

ABSTRACT

BLOSZIES, SEAN ALARIC. Soil Microbial Activity and Organic Carbon Dynamics in Low Input Agroecosystems. (Under the direction of Dr. Shuijin Hu).

Agriculture plays a prominent role in anthropogenic control over the cycle of greenhouse gases between atmosphere and soil. Furthermore, CO₂ and nitrous oxide (N₂O) lost from soil largely originate from soil C and N pools important for soil health and plant growth. Crop and soil management approaches in low input systems often depend heavily on cycling of nutrients derived from organic matter, making it critical to understand how N input, cover cropping, and tillage choices drive organic C dynamics and microbial activity. A series of experiments were developed to test how soil biological indicators responsible for soil C stabilization and N retention are affected by agricultural management. The first experiment examined the combined impact of legume cover crop selection and termination approaches on aggregate stability and other soil parameters predictive of long term changes in soil C. Soil under crimson clover (*Trifolium incarnatum*, CC) soil had higher microbial biomass C (MBC) than hairy vetch (*Vicia villosa*, HV) or non-planted (NP) plots before termination, while HV had higher MBC than CC or NP after termination. Soil under HV yielded higher MBC and mineralizable N than a non-planted control before termination, while CC soil had higher MBC and mineralizable C than either treatment after termination. Also, disk tillage likely accelerated microbial processing of cover crop residue as well as aggregate-protected organic matter compared to non-tillage termination approaches, as suggested by higher mineralizable N, higher hot water extractable carbohydrate and higher MBC in tilled plots. The next study examined how either inorganic urea ammonium nitrate (UAN) or poultry litter affects soil's N₂O emissions potential across six farming systems (conventional with tillage (CCT), conventional long rotation with hay (CLR), conventional

without tillage (CNT), organic with tillage (OCT), organic long rotation with hay (OLR), and organic reduced tillage (ORT)), relating the results to soil C and N dynamics. Over the course of incubations of the above systems' soils with added N, cumulative N₂O-N emitted was equivalent to about 1.4-3.8% of added UAN-N and 0.08-1.1% of added litter N. Conventional soil with tillage had the highest emissions rate of any system for much of the time soil was incubated following a split field application of UAN in June. However, ORT emissions rate was highest among systems' soils at several points during the incubation of soil collected after field litter application in May. Following that, experiments were performed to quantify the relative importance of soil C and N pools in determining N₂O emissions among the same six farming systems soils. In a field season without N input application, CCT soil N₂O emissions rate at times during the incubation exceeded OLR and OCT, while microbial biomass N revealed that immobilization of N may have been responsible for differences among systems. Lastly, a series of experiments was focused on how root and arbuscular mycorrhizal fungi (AMF) mediate decomposition under varying soil N availability or presence of the beneficial endophyte *Burkholderia phytofirmans* PsJN. In the greenhouse, corn's reliance on N from ¹⁵N-labelled buried residues was greater in plants given root access to the residues, compared to AMF access or no access. This was most pronounced at severely N-limited conditions. These experiments reveal several divergent links between synthetic and organic input N sources and organic matter C and N cycling.

© Copyright 2016 by Sean Alaric Bloszies

All Rights Reserved

Soil Microbial Activity and Organic Carbon Dynamics in Low Input Agroecosystems

by
Sean Bloszies

A dissertation submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

Plant Pathology

Raleigh, North Carolina

2016

APPROVED BY:

Dr. Alan Franzluebbbers

Dr. Julie Grossman

Dr. Joshua Heitman

Dr. Chris Reberg-Horton

Committee Chair
Dr. Shuijin Hu

DEDICATION

To Mom and Dad

BIOGRAPHY

Sean Bloszies is native to Seale, AL, a small community across the state line from Columbus, GA. He was born in Opelika, AL to two veterinarian parents, John and Linda Bloszies, and has an older sister, Libby. In high school, he and his family moved to Columbus. He played basketball, and was active in JROTC Raiders and Spanish Club. He enrolled in the University of Georgia's Horticulture program, and upon graduation found work in a public conservatory at Callaway Gardens in Pine Mountain, GA. Organic vegetable production was meaningful to him, so he started volunteering at a nearby farm, eventually transitioning to an apprenticeship there. That stimulated his curiosity about how alternative agricultural practices could be used to mitigate atmospheric greenhouse gas rise, so he sought out university research programs focusing on cover crops and soil C sequestration.

ACKNOWLEDGMENTS

This work would not have been possible without the assistance of many generous people at North Carolina State University, Caswell and Cherry Research Farm staff, the USDA ARS Plant Science Research Unit staff, and our collaborators at VA Tech's Institute of Advanced Learning and Research. I thank my committee members, Dr. Alan Franzluebbbers, Dr. Julie Grossman, Dr. Josh Heitman, Dr. Shuijin Hu, and Dr. Chris Reberg-Horton, for their help and guidance. I also especially would like to thank Dr. Consuelo Arellano for statistical consulting; Angel Cruz, Yu Jiang, Alex Knight, Huan Li, Yunpeng Qiu, JiJY Sooksanguan, Alana Stanley, Dr. Cong Tu, Li Zhang, and Yi Zhang for their help with field and lab procedures; Ivy Lanier, Tomas Moreno, Kerry Olive, Evan Taylor, and Sarah Seehaver for their assistance in greenhouse and field planning and logistics; and Scott Lowman and Dr. Changsheng Mei for their assistance in the switchgrass study. This work was made possible by funding from USDA National Institute for Food and Agriculture (Grant 2012-51106-20332 and grant 2010-51106-21872) and the College of Agricultural and Life Sciences at NCSU.

TABLE OF CONTENTS

| | |
|--|-------------|
| LIST OF TABLES | viii |
| LIST OF FIGURES | x |
| CHAPTER 1: OPPORTUNITIES AND CHALLENGES FOR MANAGING LOW INPUT AND ORGANIC SYSTEMS FOR TIGHTENED NUTRIENT CYCLING AND SOIL C SEQUESTRATION IN THE SOUTHEASTERN U.S..... | 1 |
| 1.1 OPPORTUNITIES AND CONSIDERATIONS IN ORGANIC AGRICULTURE | 1 |
| 1.2 CHALLENGES OF USING ORGANIC AND LOW INPUT PRACTICES IN THE SOUTHEASTERN U.S. | 2 |
| 1.3 MANAGEMENT SOLUTIONS TO CHALLENGES IN INCREASING SOIL ORGANIC MATTER AND MEETING CROP N NEEDS IN ORGANIC SYSTEMS | 3 |
| 1.4 IMPACT OF PLANT SYMBIONTS ON SOIL C AND N IN LOW INPUT BIOENERGY CROPPING SYSTEMS | 5 |
| 1.5 CONCLUSIONS..... | 7 |
| | |
| CHAPTER 2: EFFECTS OF LEGUME COVER CROPS AND SPRING TERMINATION PRACTICES ON SOIL C DYNAMICS IN AN ORGANIC SYSTEM IN THE SOUTHEASTERN U.S..... | 8 |
| 2.1 ABSTRACT | 8 |
| 2.2 INTRODUCTION | 9 |
| 2.3 MATERIALS AND METHODS | 12 |
| 2.4 RESULTS | 17 |
| 2.4.1 <i>Cover crop biomass</i> | 17 |
| 2.4.2 <i>Aggregate mean weight diameter</i> | 18 |
| 2.4.3 <i>Microbial biomass C and N</i> | 18 |
| 2.4.4 <i>Hot water extractable carbohydrate</i> | 19 |
| 2.4.5 <i>Potential C and N mineralization</i> | 19 |
| 2.4.6 <i>Bulk density and soil moisture</i> | 20 |
| 2.5 DISCUSSION | 20 |
| 2.6 CONCLUSIONS..... | 25 |
| 2.7 TABLES AND FIGURE..... | 27 |
| 2.7.1 <i>Tables</i> | 27 |
| 2.7.2 <i>Figure</i> | 32 |
| | |
| CHAPTER 3: EFFECT OF INPUT N SOURCE AND FIELD MANAGEMENT ON AGRICULTURAL SOIL N₂O EMISSIONS | 33 |
| 3.1 ABSTRACT | 33 |
| 3.2 INTRODUCTION | 34 |
| 3.3 MATERIALS AND METHODS | 36 |
| 3.3.1 <i>Site description, soil sampling and preparation</i> | 36 |

| | | |
|-------|--|----|
| 3.3.2 | <i>Cover crop biomass</i> | 37 |
| 3.3.3 | <i>N₂O and CO₂ emissions rate</i> | 37 |
| 3.3.4 | <i>Calculating cumulative N₂O and CO₂ flux</i> | 38 |
| 3.3.5 | <i>Microbial Biomass C and N, NH₄⁺-NO₃⁻-N, K₂SO₄-C</i> | 39 |
| 3.3.6 | <i>Incubations of farming systems soil with UAN or poultry litter</i> | 40 |
| 3.3.7 | <i>Potentially mineralizable N</i> | 40 |
| 3.3.8 | <i>Statistical analyses</i> | 40 |
| 3.4 | RESULTS | 41 |
| 3.4.1 | <i>Effect of farming system and field N inputs on gas emissions and soil C and N dynamics</i> | 41 |
| 3.4.2 | <i>Gas emissions and soil C and N dynamics of soil amended with synthetic or organic N</i> | 48 |
| 3.5 | DISCUSSION | 49 |
| 3.5.1 | <i>Effect of farming system and field N inputs on gas emissions and soil C and N dynamics</i> | 49 |
| 3.5.2 | <i>Gas emissions and soil C and N dynamics of soil amended with synthetic or organic N</i> | 54 |
| 3.6 | CONCLUSIONS..... | 54 |
| 3.7 | TABLES AND FIGURES | 56 |
| 3.7.1 | <i>Tables</i> | 56 |
| 3.7.2 | <i>Figures</i> | 66 |

CHAPTER 4 SOIL N₂O EMISSIONS POTENTIAL AMONG ORGANIC AND CONVENTIONAL FARMING SYSTEMS AND ASSOCIATED SOIL C AND N DYNAMICS..... 74

| | | |
|-------|--|----|
| 4.1 | ABSTRACT | 74 |
| 4.2 | INTRODUCTION | 75 |
| 4.3 | MATERIALS AND METHODS | 77 |
| 4.3.1 | <i>Site description, soil sampling and preparation</i> | 77 |
| 4.3.2 | <i>Cover crop biomass</i> | 78 |
| 4.3.3 | <i>Incubations and determination of N₂O and CO₂ emissions rate</i> | 79 |
| 4.3.4 | <i>Calculation of cumulative N₂O and CO₂ flux</i> | 80 |
| 4.3.5 | <i>Microbial Biomass C and N, NH₄⁺-NO₃⁻-N, K₂SO₄-C</i> | 80 |
| 4.3.6 | <i>Potentially mineralizable N in 60%WFPS soil, net N flux in 70% WFPS soil</i> . | 81 |
| 4.3.7 | <i>Total C and N</i> | 81 |
| 4.3.8 | <i>Statistical analyses</i> | 82 |
| 4.4 | RESULTS | 82 |
| 4.4.1 | <i>Cover crop biomass</i> | 82 |
| 4.4.2 | <i>N₂O and CO₂ cumulative emissions and rate over time</i> | 82 |
| 4.4.3 | <i>Microbial biomass C and N, NH₄⁺-NO₃⁻-N, K₂SO₄-C</i> | 84 |
| 4.4.4 | <i>Potentially mineralizable N in 60%WFPS soil, net N flux in 70% WFPS soil, total C and N</i> | 84 |
| 4.5 | DISCUSSION | 85 |
| 4.6 | CONCLUSIONS..... | 87 |
| 4.7 | TABLES AND FIGURES | 89 |

| | |
|---------------------|----|
| 4.7.1 Tables | 89 |
| 4.7.2 Figures | 96 |

CHAPTER 5: ROOT AND ARBUSCULAR MYCORRHIZAL

FUNGAL REGULATION OF DECOMPOSITION:

