & Tom Bongers, 2006; D. Neher et al., 2004). Furthermore, food webs with higher predator organisms can regulate the abundance of opportunists and plant parasites, improving ecosystem function (Ferris, 2010). In this study, incorporating oat cover crops into the relay crop system significantly increased food web structure compared to plots with no cover crop amendments, likely due to the slow decomposition of oat residues which provided a gradual supply of resources to soil food webs and increased fungal channel decomposition compared to NCC treatments.

When evaluating short-term changes in nematode populations and associated shifts in NBIs, it is important to consider that there is likely a lag following the increase of the food source and reflected changes in nematode population dynamics (Ferris et al., 1996). The differences observed in NBIs across sampling times reflect successional shifts in nematode communities in response to changing resource quality and availability. Interestingly, there was an increase in BI and simultaneous decrease in EI and SI in the first year between 4/14-5/12 which corresponded with the vegetative growth period in garlic plants and decreased soil nitrate in cover crop plots (Chapter 2). An increase in enrichment and trophic interchange corresponded with increased root biomass production in garlic and improved nitrate levels after 5/12, which could have increased carbon availability via rhizodeposition.

As a consequence of frequent disturbance, agroecosystems are dominated by ‘enrichment pulses’ which are followed by trophic succession to other guilds once the available resource pool no longer meets the requirements for growth and metabolism of a particular group of organisms; for instance, during residue decomposition, a successional
shift may occur from bacterial copiotrophs to saprophytic fungi once readily decomposable C is depleted from residues (Ferris & Matute, 2003; Howard Ferris & Tom Bongers, 2006; K.-H. Wang et al., 2004a). Typically, these shifts occur more rapidly in response to high C:N inputs, and the initial microbial population decreases as decomposition shifts from readily decomposable C to lignin and cellulose, which are slower to be decomposed (Ferris & Matute, 2003; K.-H. Wang et al., 2004b). In the first year, it is possible that food webs in cover crop plots experienced successional shifts before the sampling period, as evidenced by the primarily fungal decomposition channel, whereas in the second year, this successional shift was possibly delayed due to a higher amount of residue biomass, as evidenced by low CI values and the dominance of bacterial decomposition channels in all treatments. However, it is important to consider that many factors outside of litter quality influence fungal: bacterial decomposition dominance, including ecosystem type, soil fertility, and the abundance of higher trophic level soil fauna, and that shifts in dominance are not always consistent with expectations (Strickland & Rousk, 2010; Wardle et al., 2004). Moreover, interactions between fungi, bacteria and their associated grazers are complex and not yet fully characterized. For instance, Kane et al. (2023) reported that fungal grazing by *Aphelenchus avenae* stimulated overall microbial activity, while Mielke et al. (2022) reported increased C assimilation in saprophytic fungi in response to grazing by bacterivorous nematodes, likely due to decreased competitive pressure from bacteria.

It is also worth noting that in this study, the family *Tylenchidae* was classified as plant-parasitic, consistent with their primary recognized feeding type. However, the feeding habits of *Tylenchidae* are disputed, as members of *Tylenchidae* have been
reported as being fungal-feeding or behaving as broader generalists with dynamics similar to fungal-feeders *Aphelenchoides* and *Aphelenchus avenae* (Ferris et al., 1996; Kadota et al., 2002; Okada et al., 2005). Consequently, several studies have chosen to classify members of *Tylenchidae* as fungal-feeders when conducting nematode assays (Margenot & Hodson, 2016; K.-H. Wang et al., 2004a). As members of *Tylenchidae* are overwhelmingly abundant in soil samples, in some cases reported as constituting 30% or more of the individuals in a sample, their trophic classification can drastically influence NBI values and associated conclusions (Howard Ferris & Tom Bongers, 2006; Ruess, 2003). For instance, if *Tylenchidae* were classified as a fungal-feeder in this study, the CI values of plots containing cover crops would reflect fungal dominated decomposition in the second year, rather than bacterially-dominated decomposition.

Overall, plots containing cover crops had higher CI, SI and MI values and lower EI values than NCC plots in this study. MI values were significantly higher in cover crop plots than in the NCC treatment, indicating improved overall ecosystem condition. Similarly, food web structure was improved in cover crop systems, particularly in OT. The higher CI in cover crop systems reflected higher multi-channel decomposition in the presence of cover crop residues, which can contribute to the efficiency of nutrient cycling in agricultural systems by recycling nutrients at different rates. Thus, incorporating residues with medium or high C:N can contribute to food web structure, necessary for effective nutrient cycling in systems with decreased disturbance. Enrichment was likely limited by leaving residues on the soil surface, decreasing the rate of decomposition and nutrient release. Although incorporating crop residues into the soil profile increases
enrichment compared to leaving residues on the soil surface, it decreases soil and food web structure (Howard Ferris & Tom Bongers, 2006).