EFFECTS OF C AND N AVAILABILITY AND SOURCE 98

| | |
|---|-----|
| 5.1 ABSTRACT | 98 |
| 5.2 INTRODUCTION | 99 |
| 5.3 MATERIALS AND METHODS | 101 |
| 5.3.1 <i>Effects of N input source and rate on AMF activity and decomposition of buried litter in a field corn setting</i> | 101 |
| 5.3.2 <i>Effect of N application rate and root and mycorrhizally mediated N acquisition from decomposing residues in a greenhouse setting</i> | 105 |
| 5.3.3 <i>Effect of N application rate on root and AMF controls over decomposition in a turfgrass setting</i> | 110 |
| 5.3.4 <i>Effect of infection with the beneficial bacterial endophyte Burkholderia phytofirmans PsJN on switchgrass soil C and N cycling</i> | 111 |
| 5.4 RESULTS AND DISCUSSION | 116 |
| 5.4.1 <i>Effects of N input source and rate on AMF activity and decomposition of buried litter in a field corn setting</i> | 116 |
| 5.4.2 <i>Effect of N application rate and root and mycorrhizally mediated N acquisition from decomposing residues in a greenhouse setting</i> | 118 |
| 5.4.3 <i>Effect of N application rate on root and AMF controls over decomposition in a turfgrass setting</i> | 122 |
| 5.4.4 <i>Effect of infection with the beneficial bacterial endophyte Burkholderia phytofirmans PsJN on switchgrass soil C and N cycling</i> | 124 |
| 5.5 CONCLUSIONS..... | 127 |
| 5.6 TABLES AND FIGURES | 128 |
| 5.6.1 Tables | 128 |
| 5.6.2 Figures | 132 |

REFERENCES..... 145

LIST OF TABLES

CHAPTER 2: EFFECTS OF LEGUME COVER CROPS AND SPRING TERMINATION PRACTICES ON SOIL C DYNAMICS IN AN ORGANIC SYSTEM IN THE SOUTHEASTERN U.S.

| | |
|---|----|
| <i>Table 2.1 Field activity and sampling timeline</i> | 27 |
| <i>Table 2.2 Cover crop biomass accumulation</i> | 27 |
| <i>Table 2.3 Analysis of variance for the effects of termination approach, cover crop, sampling date during the spring through summer growing season, and soil sampling depth</i> | 28 |
| <i>Table 2.4 Effect of termination method and winter ground cover on aggregate mean weight diameter</i> | 29 |
| <i>Table 2.5 Effect of cover crop on MBC, PminC from combined 2012-2013 analysis and soil gravimetric moisture content from each year</i> | 30 |
| <i>Table 2.6 Effect of termination approach on MBC, HWC, and potentially mineralizable N from combined 2012-2013 analysis</i> | 30 |
| <i>Table 2.7 Effect of sampling date and sampling depth on MBC, MBN, and HWC from combined 2012-2013 analysis</i> | 31 |
| <i>Table 2.8 Depth × termination approach interaction effect on MBN and HWC at Mid-season sampling date</i> | 31 |

CHAPTER 3: EFFECT OF INPUT N SOURCE ON AGRICULTURAL SOIL N₂O EMISSIONS

| | |
|--|----|
| <i>Table 3.1 Field operations and sampling dates</i> | 56 |
| <i>Table 3.2 Summary of gas emissions rate ANOVA results</i> | 57 |
| <i>Table 3.3 2015 field soil incubations N₂O emissions</i> | 58 |
| <i>Table 3.4 2015 field soil incubations CO₂ emissions</i> | 60 |
| <i>Table 3.5 Means of farming systems' mg N₂O kg⁻¹ soil day⁻¹ emitted from soil wetted to 60% WFPS across four sampling dates</i> | 61 |
| <i>Table 3.6 Means of farming systems' mg CO₂ kg⁻¹ soil day⁻¹ emitted from soil wetted to 70% WFPS</i> | 61 |
| <i>Table 3.7 Means of farming systems' cumulative gas emissions over four sampling dates</i> | 62 |
| <i>Table 3.8 Means of farming systems' cumulative mg CO₂-C kg⁻¹ dry soil from 60% WFPS incubations</i> | 62 |
| <i>Table 3.9 Summary of soil extraction parameters ANOVA</i> | 63 |
| <i>Table 3.10 Soil parameter and gas emissions summary of ANOVA results (P>F) of lab incubations with added manure and UAN</i> | 64 |
| <i>Table 3.11 Means of farming systems' biomass inputs and total soil organic C and total nitrogen</i> | 65 |

CHAPTER 4: SOIL N₂O EMISSIONS POTENTIAL AMONG ORGANIC AND CONVENTIONAL FARMING SYSTEMS AND ASSOCIATED SOIL C AND N DYNAMICS

| | |
|--|----|
| <i>Table 4.1 Field operations, sampling, and dates</i> | 89 |
|--|----|

| | |
|---|----|
| <i>Table 4.2 Means of cumulative gas emissions over four sampling dates</i> | 90 |
| <i>Table 4.3 Summary of gas emissions rate ANOVA results</i> | 90 |
| <i>Table 4.4 Means separation among farming systems' incubation N₂O emissions, mg N₂O kg⁻¹ soil day⁻¹ in October.....</i> | 91 |
| <i>Table 4.5 Soil incubations CO₂ emissions.</i> | 92 |
| <i>Table 4.6 Mean CO₂ emissions rate from soils incubated at 60% WFPS.</i> | 94 |
| <i>Table 4.7 Summary of soil extraction ANOVA.</i> | 95 |
| <i>Table 4.8 Summary of plant biomass and soil C and N means separation.</i> | 95 |

CHAPTER 5: ROOT AND ARBUSCULAR MYCORRHIZAL FUNGAL REGULATION OF DECOMPOSITION: EFFECTS OF C AND N AVAILABILITY AND SOURCE

| | |
|---|-----|
| <i>Table 5.1 Greenhouse decomposition core soil parameter summary of ANOVA.....</i> | 128 |
| <i>Table 5.2 Greenhouse decomposition corn biomass N summary of ANOVA.....</i> | 129 |
| <i>Table 5.3 Switchgrass microcosm means and standard errors for soil and residue C and N of core contents.....</i> | 130 |
| <i>Table 5.4 Switchgrass plot soil summary of ANOVA of microbial parameters.....</i> | 131 |

LIST OF FIGURES

CHAPTER 2: EFFECTS OF LEGUME COVER CROPS AND SPRING TERMINATION PRACTICES ON SOIL C DYNAMICS IN AN ORGANIC SYSTEM IN THE SOUTHEASTERN U.S.

| | |
|---|-----------|
| <i>Figure 2.1 Potentially mineralizable N and potentially mineralizable C in soil as affected by cover crop plants hairy vetch, crimson clover, or a non-planted control.....</i> | <i>32</i> |
|---|-----------|

CHAPTER 3: EFFECT OF NITROGEN SOURCE ON AGRICULTURAL SOIL N₂O EMISSIONS

| | |
|---|-----------|
| <i>Figure 3.1 N₂O emissions by farming system over time during lab incubations of soil collected in April.....</i> | <i>66</i> |
| <i>Figure 3.2 N₂O emissions by farming system over time during lab incubations in May.....</i> | <i>67</i> |
| <i>Figure 3.3 N₂O emissions by farming system over time during lab incubations in June.....</i> | <i>68</i> |
| <i>Figure 3.4 N₂O emissions by farming system over time during lab incubations in September.....</i> | <i>69</i> |
| <i>Figure 3.5 Effect of farming system on extractable N.....</i> | <i>70</i> |
| <i>Figure 3.6 Effect of farming systems on potentially mineralizable N.....</i> | <i>70</i> |
| <i>Figure 3.7 Effect of farming systems on K₂SO₄—extractable C.....</i> | <i>71</i> |
| <i>Figure 3.8 Effect of farming systems on cumulative mineralizable C.....</i> | <i>71</i> |
| <i>Figure 3.9 Effect of farming systems on microbial biomass N.....</i> | <i>72</i> |
| <i>Figure 3.10 Effect of farming systems on cumulative mean N₂O emissions over the full season.....</i> | <i>72</i> |

CHAPTER 4: SOIL N₂O EMISSIONS POTENTIAL AMONG ORGANIC AND CONVENTIONAL FARMING SYSTEMS AND ASSOCIATED SOIL C AND N DYNAMICS

| | |
|--|-----------|
| <i>Figure 4.1 N₂O emissions by farming system over time during lab incubations of soil collected in October at soy harvest.....</i> | <i>96</i> |
| <i>Figure 4.2 Effect of farming system on extractable inorganic N.....</i> | <i>97</i> |
| <i>Figure 4.3 Effect of farming systems on microbial biomass N.....</i> | <i>97</i> |

CHAPTER 5: ROOT AND ARBUSCULAR MYCORRHIZAL FUNGAL REGULATION OF DECOMPOSITION: EFFECTS OF C AND N AVAILABILITY AND SOURCE

| | |
|---|------------|
| <i>Figure 5.1 Microcosm root + AMF ingrowth core, and AMF ingrowth core.....</i> | <i>133</i> |
| <i>Figure 5.2 Mean isotopic signature of root + AMF and AMF cores following burial.....</i> | <i>133</i> |
| <i>Figure 5.3 Corn yield.....</i> | <i>133</i> |
| <i>Figure 5.4 Proportion of corn biomass N originating in core residues at first destructive sampling.....</i> | <i>134</i> |
| <i>Figure 5.5 Proportion of corn biomass N originating in core residues at second destructive sampling.....</i> | <i>135</i> |

CHAPTER 1

OPPORTUNITIES AND CHALLENGES FOR MANAGING LOW INPUT AND ORGANIC SYSTEMS FOR TIGHTENED NUTRIENT CYCLING AND SOIL C SEQUESTRATION IN THE SOUTHEASTERN U.S.

1.1 Opportunities and considerations in organic agriculture

Demand for organic food has grown tremendously in recent years, driving a doubling in organic cropping acreage in the U.S. between 1997 and 2012 (Greene et al., 2009). These farming systems rely heavily on mineralization of nutrients from decomposing residue additions like animal manures and plant biomass to meet crop needs. Among surveyed farmers, cover crops were most commonly viewed as beneficial to soil organic matter levels and N delivery to subsequent crops, with 79% of this group of mostly small farmers in North Carolina having grown them (O'Connell et al., 2015a). However, the advantage cover crops supply must outweigh their financial and time costs in order to justify their planting and management (Bergtold et al., 2012). Manure application is often constrained by high transportation costs (Ribaud et al., 2003), and as such it is necessary to maximize returns from this input in the form of crop nutrient delivery or maintaining soil organic matter levels.