4.5.3 Relationships of NBIs to soil health indicators

In this study, POXC was not a reliable indicator of food web condition. As POXC is positively correlated to other carbon pools and soil health indicators, these results suggest that it is unlikely that NBIs can be readily integrated into the existing soil health framework, as they provide insight into soil food web processes that cannot be easily inferred by proxy indicators. These results are consistent with the findings of Martin et al. (2022), who reported that biological indicators of soil health were more integrated with nematode feeding types than NBIs. Our results support the findings of Margenot and Hodson (2016), who reported a negative correlation between POXC and CI in woodland soils, indicating lower proportions of opportunistic fungal feeders relative to enrichment opportunist bacterial feeders.

By contrast, microbial respiration was moderately and positively related to CI in this study, suggesting that higher relative proportions of fungal to bacterial decomposition are associated with increased respiration. These results are inconsistent with respiration trends reported in the literature, as bacterially dominated systems typically have higher respiration rates due to reduced carbon use efficiency and the high metabolic rate of bacteria and their associated grazers (Freckman, 1988; Neely et al., 1991; Witkamp, 1966). As the respiration rates of all plots were significantly lower in the second year than in the first and no significant differences were observed between treatments, it is likely that respiration dynamics in this study were more closely
associated with environmental factors than microbial community composition. Thus, we conclude that microbial respiration cannot be reliably used as a predictor of the decomposition dynamics in annual cropping systems, and that further research is necessary for NBIs to be effectively integrated into the existing framework developed for measuring soil health.

4.6 Conclusions

This study demonstrated that annual, planted cover crops can improve soil food web condition by increasing higher trophic complexity after two years in a garlic relay cropping system. Surface residues from frost terminated cover crops increased fungal decomposition activity and decreased food web enrichment as measured by nematode faunal analysis compared to a no cover crop control. In this study, biological activity was influenced by climatic conditions rather than cover crop or mycorrhizal amendments, and neither POXC nor respiration were sensitive to cover crop or mycorrhizal amendments in the two-year period. However, POXC levels were significantly higher in the second year of the study, suggesting overall soil health improvements. Relationships between nematode-based indicators (NBIs) of food web condition and the soil health indicators were not strong enough to be effective indicators of soil food web condition, thus it is unlikely that these parameters could be helpful in integrating food web analysis into current soil health testing. This study emphasized the importance of incorporating food web analysis into the soil health framework, particularly in agroecosystems transitioning to reduced tillage, high-residue systems, as it can provide unique insight into ecosystem
recovery and nutrient cycling processes before these changes are reflected by other soil health indicators.
4.7 Chapter References


https://doi.org/10.1016/0038-0717(91)90175-J


https://doi.org/10.1016/j.soilbio.2004.11.010


https://doi.org/10.3390/agronomy11010119

https://doi.org/10.3389/fpls.2016.01625


https://doi.org/10.3389/fmicb.2023.1155088


https://doi.org/10.1007/s11356-017-8371-4


CHAPTER 5

GENERAL CONCLUSIONS

Environmental degradation, exacerbated by poor soil management and inefficient resource use associated with intensive agriculture, poses a dire and immediate threat to the longevity of human existence. Transition of world farming to conservation agriculture systems that prioritize soil health and ecological regeneration is critical to ensuring global food security and the environmental preservation of our planet. As such, applied agricultural research should focus on developing alternative, regionally specific production systems that promote sustainable intensification and evaluating their impacts on soil chemical, physical, and biological processes.

This purpose of this project was to evaluate the short-term impacts of an innovative garlic production system in which garlic is relay planted into annual, fall planted cover crops and amended with commercially available arbuscular mycorrhizal (AM) fungi inoculant at time of planting, on garlic yield and quality, nitrogen use efficiency, soil health, and food web dynamics. Overall, this study found that incorporating cover crops and AM fungi into garlic production can increase the sustainability of garlic cultivation in northern climates, although additional research is required to ensure the economic feasibility of the proposed system. Oat cover crops show the most promise for adoption, as their incorporation into garlic production enhanced NUE, significantly improved soil food web structure, and led to the highest garlic yield among cover crops established in the relay-cropping system in the second year. However, it is important to note that the benefits of oat cover crops were only realized after two years of production, pointing to the cumulative effects of cover crops over multiple years.
The results of this study suggest that interspecies competition between cover crops and garlic roots during the competition phase of intercropping can result in significant yield penalties of field grown garlic when cover crops are seeded at the recommended rate. Yield reduction was not observed in garlic bulbs when the fall biomass production of cover crops was low, suggesting that reducing the seeding rate of cover crops can alleviate competition and improve garlic yield. As such, future research should focus on optimizing cover crop seeding rates with the goal of increasing garlic yield without sacrificing the ecological benefits provided by fall cover crops.