1.2 Challenges of using organic and low input practices in the Southeastern U.S.

The Southeastern U.S. is a challenging environment in which to manage manures and soil for optimum nutrient delivery and increased soil C levels. Due to high temperatures and

precipitation, soils in the region are highly weathered and mineralization of applied residues is rapid. On the other hand, warm winters allow for high aboveground cover crop biomass productivity, over 9,000 kg ha⁻¹ from rye (Smith et al., 2011), demonstrating their potential as a key soil C source. Still, it is often difficult to match crop nutrient needs with what is released from cover crops or animal manures used as fertility sources. Asynchrony between nutrient concentrations and crop uptake can be even larger than with inorganic N applications (Crews and Peoples, 2005). In addition, phosphorus can easily be over-applied in animal manures when attempting to meet N needs (Daniel et al., 1998), making management and calculation of ideal application rates and timing crucial.

Reducing soil greenhouse gas emissions is another environmental challenge arising from implementing organic soil fertility strategies. Soil C is converted to CO₂, and N can be lost as N₂O under reducing soil conditions. Agriculture is responsible for close to 9% of total U.S. greenhouse gas emissions, and approximately 79% of anthropogenic N₂O emissions stems from agricultural soil management (EPA, 2010). Appropriate management and selection of cover crops and manures are important because both inputs have been shown to increase N₂O emissions under the right circumstances (Aguilera et al., 2014; Diba et al., 2011).

Reducing weed competition is a major issue in systems restricted from herbicide use, and crops originating in cooler regions, like soybean, suffer unique weed competition challenges at the high temperatures common in summer in the Southeast (Wright et al., 1999). Weed control in Southeastern organic systems most typically consists of frequent tillage and cultivation. These operations create additional challenges for maximizing soil C sequestration

and slowing nutrient mineralization, because increasing tillage intensity is linked to lower SOC (Halvorson et al., 2002).

Farming without synthetic pest control and inorganic N presents a number of challenges, several of which are exacerbated in the Southeast. These include regulating soil C and N mineralization, controlling weeds, and meeting crop nutrient demands. Partial solutions to all of these problems may lie in optimizing cover crop selection and management, reducing the frequency or extent of tillage, and encouraging plant symbionts like diazotrophs, arbuscular mycorrhizal fungi, and plant endophytes.

1.3 Management solutions to challenges in increasing soil organic matter and meeting crop N needs in organic systems

Pools of organic C resistant to mineralization can develop in a number of ways. Levels of biogenic soil aggregation, either measured as physical aggregate stability or concentration in soil, are tightly linked with SOC accrual over time (Chung et al., 2008). Aggregates are organomineral assemblages that form as decomposing organic matter is occluded from further microbial degradation and as organic compounds adsorb to and bind together mineral surfaces. This physical protection, together with hydrophobicity, are most responsible for stabilizing soil C from further loss (von Lützow et al., 2008). Aggregates are both composed of and held together by aromatic C compounds, polysaccharides, and fungal hyphae and roots at different size scales. This gives rise to a hierarchy of both size distribution and rates of turnover of aggregate-protected C (Tisdall and Oades, 1982). Aggregate stabilization of C is moderated by management practices including tillage (Roldán et al., 2003) cover crop use and species selection (Kong et al., 2005), and residue biomass inputs (Karlen et al., 1994).

Cover crops are especially useful to the goal of increasing aggregation. Improvements in aggregate stability due to crop presence begin with root growth (Reid and Goss, 1980), as water flow, clay mineral alignment around roots, and their C contributions are fundamental to aggregation (Oades, 1984). Because of this, planting cover crops every year can improve aggregation over the long term compared to winter fallow (Calegari et al., 2013). Furthermore, differences in growth and biomass quality distinguish cover crop species in terms of their ability to increase aggregation (Nguyen et al., 2011).

In organic systems, a lack of effective registered herbicides necessitates that cover crops be terminated mechanically. Tillage has been the conventional method of termination, but tillage is well known to decrease aggregation (Haynes and Swift, 1990). Therefore, alternatives to tillage for terminating cover crops are gaining in popularity. Cover crops can be terminated with a roller-crimper, for instance, which leaves a surface mulch layer that serves to diminish weed pressures through mechanisms of light impedence, N immobilization, and allelopathy (Reberg-Horton et al., 2012). It remains to be seen whether this method can maximize SOC accrual, especially since challenges remain with perennial weed control using this technique (Mirsky et al., 2012).

Although cover crops and reduced tillage have the potential to limit C mineralizaion to increase SOC sequestration, they also may impact N₂O emissions. Soil C content is often positively correlated with N₂O emissions, to the extent that high SOC natural grasslands can have higher emissions than agricultural lands (Braker et al., 2015). Others have found no relationship between total C and N₂O emissions (Akinsete and Nkongolo, 2016). A recent meta-analysis by Basche et al. (2014) found that cover crops can either increase or decrease

emissions depending on the circumstances. With limited N applications or when residues are left on the surface, cover crops can decrease emissions. However, legumes, cover crops plus large N additions, and incorporation of biomass can all increase emissions relative to no cover crop. Tillage is more widely understood to reduce N₂O emissions under most circumstances, apparently by decreasing soil water content (Dong et al., 2012) or by distributing higher-emissions surface layers of soil throughout the soil profile (Attard et al., 2011). On the other hand, tillage has been shown to either not affect or increase N₂O emissions. Passianoto et al. (2003), who found increased emissions in a tilled tropical pasture soil than one sprayed with herbicide, suggested their differing results may have been due to the fact that most studies have been carried out in temperate soils.

1.4 Impact of plant symbionts on soil C and N in low input bioenergy cropping systems

In addition to more directly modulating soil C inputs and mineralization through cover crop selection and tillage, management can indirectly alter soil C dynamics via the activity of plant-associated microbes like arbuscular mycorrhizal fungi (AMF) and bacterial endophytes. Switchgrass, which forms associations with not only AMF but also an endophytic *Burkholderia*, is an ideal case study in which to understand the impacts of both groups of microorganisms.

While switchgrass has been named a model energy crop (McLaughlin et al 2005), a significant challenge remains in improving its production (Mitchell et al 2008). Notably, successful stand establishment during early years (Schmer et al 2006) and improving crop N utilization (Muir et al. 2001) are key to increasing and sustaining productivity, particularly in

low-fertility marginal soils. Although switchgrass requires substantially lower inputs than many annuals, efforts are still underway to devise optimal switchgrass nutrient management. While a lack of available N is implicated as a limiting factor in switchgrass growth (Brejda 2000, Wang et al 2010), reducing costly fertilizer N inputs not only increases economic competitiveness (Giannoulis et al 2014) but also promotes environmental sustainability (Pedroso et al 2014). Therefore, it is necessary to develop alternative soil fertility management strategies that don't offset switchgrass's ecosystem and provisioning services.

Endophytic beneficial microorganisms may provide unique opportunities to sustainably enhance plant growth and N acquisition. Beneficial microbes such as bacterial endophytes provide unique opportunities to enhance plant growth without compromising long-term sustainability (Farrar et al., 2014; Ker et al., 2014). Mycorrhizal fungi are already known to play a major role in switchgrass growth (Parrish and Fike, 2005). Recently, more attention has been directed toward understanding and capitalizing upon the ability of bacterial endophytes to benefit crop production (Ker et al., 2014; Singh et al., 2013). In particular, a bacterial endophyte, *Burkholderia phytofirmans* strain PsJN, has been shown to increase switchgrass seedling growth, vigor, and biomass yield in both greenhouse and field settings (Kim et al., 2012). PsJN is a uniquely promising strain because it has properties suited to increasing plant resistance to environmental stresses such as low fertility and drought (Barka et al., 2006) and regulating plant hormones (Weilharter et al., 2011).

In addition to switchgrass's promise as a low input biofuel crop, it also has the potential to sequester large amounts of soil C (Qin et al., 2012). Presumably, boosting switchgrass growth via inoculation with PsJN would also increase root contribution to soil organic matter.

This suggests that the endophyte may stimulate soil C cycling, but its consequence is unknown. It is possible that this C mineralization may come at the expense of extant soil C if more decomposition is occurring due to more active microbial metabolism spurred by labile C inputs from switchgrass roots, i.e. a priming effect (Cheng et al., 2003; Kuzyakov et al., 2000). On the other hand, PsJN may increase switchgrass's ability to sequester C if higher C:N ratio root residues replace low C:N old organic matter and fine roots and increased AMF growth facilitate the formation and stability of soil aggregates. Endophyte switchgrass likely contributes large amounts of C to soil, and may increase C sequestration. Others have found the tall fescue endophyte *Epichloe coenophiala* to have this effect through improving aggregation under infected fescue (Hosseini et al., 2015).

1.5 Conclusions

Organic and low input systems in the southeast rely heavily on residue inputs to deliver a number of services. These include nutrient mineralization, weed control, soil C sequestration, and even disease suppression (Larkin et al., 2011; Liu et al., 2007). However, meeting each goal depends on proper cover crop and input selection, judicious use of tillage, and enacting practices that encourage beneficial microorganisms. Agriculture plays a prominent role in control over the cycle of greenhouse gases between the atmosphere and soil, and soil health is critical to farm profitability. Research linking farm management and soil microbial activity responsible for gas emissions and nutrient mineralization helps low input farms become more sustainable and informs efforts to mitigate rising atmospheric greenhouse gas.

CHAPTER 2

EFFECTS OF LEGUME COVER CROPS AND SPRING TERMINATION PRACTICES ON SOIL C DYNAMICS IN AN ORGANIC SYSTEM IN THE SOUTHEASTERN U.S.

2.1 Abstract

The use of legume cover crops for both crop nutrient delivery and maintaining or improving soil organic matter is a key part of organic farming practices. Furthermore, reducing tillage systems using cover crops may maximize organic systems' ability to augment stable soil organic matter. Therefore, we examined how soil C and N dynamics were affected by different winter legume cover crop species and two no-till and one conventional tillage spring termination approaches in a factorial experiment. Of particular interest is how indicators of C and N status are affected during the first year of transition to using these approaches. Crimson clover (*Trifolium incarnatum*), hairy vetch (*Vicia villosa*), and a non-planted control were compared in concert with disk harrow, flail mower, or roller-crimper termination for their ability to influence short term indicators for soil organic matter trends, including microbial biomass C and N (MBC, MBN), hot water extractable carbohydrates, mineralizable C and N, and soil aggregate mean weight diameter (MWD). In two years of study, microbial activity was increased by planting a cover crop, and affected at different times throughout the growing season by species selection, with hairy vetch soil yielding higher MBC and mineralizable N than non-planted soil pre-termination, and crimson clover resulting in higher MBC and mineralizable C than either hairy vetch or control after termination. Also, disk tillage likely

accelerated microbial processing of cover crop residue as well as aggregate-protected organic matter, as suggested by higher mineralizable N, higher hot water extractable carbohydrate and higher MBC in disked plots. There were no main effects of either cover crop or termination method on aggregate mean weight diameter; however, tillage decreased MWD in soil without cover crops. Our findings demonstrate that these legume species may differ in their effects on C and N dynamics as a function of residue quality, and add further evidence that alternatives to tillage in organic systems may preserve soil organic matter, even within the first year of management adoption.