This study demonstrated that fall planted, annual cover crops contribute a significant amount of carbon (C) to agroecosystems and can recycle substantial amounts of N via residue decomposition after frost termination. Daikon radish has the potential to supply the greatest amount of early season N, as it accumulates high amounts of N in the fall and decomposes rapidly in the spring months. However, it is unlikely that the amount of N contributed by cover crops is enough to reduce the fertilization requirements of hardneck garlic. Thus, the benefits of incorporating cover crops into garlic production should be primarily evaluated by their contributions to soil health.

The benefits of fall planted cover crops to the relay crop system varied by crop type in this study, reinforcing the idea that species selection should contribute to management decisions. The high amounts of C accumulated by cover crop mixtures in the fall coupled with an intermediate decomposition rate can promote C stabilization in soil and build SOM in the long-term, thus improving N retention and enhancing ecological services. In this study, planting garlic into oat residues improved nitrogen use
efficiency and enhanced soil food web structure after two consecutive years. In the first year, the nutrient concentrations of garlic bulbs were lower in relay cropped treatments; however, by the second year, the nutrient concentrations had normalized, emphasizing the cumulative effects of cover crops over multiple years. These results highlight the importance of long-term research in evaluating the benefits of sustainable management systems.

Although amending garlic with AM fungi at time of planting did not have significant impacts on garlic yield or soil food web dynamics in this study, our results indicated that AM fungi can improve micronutrient uptake in nutrient limited soils, as evidenced by higher Fe concentrations in garlic bulbs amended with AM fungi inoculant. As such, including AM fungi amendments can be a feasible management strategy for sustainable intensification in cropping systems with limited nutrient availability.

In addition to evaluating an alternative garlic production system, this project provided evidence that the decay rate constants affiliated with biomass decomposition \(k\), N release \(k_n\), and C release \(k_c\) are associated with different litter quality parameters in root and aerial residues. Our results indicated that root cellulose content is the strongest predictor of \(k\), \(k_n\) and \(k_c\), while moisture content is the strongest predictor of \(k\) in aerial residues. Future research should focus on investigating the influence of root residue quality, edaphic and climate interactions on the rate of root decomposition.

The development of alternative, regionally specific production systems that prioritize environmental conservation in addition to productivity is a necessary part of sustainable intensification and a major focus of applied agricultural research. Thorough evaluation of sustainable management practices is critical for their adoption and requires
quantifying their contributions to both crop productivity and soil health. In the proposed relay cropping system, fall planted cover crops enhanced ecological sustainability by improving soil food web structure, recycling N, and contributing substantial amounts of C to soil food webs via residue decomposition in the spring. This project emphasized the need for long-term research when evaluating production systems that incorporate sustainable management practices, as their benefits may be cumulative and must be assessed accordingly. As global populations continue to increase annually and global resources grow scarce, there is increased urgency for researchers, farmers, and policymakers to prioritize sustainable intensification as a means towards a food secure and ecologically sound future.


https://doi.org/10.1016/j.agsy.2008.11.001


https://doi.org/10.1016/j.agee.2005.09.009


https://doi.org/10.1007/BF00361612


www.uvm.edu/vtvegandberry/factsheets/garlic.html


https://doi.org/10.1155/2019/9106395


Performance under Organic Management. *PLOS ONE, 10*(6), e0129597.

https://doi.org/10.1371/journal.pone.0129597


https://doi.org/10.1016/j.apsoil.2011.11.008


https://doi.org/10.2134/agronj15.0146


https://doi.org/10.1016/j.apsoil.2016.08.004


https://doi.org/10.2134/agronj2001.932299x


https://doi.org/10.1016/j.soilbio.2004.11.010


https://doi.org/10.2134/agronj2018.04.0277


https://doi.org/10.2134/agronj15.0065

https://doi.org/10.3390/agronomy11010119

https://doi.org/10.1007/s10533-010-9439-0


https://doi.org/10.1016/j.still.2015.09.004


237


https://doi.org/10.21273/HORTSCI.27.7.749


https://doi.org/10.3389/fmicb.2023.1155088


https://doi.org/10.1111/ejss.13232


ewxtension.unh.edu/resource/growing-garlic-new-hampshire-fact-sheet.


https://doi.org/10.2136/sssaj2017.05.0139


https://doi.org/10.1007/s004420100740