2.2 Introduction

Farms utilizing organic practices depend heavily on organic matter additions such as animal manures and cover crops to maintain soil fertility and productivity. Soil organic matter is a key component of soil fertility and augmenting soil organic C (SOC) is central, especially during the transition phase from a conventional to an organic management system. Farmers therefore are particularly interested how soil organic matter may be affected during the initial year of implementation of a new practice. Cover crops planted in the fall can contribute to SOC on organic farms by taking advantage of early spring days to produce plant biomass that is eventually added to soils (Larsen et al., 2014), although the frequent tillage common to these systems limits their potential. Such winter annuals have been shown to tremendously improve winter biomass production compared to weedy fallow (Kuo et al., 1997), serving as a large source of C and as fuel for microbial activity. Residue C derived from cover crops is transformed by soil microorganisms and partially incorporated into more stable C pools, thereby increasing soil organic C (Maughan et al., 2009; Sainju et al., 2003; Venkateswarlu et al., 2007)

Cover crop species and variety selection can strongly affect SOC contributions. When compared with grasses, cover crop legumes differ in terms of root C mineralization (Formowitz et al., 2009), effects on slower-cycling soil C (TerAvest et al., 2011), potential C mineralization (Sainju et al., 2007), microbial utilization of aromatic C (Ding et al., 2006), and even SOC impacts (Conceição et al., 2013). While much research has focused on the difference between legumes and grasses, fewer efforts have been made to compare the effects of different legume species on SOC.

Within the *Fabaceae* family, there exists a range of plant physiologies and biomass qualities associated with different species, greatly affecting N mineralization dynamics (Asagi and Ueno, 2009). Crimson clover (*Trifolium incarnatum*) and hairy vetch (*Vicia villosa*) are two commonly planted legume cover crops in the Southeast, both producing large amounts of biomass over the winter and early spring (Parr et al., 2011). These two species have shown stark contrasts in physiology and plant biomass quality (Ban et al., 2008), with clover contributing more aboveground biomass and more N ha⁻¹ but lower biomass N, P, and K concentrations. Others have found that biomass quality is a controlling factor determining decomposition rate and nutrient cycling (Cattanio et al., 2008; Nicolardot et al., 2001; Palm and Sanchez, 1990), meaning that these species may differ markedly in their biomass C stabilization potential.

Even when appropriate cover crop species selections are made for SOC accrual, frequent soil disturbance precludes maximum incorporation of biomass C into stable soil C pools due to disruption of soil macroaggregates. This results in degradation of previously physically protected aggregate C, and moreover prevents C sequestration in new microaggregates (Six et al., 2000). Reducing disturbance is a significant hurdle in organic

production, which typically relies on cultivation both to kill cover crops and to control weeds. It has long been known that higher intensity (Halvorson et al., 2002) or increased frequency (Bowman et al., 1990) of tillage decreases SOC levels, such that limiting the degree of soil disturbance presents a major challenge to enhancing C sequestration in agroecosystems (Torbert et al., 1999).

As an alternative to cultivation-based weed control and cover crop termination practices, high-residue, weed-suppressive cover crop mulches that are killed mechanically have become a promising practice for organic systems (Mirsky et al., 2012). In this approach a tractor mounted roller crimper kills winter annuals in the spring to create a thick residue barrier on the soil surface. In comparison to cultivation-based termination, the roller crimper leaves both cover crop residues and soil structure intact, potentially slowing mineralization of accrued biomass C. However, the effects of this practice on organic matter dynamics have yet to be assessed.

In an agronomic field setting, we compared several legume cover crop species plus a non-planted control, as well as two no-till spring termination techniques (flail mowing and roller crimping) and one tillage approach (disk termination) for their effects on labile soil C pools. Because soil aggregates and organomineral associations serve to protect C from microbial attack (Beare et al., 1994; Gregorich et al., 1989) and are themselves the result of microbial activity (Jastrow, 1996), we assessed aggregate stability as an indicator of a species' or termination approach's ability to increase SOC. Changes in aggregate stability in particular precede changes in SOC (Haynes et al., 1991), thus serving as a predictor for longer term change in SOC expected from adoption of a practice. We related observed changes to differences in measurements of microbial activity: Microbial biomass C (MBC) and N (MBN),

hot water extractable carbohydrates (HWC), and potentially mineralizable C and N (PminC, PminN).

The objectives of this project were to examine the effect of 1) legume cover crop species and 2) termination approaches on sensitive soil organic matter pools during the first season of implementation. We chose pools previously shown to be more responsive to changes in management than total SOC. We hypothesized that limiting soil disturbance with no-till spring termination practices would increase these sensitive pools. Furthermore, we predicted that the difference in cover crop species and their associated microbial processing would lead to differences in the size of these pools.

2.3 Materials and Methods

2.3.1 Site description

Experiments were located on a field that had been certified USDA Organic for 2 years at Cherry Research Farm in Goldsboro, NC, part of the North Carolina Department of Agriculture and Consumer Services system of research stations. In both the 2011-2012 and 2012-2013 seasons, plots were located on a Wickham loamy sand cultivated as an annual cropping system. Particle size analysis was performed according to the method of Gee and Bauder (1986). Soils contained 21.7-27.4% sand, 60.0-63.7% silt, and 12.3-18.2% clay. The plots were re-located within the field between the first and second seasons to mimic transition to given practice, represented by each treatment.

2.3.2 Experimental design

The experiment was established as a randomized block, split plot design, with termination method serving as the main plot and cover crop species arranged randomly as 6.10 m × 9.14 m split plots within the 18.3 m × 27.4 m main plots. Four replicate blocks were used

for all soil sampling and microbial biomass C and N, hot water extractable carbohydrate, aggregate stability, soil moisture, and bulk density measurements and a total of 6 replicate blocks were used for cover crop biomass sampling once each year. Analysis of variance and Fisher's LSD means separations were performed using the Mixed procedure in SAS 9.3.

2.3.3 Field operations

Crimson clover (*Trifolium incarnatum* L., var. 'Dixie'; CC) and hairy vetch (*Vicia villosa* Roth, var. 'AU Early Cover'; HV) were planted in early October of each year following disk harrow cultivation (10 cm depth) using a cone planter mounted on a tractor at seeding rates of 25 and 28 kg ha⁻¹ respectively (Table 2.1). Non-planted control (NP) plots also received cultivation plus a disking pass, also to a depth of 10cm. Weed growth was controlled in the non-planted plots by mowing monthly over the winter each year. All cover crops received one of three termination treatments on the same day, in mid-May both years (Table 2.1). Conventional disk tillage (CT) termination consisted of cultivation to a depth of 8cm with a disk harrow, while roller crimping (RC) occurred via rear-mounted roller with blades arranged in a chevron pattern. Flail mowing was performed prior to disk tillage in the disked plots as well as in the no-till flail mowing treatment (FM). Corn (*Zea mays* var. 'N630') was planted in late May each year on 76cm rows at a planting density of 82,000 seeds ha⁻¹ using a conservation planter.

2.3.4 Cover crop biomass

No more than one week before cover crop termination, aboveground HV and CC biomass was collected within a representative 0.5 m² quadrat within each plot. Tissues were harvested with shears and dried to a constant weight at 40°C in order to calculate biomass production in Mg ha⁻¹ dry biomass.

2.3.5 Soil sampling

Ten subsamples per plot were taken to depths of 0-5 and 5-15 cm with a 2 cm diameter push probe soil sampler, and each depth was mixed uniformly into one composite sample per plot. Sampling occurred one week before termination (pre-kill) then one (post-kill) and four weeks (mid-season) after cover crop termination (Table 2.1). Soils were sieved to pass a 2.8 mm mesh and stored in sealed plastic bags at 4°C. These samples were used for MBC, MBN, HWC, PminC, PminN, and soil gravimetric water content.

2.3.6 Aggregate mean weight diameter

Three subsamples per plot were taken to depths of 0-5 cm and 5-15 cm using a narrow blade shovel in a 10cm diameter area, and each depth combined into one composite sample per plot, taking care to disturb the sample as little as possible. Sampling occurred 1 week before termination and 4 weeks after termination (Table 2.1). Samples were air dried at 25°C until a constant weight was reached, then gently teased apart by hand along natural planes of weakness to pass an 8 mm sieve. This diameter served as the upper limit for aggregates used for study. The wet sieving procedure of Yoder (1936) as modified by Haynes (1993) was used for aggregate stability analysis. Using this approach, a 30 g sample of air dried 4.75-8 mm diameter aggregates was obtained by gently sieving the soil with a 4.75mm sieve. This 4.75-8mm size class represented approximately 60-70% of the bulk soil. These aggregates were placed on the uppermost of a stack of four sieves with 4.75, 2.0, 1.0, and 0.25 mm aperture mesh. Aggregates first were allowed to wet by capillary action for 10 minutes, and then were mechanically raised and lowered a distance of 3.5 cm in a column of water at 34 oscillations min⁻¹ for 10 minutes. Afterward, soil falling through the 0.25 mm sieve was gently poured out onto a 0.053 mm mesh sieve. Soil passing through the 0.053 mm sieve was discarded and was not weighed.

Materials remaining on the five sieves were oven dried at 105°C for 24 hr in order to determine the mass in each aggregate fraction. To obtain a quantification of the sample's resistance to dispersion, aggregate stability was expressed as aggregate mean weight diameter (MWD) and calculated as: $x_1(m_1/m_t) + x_2(m_2/m_t) + x_3(m_3/m_t) + x_4(m_4/m_t) + x_5(m_5/m_t)$ where m_i = mass of soil in a particular size fraction, m_t = total mass recovered from the sample, and x_i = the respective mean diameter of each fraction, calculated as the average of the aperture of the sieves directly above and directly below that fraction.

2.3.7 Microbial biomass C and nitrogen (MBC/MBN)

Microbial biomass C (MBC) and microbial biomass N (MBN) were determined using the chloroform-fumigation–extraction method (Ross, 1992; Vance et al., 1987) where 20.0 g of field moist soil was fumigated with ethanol–free chloroform for 48 h. Both fumigated and non-fumigated soils were extracted with 50 mL of 0.5 mol L⁻¹ K₂SO₄ by shaking for 30 min on an orbital shaker. A Shimadzu TOC-5050 analyzer was used to determine the organic C concentration (C_{org}) in the extractants. MBC was calculated as follows: $[(C_{org} \text{ in fumigated soil}) - (C_{org} \text{ in nonfumigated soil})] / k_{ec}$, where $k_{ec} = 0.33$ and serves to convert the extracted organic C to MBC (Sparling and West, 1988). The concentration of N in the extractant was determined on a Flow Injection Analysis Autoanalyzer (Lachat Omnion 8000, Hach Company, Loveland, CO) after digestion with alkaline persulfate oxidation (Cabrera and Beare, 1993), and calculated using the equation: $(total N \text{ extracted from fumigated soil} - total N \text{ extracted from nonfumigated soil}) / k_{en}$, where $k_{en} = 0.45$ and is used to convert the extracted organic N to MBN (Jenkinson, 1988). Both MBC and MBN are expressed on a soil dry weight basis.

2.3.8 Hot water extractable carbohydrates

Hot water extractable carbohydrates were measured as an approximation of soil carbohydrate of microbial origins (Bol et al., 2009), which is especially critical for binding clay particles into microaggregates around a core of organic matter (Oades, 1984). Three grams of soil were extracted by vortexing in a centrifuge tube with deionized water before heating in a hot water bath (80°C) for 24 h (Haynes and Swift, 1990). Extracts were then centrifuged before mixing 2mL supernatant with 10mL anthrone reagent (1.0g anthrone crystals in 75% H₂SO₄) and analyzing colorimetrically to compare with glucose standards from 0 to 100 mg L⁻¹ at 20 mg L⁻¹ intervals (Cheshire and Mundie, 1966).

2.3.9 Potentially mineralizable C

Soil C mineralization potential was measured as CO₂ evolution in the absence of plant roots using a lab incubation-alkaline absorption method (Coleman et al., 1977) as a proxy for heterotrophic soil respiration. Field-moist soil subsamples of 20g were placed in flasks and brought to 60% water holding capacity (Alef, 1995). These flasks as well as five blanks consisting of only empty flasks were placed in 1-L mason jars along with an alkaline trap consisting of a 50 mL beaker containing 5mL 0.5 mol L⁻¹ NaOH. Jars were sealed gas-tight and NaOH traps were removed after 7 days. Traps were replaced with fresh NaOH solution and these were removed after another 7 days. All traps were titrated with 1.0mol L⁻¹ HCl and C mineralization was calculated as the average rate of CO₂ evolution between the two 7-day periods, and expressed as mg CO₂ kg⁻¹ soil d⁻¹ (soil dry weight basis).

2.3.10 Potentially mineralizable N

A separate 20 g subsample of each sample was used for immediate N extraction following establishing the incubation soils. The same soil subsamples used for respiration were incubated a further 14 days (28 days total) at 22±1°C. At the end of 28 days, N was

extracted using 50 mL of 0.5 mol L⁻¹ K₂SO₄ in order to determine potentially mineralizable N (Hart et al., 1994). Extractant N concentrations of NH₄⁺ and NO₃⁻ were determined using a Lachat flow-injection analyzer. Potentially mineralizable N was calculated as the difference between incubated and non-incubated (immediate extraction) soil samples.

2.3.11 Bulk density

Plots were sampled once a season on the mid-season sampling date for bulk density determination, with a total of three subsample cores per plot sampled. These were obtained by driving a 7.62 × 7.62cm Uhland slide hammer corer into the soil at the soil surface for a depth range of 0-7.62cm. Soils were dried and weighed to determine bulk density in g soil cm⁻³ and triplicate subsample measurements averaged for one bulk density observation per plot.

2.3.12 Statistical Analysis

Analysis of variance was performed using the Mixed procedure in SAS 9.4, with data analyzed as a randomized complete block, split plot design with repeated measures. The repeated measures subject was the plot, with the three sampling periods serving as the repetition.

2.4 Results

2.4.1 Cover crop biomass

Cover crop biomass was similar between clover and vetch in 2012 (Table 2.2). Biomass accumulation was lower in 2013 and in that year vetch produced approximately 1 Mg ha⁻¹ more biomass than clover. Due to periodic close mowing in both years of weeds in the NP controls, biomass productivity was minimal and so was not recorded.

2.4.2 Aggregate mean weight diameter

Aggregate stability, expressed as mean weight diameter was strongly affected by the presence of cover crops on the plot in 2012 (Table 2.3). Surprisingly, MWD was larger in NP control plots, than either CC ($P < 0.001$) or HV ($P < 0.05$) combining both years. When comparing species selection, HV MWD was higher than that in CC ($P < 0.05$) combining 2012 and 2013 (Table 2.4). There was a cover crop by termination approach interaction ($P < 0.001$) combining the two years (Table 2.4), with FM and RC having higher MWD only in NP plots but not in either cover crop ($P < 0.001$). Aggregate MWD had also decreased between pre-kill and mid-season sampling points ($P < 0.001$) both years.

2.4.3 Microbial biomass C and N

Planting a cover crop had a positive impact on MBC combining both years ($P < 0.05$), with both HV ($P < 0.05$) and CC ($P < 0.01$) increasing MBC levels compared to the NP treatment with no cover crop (Table 2.5). Particular legume species were associated with elevated MBC at certain dates (significant cover x time interaction, $P < 0.01$), with HV increasing MBC relative to NP at the pre-kill sampling time ($P < 0.05$) and CC increasing MBC relative to NP and HV at the one week post-kill sampling time ($P < 0.001$, $P < 0.01$ respectively), combining both years. Termination approach also played a role in altering MBC levels ($P < 0.05$). Calculated MBC was higher in CT plots than RC plots ($P < 0.01$) across both years (Table 2.6). The surface 0-5cm soil in all treatments had higher MBC concentrations ($P < 0.001$) than 5-15 cm soil (Table 2.7). Microbial C was lower at the post-kill sampling date compared to pre-kill or mid-season ($P < 0.001$ for each). Over the two post-kill sampling dates in 2012, there was also a termination x cover interaction in MBC, in that MBC was affected by termination approach in plots planted

with cover crops but not in NP checks ($P < 0.05$), and cover crop species made no difference in rolled plots. In both of the termination treatments with the most aboveground biomass disturbance, CC gave rise to higher MBC than HV ($P < 0.05$ for CT and $P < 0.01$ for FM).

All measures of MBN were lower four weeks following CT termination, when compared to either FM treatment ($P < 0.05$) or RC ($P < 0.05$) at 0-5 cm. However, the opposite occurred at lower depths, where CT plot showed increased MBN over FM or CC ($P < 0.05$) (Table 2.8). In addition, MBN concentrations were higher in soil from the surface 5cm than below that ($P < 0.001$) (Table 2.7). Lastly, when analyzing across both years, MBN was affected by sampling date ($P < 0.001$), where pre-kill MBN levels were highest, followed by mid-season and finally post-kill (Table 2.7).

2.4.4 Hot water extractable carbohydrates

Hot water extractable carbohydrates remained largely unaffected by cover crop species or termination approach, with the exception of higher HWC in CT compared to FM ($P < 0.05$) or RC ($P < 0.05$) (Table 2.6). In addition, HWC were notably higher at the 5-15 cm depth mid-season in CT relative to FM ($P < 0.01$) or RC ($P < 0.001$) combining both years (Table 2.8). Similar to MBC, HWC concentrations were consistently higher in the surface 0-5cm of soil relative to 5-15cm ($P < 0.001$). (Table 2.7). HWC decreased from pre-kill to post-kill sampling ($P < 0.01$) and again from post-kill to mid-season ($P < 0.001$) (Table 2.7).

2.4.5 Potential C and N mineralization

Sampling date drove the observed differences in soil C mineralization for both cover crop and termination factors. As was the case with MBC, higher mineralization rates were found in cover cropped plots either at pre-kill or post-kill sampling times. Prior to termination, soil sampled from HV plots emitted CO_2 at a greater rate than CC ($P < 0.05$), while after termination,

CC soil C mineralization was significantly higher than that of NP ($P < 0.01$) or HV ($P < 0.01$) (Fig. 1.a). Hairy vetch increased potential N mineralization relative to both NP and CC at pre-kill and 1 week post-kill sampling times, while CC had higher N mineralization than NP at 1 week post kill (Fig. 1.b.). Both disking and rolling caused higher potential N mineralization compared with flail mowing (Table 2.6).

2.4.6 Bulk density and soil moisture

Disk tilling significantly decreased bulk density in both years compared to FM ($P < 0.01$) or RC ($P < 0.05$) (Table 2.5). Cover crop plots had higher soil moisture in 2012 than non-planted plots (CC > NP: $P < 0.001$; HV > NP $P < 0.01$). Conversely, NP plots were wetter than either CC ($P < 0.001$) or HV ($P < 0.05$) only at the pre-kill sampling date in 2013.

2.5 Discussion

Observed differences in microbially-driven C and N pool sizes (MBC, MBN and C and N mineralization) between cover cropped plots and the non-planted control plots were likely driven by biomass contributions from cover crops via shoots, roots, or rhizodeposition, as compared to controls with no cover crops and minimal weed biomass kept in check by frequent mowing. This was expected, as cover crops, and especially legumes, have been found to increase microbial biomass and respiration, especially at longer time scales (Steenwerth and Belina, 2010; TerAvest et al., 2011). We also found that in some cases cover crop species choice resulted in pool size differences, where either hairy vetch or crimson clover in combination with termination approach resulted in increased or reduced levels of our measured parameters. This impact of cover crop species varied by sampling time.

Of particular interest to this study was that cover crop species choice affected these pools at different points in the cover crop production season when compared to plots where cover

crops were not grown, with crimson clover plots showing elevated C mineralization and MBC one week following termination, and hairy vetch having increased MBC and N mineralization prior to termination while the crop was still alive. No consistent difference in biomass production between CC and HV was observed, indicating that differences between the species in their effect on soil C dynamics likely were due to differences in residue quality, such as C:N ratios or biomass chemistry (Rivas et al., 2014). Crimson clover, with a C:N ratio of 18.5:1-24.9:1, compared to vetch's 13:1-15.7:1 (Brown, 2012), likely affected decomposition kinetics with higher C:N crimson clover stimulating microbial biomass following termination when compared to lower C:N vetch, when this slightly higher C:N shoot material may have also served as a readily metabolizable C source (Sainju et al., 2003). This is evidenced by the higher MBC for CC than HV in treatments where aboveground residues were chopped or tilled in compared to the rolling method. Mowing and CT both would have left the stems relatively available as substrate for microbial growth (Liang et al., 2014; Ngwira et al., 2012). Crimson clover also raised soil respiration regardless of termination after cover crops were killed. It may also be that the simultaneous increase in MBC in CC may have been subject to rapid turnover and release as CO₂ in our incubations, which may explain the lack of an effect of species later in the season on these parameters, as well as a lack of an effect on aggregate stability if much of the higher C:N clover residue fueled only a shorter term growth in microbial activity followed by decline. Higher respiration has been demonstrated in some circumstances with higher C:N residues (Duong et al., 2009; Rivas et al., 2014; Zhou et al., 2012), as has accelerated turnover of mineral-associated organic matter (Mazzilli et al., 2014), which may explain the difference in MWD between HV and CC as well. In addition, there has been shown to be an increase in MBC as mineralizable N decreases (Franzluebbers et al., 1995), which is

in line with our results. Typically, however, research has shown that among non-legumes, high C:N biomass can lower soil respiration compared to lower C:N residues (Laudicina et al., 2014; Mbuthia et al., 2015). It was interesting that vetch presence alone resulted in higher MBC and potentially mineralizable N prior to termination compared to either the crimson clover or plots, or the plots with no cover crop planted, suggesting that vetch stimulated increases in soil microbial activity through rhizodeposition or associated processes. Vetch is known to contribute substantially to soil organic matter (Kong and Six, 2012) and vetch roots have been shown to have a narrower C:N ratio and release more N in incubations compared to clover (Jani et al., 2015). As a caveat, it is important to note that the time of killing impacts cover crop biomass accumulation (Higashi et al., 2014), and killing cover crops on the same day may have resulted in less than the maximum biomass accumulation for either HV or CC.

Elevated microbial activity observed one week after termination in plots where cover crops were killed and then incorporated via CT likely was fueled by a combination of two C sources. First, high HWC and MBC as a result of tillage are likely explained by increased cover crop residue-soil contact, as both are sensitive to biomass additions (Benbi et al., 2015; D'Hose et al., 2012). Elevated MBN and HWC at the lower depth of 5-15cm in tilled plots likely was derived from incorporated aboveground residues, which was not a factor in the other two termination approaches where biomass remained on the soil surface. Second, tillage is well known to disrupt soil aggregates and expose soil organic matter to microbial degradation (Mikha and Rice, 2004), and is possible that a portion of the increase in these pools may originate from previously aggregate-protected C and N that was now available for microbial degradation. Nitrogen availability also appeared to be affected by tillage disturbance throughout the soil profile, as MBN and mineralizable N both increased when tillage was a

factor, regardless of cover crop presence. This supports the idea that tillage hastened mineralization and release of previously protected soil organic matter to decomposition, described in many other studies (Conant et al., 2007; Six et al., 2000). New access to particulate organic matter within destroyed aggregates is a major driver of mineralizable N and microbial growth in the absence of residue additions (Wander and Bidart, 2000). However, this C and N release is still small compared to the enhanced mineralization of buried cover crop residues (Bajgai et al., 2014). Furthermore, the plots used in the current study were on certified organic land that would have received tillage and cover crops in previous years, limiting the amount of aggregation that would occur and therefore the amount of aggregate-protected organic matter capable of mineralizing N upon new disturbance.

In contrast to our hypothesis, tillage-based termination alone did not significantly affect aggregate stability. Our data suggests that factors such as lack of soil cover over the summer may have influenced aggregate stability, as all plots where cover crops were planted showed low MWD similar to tilled plots where no cover crops were planted while non-tilled NP plots exhibited elevated MWD. Because the NP plots were managed for minimal weed biomass production via frequent mowing over the winter and early spring, this increase in MWD was not likely due to the action of organic matter from weeds in the NP plots. Research documenting direct changes in aggregation due to soil tillage is often carried out over much longer intervals than two site years, as was studied here. However, short-term changes can serve as powerful indicators of longer term SOC stabilization as the effect the soil mineral fraction plays in determining aggregate stability. Over a longer time frame, this physical disruption combined with the above changes in microbial activity may have an effect on soil aggregation in both cover cropped and non-planted soils, as fields not exposed to tillage for

long periods of time have been found to have highly increased levels of stable aggregates. Tillage has been found to reduce aggregate stability by numerous researchers (Bhattacharyya et al., 2009; Chan et al., 1994; Roldan, 2003; Ryan et al., 2011; Tivet et al., 2013). Our data may reflect the lack of a substantial organic C source in no-cover crop plots to create aggregates resistant to tillage over the winter and spring. Such an effect has been suggested before, although others noticed this effect after treatments may be even on a longer time frame-up to 10 years (Abdollahi and Munkholm, 2014). Interestingly, NP soil that was not tilled had higher aggregate MWD than any cover cropped soil in at mid-season in 2012, while all NP plots had MWD similar to HV and CC prior to cover crop termination. In 2012, when this difference was apparent, NP plots were drier than either HV or CC pre-kill and post-kill (Table 2.5), possibly due to lack of ground cover to conserve soil moisture. It has been found that drying contributes substantially to increases in aggregate stability in the short term (Cosentino et al., 2006). Such results highlight the importance of within-season variability in explaining some soil physical phenomena at shorter time scales.

There is some indication that the increased microbial biomass in CT plots may have been a transient phenomenon, because differences in NP MBC driven by termination approach were no longer different at the mid-season sampling in 2013. A similar phenomenon was observed by Zhang et al., where CT MBC spiked to surpass a NT treatment before again subsiding (2012). Enhanced N mineralization only shortly after termination in the disk treatment also suggests that the disruptive process of tillage would decrease soil organic matter long term. This increase in mineralization likely came as a result of exposing previously aggregate-protected OM to microbial breakdown, as well as from more complete mineralization of plowed in aboveground residues. The fact that flail mowing did not exhibit a similar spike

suggests that simply increasing residue surface area through mowing would not cause breakdown to hasten significantly.

In summary, tillage was a highly disruptive action that drastically changed soil MBC, MBN, and respiration, indicating that over the long term this treatment would likely decrease soil C overall. Interestingly, planting a cover crop enhanced many measures of microbial activity, but not aggregation, suggesting their biomass may take a longer time to have a significant impact on soil C and structure.

2.6 Conclusions

Microbial processing of residues is key to stabilization of soil C, both through the physical protection coming with increased aggregation and transformation into more stable soil organic matter. Several of the measured indicators of microbial growth and activity point to the potential of using legume cover crops to improve soil C long term. In addition, individual species displayed differing C and N mineralization potentials, likely due to differences in biomass quality and possibly biomass quantity. Disk tillage stood out in its ability to increase potentially mineralizable C, hot water extractable carbohydrate, and microbial biomass, although the permanence of these changes appear to be limited by the amount of soil or residue C made available through disturbance and turning under residues. Aggregate stability dynamics may have been dominated by factors other than microbial activity at the within-season timescale, although aggregate stability was no lower in cover cropped plots that were disked than ones with no-till termination. This study highlights the short-term soil C and N dynamics associated with cover crops and alternative termination methods.

Acknowledgements

This work was partially supported by USDA's National Institute of Food and Agriculture [Grant 2010-51106-21872 and Grant 2012-51106-20332]. Thanks to Cherry Research Farm staff and Sarah Seehaver for field maintenance and sampling assistance.

2.7 Tables and Figure

2.7.1. Tables

Table 2.1 Field activity and sampling timeline

| Activity | Corn growing season | |
|-----------------------------|---------------------|-----------|
| | 2012 | 2013 |
| Cover crop planting | 3-Oct-11 | 10-Nov-12 |
| Pre-kill soil sampling | 7-May-12 | 23-Apr-13 |
| Cover crop biomass sampling | 11-May-12 | 2-May-13 |
| Cover crop termination | 11-May-12 | 9-May-13 |
| Post-kill soil sampling | 17-May-12 | 15-May-12 |
| Corn planting | 18-May-12 | 16-May-13 |
| Mid-season soil sampling | 7-Jun-12 | 13-Jun-13 |
| Bulk density sampling | 16-Aug-12 | 5-Sep-13 |

Table 2.2 Cover crop biomass accumulation, Mg ha⁻¹

| Cover crop | Corn growing season | |
|------------|---------------------|-------|
| | 2012 | 2013 |
| CC | 9.66a ¹ | 4.42b |
| HV | 8.83a | 5.46a |

¹Means followed by the same letter in each year column are not significantly different at $P=0.05$.

Table 2.3 Analysis of variance for the effects of termination approach, cover crop, sampling date during the spring through summer growing season, and soil sampling depth.

| Source of variation | 2012 | | | | 2013 | | | | | |
|---------------------|------------------|-----|-----|-------------------|------|-----|-----|-------------------|-------|------|
| | MBC ¹ | MBN | HWC | AS | MBC | MBN | HWC | PminC | PminN | AS |
| Termination (T) | * ² | NS | NS | NS | * | NS | * | NS | * | NS |
| Cover (C) | *** | NS | NS | *** | NS | NS | NS | NS | *** | NS |
| T × C | * | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| Sampling date (S) | *** | *** | *** | * | *** | *** | *** | * | *** | * |
| S × T | NS | NS | NS | NS | * | NS | NS | NS | * | NS |
| S × C | ** | NS | NS | NS | NS | NS | NS | ** | *** | * |
| S × C × T | NS | NS | NS | NS | * | NS | NS | NS | NS | NS |
| Depth (D) | *** | *** | *** | ---- ³ | *** | *** | *** | ---- ⁴ | ---- | ---- |
| D × T | NS | ** | NS | ---- | NS | *** | NS | ---- | ---- | ---- |
| D × C | NS | NS | NS | ---- | NS | NS | NS | ---- | ---- | ---- |
| D × S | NS | *** | *** | ---- | NS | * | NS | ---- | ---- | ---- |
| D × C × T | NS | NS | NS | ---- | NS | NS | * | ---- | ---- | ---- |
| D × T × S | NS | * | NS | ---- | NS | NS | NS | ---- | ---- | ---- |
| S × C × D × T | NS | NS | NS | ---- | NS | NS | NS | ---- | ---- | ---- |

¹MBC microbial biomass C; MBN microbial biomass N; HWC hot water extractable carbohydrates; AS aggregate stability; PminC and PminN potentially mineralizable C and N.

²NS not significant at $P=0.05$; * significant at $P=0.05$; ** significant at $P=0.01$; *** significant at $P=0.001$

³Aggregate stability only measured for soil from 0-5cm

⁴Pmin C and PminN measured in 2013 only

Table 2.4 Effect of termination method and winter ground cover on aggregate mean weight diameter (mm).

| Cover | 2012 | | | | | | Termination | | |
|-----------------|------|----------------|------|---|------|---|-------------|------|---|
| | Pre | | 4wk | | Mean | | 4wk | | |
| NP ¹ | 5.23 | a ² | 4.80 | a | 5.02 | a | CT | 3.26 | a |
| CC | 4.08 | a | 2.35 | b | 3.22 | b | FM | 3.80 | a |
| HV | 4.02 | a | 3.25 | b | 3.64 | b | RC | 3.62 | a |
| 2013 | | | | | | | | | |
| NP | 3.25 | ab | 2.74 | a | 2.99 | a | CT | 2.35 | a |
| CC | 2.55 | b | 2.10 | a | 2.33 | a | FM | 2.31 | a |
| HV | 4.06 | a | 2.28 | a | 3.17 | a | RC | 2.46 | a |
| Combined years | | | | | | | | | |
| NP | 4.23 | a | 3.77 | a | 4.00 | a | CT | 3.35 | a |
| CC | 3.13 | a | 2.22 | a | 2.68 | b | FM | 3.20 | a |
| HV | 4.03 | a | 2.77 | a | 3.40 | a | RC | 3.39 | a |

¹NP non-planted, CC crimson clover, HV hairy vetch, CT conventional tillage, FM flail mow, RC roller crimper, Pre one week before cover crop termination, 1wk one week after termination, 4wk four weeks after termination, Mean the within season mean.

²Means followed by the same letter in each column within a factor in a given year are not significantly different at $P=0.05$.

Table 2.5 Effect of cover crop on MBC, PminC from combined 2012-2013 analysis and soil gravimetric moisture content from each year

| Sampling date | Cover | MBC ³ | | PminC | | Moisture (2012) | | Moisture (2013) | |
|---------------|-----------------|------------------|----------------|--------|----|-----------------|---|-----------------|---|
| Pre-kill | NP ¹ | 303.79 | b ² | 388.35 | ab | 0.1912 | b | 0.1423 | a |
| | CC | 318.76 | ab | 367.19 | b | 0.2123 | a | 0.1188 | b |
| | HV | 342.86 | a | 532.86 | a | 0.2121 | a | 0.1273 | b |
| Post-kill | NP | 203.35 | b | 494.88 | b | 0.1918 | b | 0.08315 | a |
| | CC | 281.9 | a | 749.64 | a | 0.2041 | a | 0.08771 | a |
| | HV | 232.9 | b | 491.33 | b | 0.2209 | a | 0.09636 | a |
| Mid-season | | | | | | 0.1618 | | | |
| | NP | 329.48 | a | 501.83 | a | | a | 0.1163 | a |
| | CC | 341.32 | a | 441.12 | a | 0.1666 | a | 0.1176 | a |
| | HV | 337.39 | a | 477.97 | a | 0.1472 | b | 0.1262 | a |

¹NP non-planted, CC crimson clover, HV hairy vetch

²Means followed by the same letter in each column within a factor in a given year are not significantly different at $P=0.05$.

³MBC microbial biomass C, mg C kg⁻¹ soil; PminC potentially mineralizable C, mg CO₂ kg⁻¹ soil d⁻¹; Moisture soil gravimetric water content, g water g⁻¹ dry soil.

Table 2.6 Effect of termination approach on MBC, HWC, and potentially mineralizable N from combined 2012-2013 analysis

| Termination | MBC ³ | | HWC | | PminN |
|-------------------|------------------|----------------|--------|---|---------|
| Disk ¹ | 341.5 | a ² | 434.95 | a | 31.17 a |
| Flail | 324.31 | ab | 400.03 | b | 21.34 b |
| Roll | 308.77 | b | 392.1 | b | 28.26 a |

¹Disk disk tillage; Flail flail mowing; Roll roller-crimper

²Means followed by the same letter in each column within a factor in a given year are not significantly different at $P=0.05$.

³MBC microbial biomass C, mg C kg⁻¹ soil; PminN potentially mineralizable N, mg NO₃⁻+NH₄⁺-N kg⁻¹ soil d⁻¹; HWC hot water extractable carbohydrate, mg C kg⁻¹ soil

Table 2.7 Effect of sampling date and sampling depth on MBC, MBN, and HWC from combined 2012-2013 analysis

| Sampling date | MBC | MBN | HWC |
|---------------|----------|-----------|----------|
| Pre-kill | 321.8 a | 54.338 a | 474.26 a |
| Post-kill | 239.38 b | 43.176 c | 442.53 b |
| Mid-season | 336.06 a | 50.0165 b | 369.06 c |
| Depth | | | |
| 0-5cm | 369.46 a | 61.1759 a | 498.63 a |
| 5-15cm | 228.71 b | 37.1779 b | 358.6 b |

¹*Pre-kill* one week before cover crop termination; *Post-kill* one week after termination; *Mid-season* four weeks after termination

²Means followed by the same letter in each column within a factor in a given year are not significantly different at $P=0.05$.

³*MBC* microbial biomass C, mg C kg⁻¹ soil; *MBN* microbial biomass N, mg N kg⁻¹ soil; *HWC* hot water extractable carbohydrate, mg C kg⁻¹ soil

Table 2.8 Depth × termination approach interaction effect on MBN and HWC at Mid-season sampling date

| Depth | Termination | MBN ³ | HWC |
|--------|--------------------|----------------------|----------|
| 0-5cm | Flail ¹ | 70.57 a ² | 423.91 a |
| | Roll | 70.02 a | 417.32 a |
| | Disk | 60.57 b | 423.39 a |
| 5-15cm | Flail | 36.50 b | 295.92 b |
| | Roll | 35.81 b | 285.89 b |
| | Disk | 46.40 a | 371.61 a |

¹*Disk* disk tillage; *Flail* flail mowing; *Roll* roller-crimper

²Means followed by the same letter in each column within a factor in a given year are not significantly different at $P=0.05$.

³*MBN* microbial biomass N, mg N kg⁻¹ soil; *HWC* hot water extractable carbohydrate, mg C kg⁻¹ soil

2.7.1. Figure

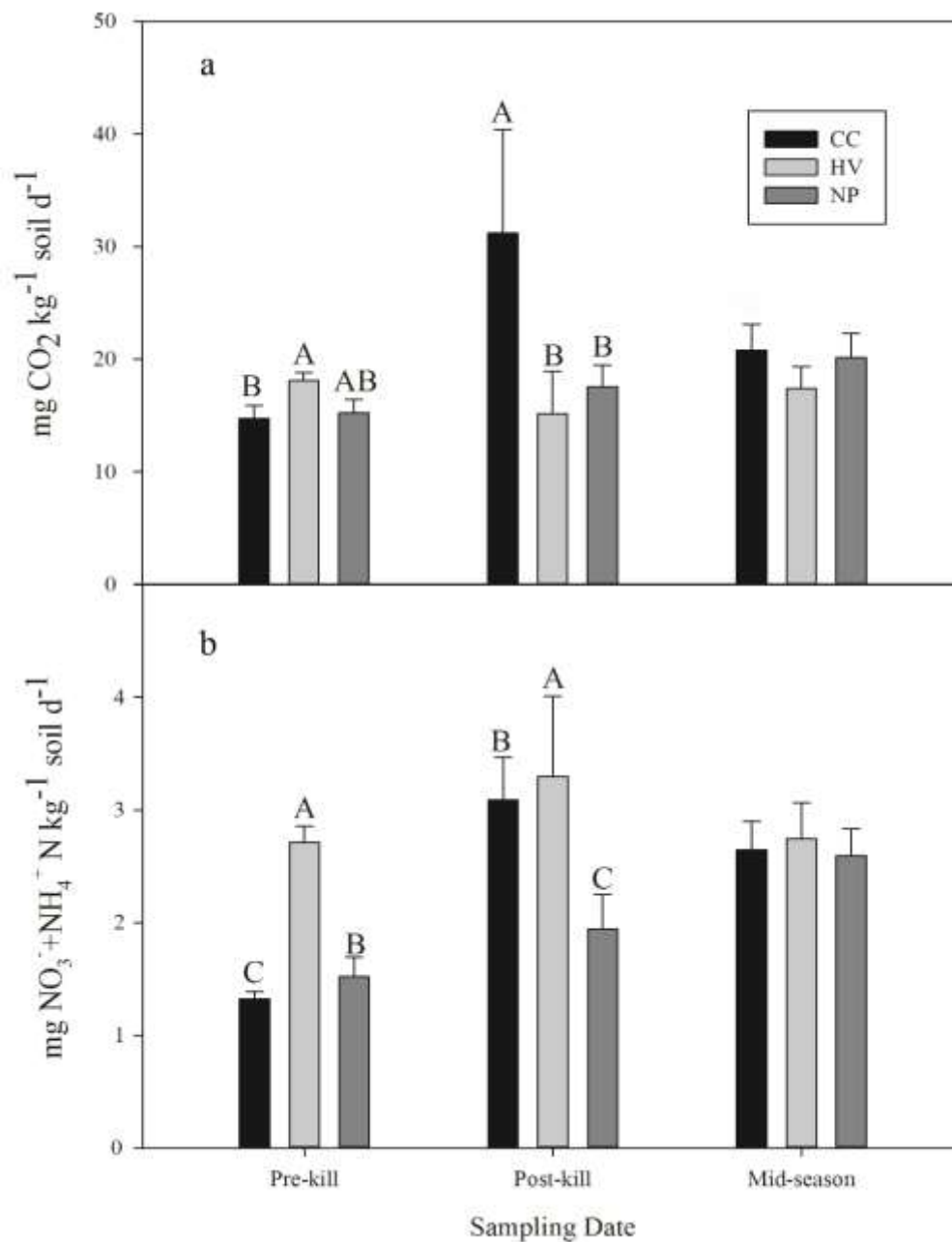


Figure 2.1 Potentially mineralizable N (a) and potentially mineralizable C in soil as affected by cover crop plants hairy vetch (HV), crimson clover (CC), or a non-planted control (NP) in 2013. Similar letters within a sampling date grouping indicate no difference between cover crops ($P > 0.05$).

Chapter 3

EFFECT OF NITROGEN SOURCE AND FIELD MANAGEMENT ON AGRICULTURAL SOIL N₂O EMISSIONS

3.1 Abstract

Levels of synthetic fertilizer and manure N inputs are each known to drive the rate of nitrous oxide (N₂O) production in agricultural soils. However, the relative importance of these inputs in determining emissions from soils spanning different tillage regimes, fertilization histories, and rotations is unknown. A series of experiments was conducted to examine the N₂O emissions potential of different farming systems' soils at multiple times throughout a corn field season, as well as the impact of either synthetic or organic N on N₂O emissions. Additionally, linkages were made between observed N₂O emissions and soil N cycling and field management practices. Surveyed farming systems included conventional with tillage (CCT), conventional long rotation (CLR), conventional without tillage (CNT), organic with tillage (OCT), organic long rotation (OLR), and organic with reduced tillage (ORT). Soils were analyzed for their potential to emit N₂O and CO₂ over a three week incubation, as well as microbial biomass C and N, mineralizable N, and K₂SO₄-extractable C. Over the course of the incubations where N was added in the lab, cumulative N₂O-N emitted was equivalent to about 1.4-3.8% of added urea N and 0.08-1.1% of added litter N. When litter was applied, N₂O emissions rate was highest in CCT soil, but when UAN was applied, OLR was the highest. In field soils that had not received amendments in the lab, CCT had the highest emissions rate among systems after field applications of UAN, while ORT emissions rate was highest among systems' soils at several points during the incubation after field litter

application. Organic reduced tillage soil K_2SO_4 -extractable C was also higher than in any conventional system at this time. After fertilization of field plots in May, potentially mineralizable N was higher in all organic system than any conventional system. These results indicate that certain treatment rotations with ORT, conventional tillage and synthetic N, and excess N at corn harvest are susceptible to larger N_2O emissions under either organic or synthetic N inputs.

3.2 Introduction

Nitrous oxide is a major greenhouse gas with 300 times the warming potential of CO_2 (IPCC 2007); 40% of global N_2O production comes from human activities (EPA 2010). This amounts to 5.3 Tg N_2O-N yr^{-1} , with 2/3 of this coming from the agricultural sector (Davidson and Kanter, 2014). The majority of this comes from soil management, where N fertilizer inputs are the single largest contributor to total emissions (de Klein et al., 2006). A meta-analysis indicates that N_2O emissions' response to fertilizer rate are exponential, rather than linear (Shcherbak et al., 2014). Although farms rely on large quantities of input or biologically fixed N to maximize productivity and return on investment, this N may also serve as a substrate for bacteria and fungi in the soil which reduce NO_3^- to N_2O when soil O_2 is limiting (Robertson and Groffman, 2014). There is therefore a major impetus to understand how to better moderate fertilizer N availability to nitrifiers and denitrifiers to not only reduce greenhouse gas emissions, but also to curb N loss from agricultural systems. Close to 1% of fertilizer N can be lost as N_2O in typical field settings, and even more may be lost under conditions of high C and limited oxygen availability (Chadwick et al., 2011).

Because N_2O production depend on a number of factors, it remains unclear how farming history and integrated management approaches affect emissions linked to N sources.

Furthermore, there is contrasting information on whether and under what conditions using synthetic vs. organic N may lower emissions. On one hand, organic N sources such as legume cover crops and manures often more slowly release N to the soil solution than inorganic forms like urea ammonium nitrate (UAN) and can even immobilize N in the short term (Paul and Beauchamp, 1994), possibly lowering emissions. Meanwhile, organic amendments may also serve as the energy source in denitrification of soil NO_3^- and promote nitrification of NH_4^+ (Koester et al., 2015). Moreover, the influence either synthetic or organic N has on N_2O may not be universal across farming systems with varying C and N availability. Alternative farming systems, whether organic, reduced tillage, or rotations including perennials, are often touted for their environmental benefits (Cavigelli et al., 2013; Kantar et al., 2016; Zandersen et al., 2016). In addition to other services such systems may provide, it is imperative to simultaneously developing more N-use efficient practices to mitigate GHG emissions and maintain yield (Eagle and Olander, 2012a). With this in mind, we sought to determine 1) N_2O emissions potential of different farming system soils with a history of various fertility management and weed control strategies when amended with either a mineral or organic N source and 2) the effect of fertilizer sources on associated soil C and N cycling that may in return affect for N_2O emissions. We hypothesized that adding mineral N inputs to systems with high soil levels of mineralizable C would lead to enhanced N_2O emissions, and microbial N would be less, thereby acting as a weaker N sink to prevent N loss in systems without manure and cover crop inputs.

3.3 Materials and Methods

3.3.1 Site description, soil sampling and preparation

The fields used for soil sampling and biomass collection in this study were located on the North Carolina Department of Agriculture and Consumer Services Cherry Research Farm, located in Goldsboro, NC (35°23'N, 78°02'W, elevation 35 m above sea level). The plots, as part of the Farming Systems Research Unit (FSRU), have been under the same management regime since establishment of the long-term study in 1999. The plots are also relatively large, varying from 1.2-3.6ha. The FSRU is divided into three replicate blocks according to intensive soil mapping and consists of various organic and conventional best management practice (BMP) systems. Predominant soil series were Tarboro loamy sands (Typic Udipsamments) in block A, Wickham sandy loams (Typic Hapluduts) in block B, and Tarboro loamy sands in block C. The 6 systems selected for use in this study were as follows: BMP with conventional synthetic N fertilizer and conventional tillage (CCT), BMP with conventional synthetic N fertilizer in a long rotation of three years of annual crops in rotation with three years of perennial grass hay (CLR), BMP with conventional synthetic N fertilizer and no tillage using herbicides (CNT), organic poultry litter fertilizer with conventional tillage (OCT), organic long rotation (OLR), and organic reduced tillage using cover crops and fallow for weed control (ORT). More information about the FSRU long term study can be found in Mueller et al., 2002. Soils were sampled June 4, 2014 in a soybean year of the rotation to avoid recent N additions and differences in cover crop species for use in incubations with added mineral or litter N (Table 3.1). All plots were planted with rye (*Secale cereal* L.) the previous fall, which was then terminated by herbicide in the CNT and CLR systems, tillage in the CCT, OCT, and OLR systems, and roller-crimper in the ORT

system. All plots were planted with soybeans following termination. The following fall, conventional plots were planted with a wheat cover crop, while organic plots were planted with either wheat, clover, and vetch (OCT and OLR) or vetch and rye (ORT). The following year, soils were sampled to coincide with key field operations events. Soils were collected on April 1, 2015, immediately before cover crop termination and corn planting; on May 4, immediately after cover crop termination, first cultivation in tilled plots, fertilization with either uncomposted turkey litter or UAN, and corn planting; on June 11, immediately following a second application of UAN, and September 8, soon after corn harvest.

3.3.2 Cover crop biomass

No more than five days before cover crop termination, aboveground biomass was collected within a representative 0.5m² quadrat. Tissues were harvested with shears and dried to a constant weight at 40°C to calculate biomass production in Mg ha⁻¹ dry biomass.

3.3.3 N₂O and CO₂ emissions rate

After collection on each of the dates mentioned above, soils were sieved to pass a 2mm mesh, and moisture content was determined by mass loss upon drying at 105°C. One hundred grams of soil (dry weight equivalent) was placed into each of four 237mL glass canning jars with screw-top lids and packed to a bulk density of 1.1g cm⁻³ (Chen et al., 2014). Three of these jars were wetted to 70% water-filled pore space (WFPS) with DI water, while the remaining one was wetted to 60% WFPS. Three jars were used for 70% WFPS measurements due to variability in N₂O emissions rates between jars. These moisture contents have previously been shown to be ideal for measuring N₂O produced by denitrification (70% WFPS) and C and N mineralization as well as N₂O produced by nitrification (60% WFPS) (Baral et al., 2016; Franzluebbers, 1999). Jar lids were perforated

with rubber stoppers to allow gas sampling with a needle and syringe. Soils were sampled for N₂O and CO₂ at 0.5, 1, 1.5, 2.5, 4.5, 8.5, and 16.5d after adding water. Gases were stored in 12mL glass vials with crimp-top lids until analyzing on a Shimadzu GC-2014 gas chromatograph equipped with an electron capture detector for N₂O and a flame ionization detector for CO₂ (Shimadzu Corporation, Kyoto, Japan). All GC gas analyses were completed within 48 hours after headspace sampling. Lids were capped tightly for 90 minute intervals at each sampling point, and then uncapped and loosely covered to allow gas exchange in the interim periods. Soils were monitored for moisture loss and water was added as necessary to maintain original moisture content. Nitrous oxide emissions rates are expressed on a dry weight equivalent basis and calculated as follows:

$$\text{mg N}_2\text{O kg soil}^{-1} \text{ d}^{-1} = \frac{\left[\left(\frac{3.2 \times N_2O_b}{h-5\text{mL}} - 3.2 \times N_2O_a \right) \times a \times m \right]}{1000 \times R \times ^\circ K \times t \times g \text{ soil}}$$

where N_2O_a and N_2O_b represent concentrations of N₂O in the jar headspace at the time of sealing the jar and of gas sampling, respectively; m = molecular mass of N₂O (44.013 g/mol); a = atmospheric pressure in the lab in Raleigh, NC = 0.965 atm; t = time in days jar was closed; h = jar headspace in mL; R = the gas constant, 0.08206 L atm mol⁻¹ °K⁻¹; °K = 273+22 (Lab temperature of 22°C); and $g \text{ soil}$ = dry weight equivalent soil in the jar.

Carbon dioxide production rates, as a measurement of mineralizable C, are expressed on a CO₂-C per soil dry weight equivalent basis and calculated similarly to N₂O, as follows:

$$\text{mg CO}_2\text{-C kg soil}^{-1} \text{ d}^{-1} = \frac{\left[\left(\frac{3.2 \times CO_2b}{h-5\text{mL}} - 3.2 \times CO_2a \right) \times a \times m_1 \right]}{m_2 \times 1000 \times R \times ^\circ K \times t \times g \text{ soil}}$$

where CO_{2a} and CO_{2b} represent concentrations of CO₂ in the jar headspace at the time of sealing the jar and of gas sampling, respectively; m_1 = molecular mass of C (12.011g /mol);

m_2 = molecular mass of CO₂ (44.01 g /mol); and a , t , h , R , °K, g soil are as defined above for N₂O.

3.3.4 Cumulative N₂O and CO₂ flux

Cumulative N₂O and CO₂ flux were estimated using an area under the curve approach based on the hours of incubation X rate of mg N₂O kg⁻¹ soil day⁻¹ or mg CO₂-C kg⁻¹ soil day⁻¹.

$$\text{mg N}_2\text{O kg}^{-1} = \sum \left[\left((d_2 - d_1) \times \frac{N_2O_{d1} + N_2O_{d2}}{2} \right) + \dots + \left((d_k - d_j) \times \frac{N_2O_{dj} + N_2O_{dk}}{2} \right) \right]$$

$$\text{mg CO}_2\text{-C kg}^{-1} = \sum \left[\left((d_2 - d_1) \times \frac{CO_2C_{d1} + CO_2C_{d2}}{2} \right) + \dots + \left((d_k - d_j) \times \frac{CO_2C_{dj} + CO_2C_{dk}}{2} \right) \right]$$

3.3.5 Microbial Biomass C and N, NH₄⁺-NO₃⁻-N, and extractable C (K₂SO₄-C)

Microbial biomass C (MBC) and microbial biomass N (MBN) were determined using the chloroform-fumigation–extraction method (Ross, 1992; Vance et al., 1987) where 20 g of field moist soil was fumigated with ethanol–free chloroform for 48 h. Both fumigated and non-fumigated soils were extracted with 50 mL of 0.5 mol L⁻¹ K₂SO₄ by shaking for 30 min on an orbital shaker. A Shimadzu TOC-5050 analyzer was used to determine the organic C concentration (C_{org}) in the extractants. MBC was calculated as follows: $[(C_{org} \text{ in fumigated soil}) - (C_{org} \text{ in nonfumigated soil})] / k_{ec}$. where $k_{ec} = 0.33$ and serves to convert the extracted organic C to MBC (Sparling and West, 1988). The concentration of N in the extractant was determined on a Lachat flow injection analyzer after digestion with alkaline persulfate oxidation (Cabrera and Beare, 1993), and calculated using the equation: $(total \ N \ extracted \ from \ fumigated \ soil - total \ N \ extracted \ from \ nonfumigated \ soil) / k_{en}$, where $k_{en} = 0.45$ and is used to convert the extracted organic N to MBN (Jenkinson, 1988). K₂SO₄-extractable C was determined from the organic C concentration in extractant from non-fumigated soil. NH₄⁺-

NO_3^- -N was determined from the total inorganic N extracted from non-fumigated soil. Both MBC and MBN are expressed on a soil dry weight basis.

3.3.6 Incubations of farming systems soils with UAN or poultry litter

Incubations similar to those described above were established with fresh soil less than one week after sampling in June 2014. Seven jars were filled with 100g for each field plot's soil: two for UAN (UN) additions at 70% WFPS, two for pelleted poultry litter (LN) additions at 70% WFPS, two for non-amended soil at 70% WFPS, and one for non-amended soil at 60% WFPS. To simulate a single 168 kg ha^{-1} fertilizer application, 16mg of either UAN-N or litter N (250mg total litter) was added to each 100g subsample of soil. Pelleted poultry litter with an analysis of 3-2-3 (% N - % P_2O_5 - % K_2O) was ground to pass a 1mm mesh and mixed thoroughly into the soil. Poultry litter consisted of uncomposted chicken litter and was different from the litter used in field applications.

3.3.7 Potentially mineralizable N

A separate 20 g subsample of each sample was used for immediate N extraction following establishing the incubation soils. The same soil subsamples used for respiration were incubated a further 14 days (28 days total) at $22 \pm 1^\circ\text{C}$. At the end of 28 days, N was extracted using 50 mL of $0.5 \text{ mol L}^{-1} \text{ K}_2\text{SO}_4$ in order to determine potentially mineralizable N (Hart et al., 1994). Extractant N concentrations of NH_4^+ and NO_3^- were determined using a Lachat flow-injection analyzer. Potentially mineralizable N was calculated as the difference between incubated and non-incubated (immediate extraction) soil samples.

3.3.8 Statistical analyses

The six farming systems were randomly assigned to one of three replicates for a total of 18 plots arranged into blocks based on soil type. Analysis of variance statistical analysis was

carried out using the MIXED procedure in SAS 9.3 (Cary, NC), and multiple comparisons between dates and farming systems were also completed using Fisher's Protected LSD. For ANOVA among farming systems' gas emissions data for soil sampled on a particular date, a repeated measures analysis was used, with incubation jars serving as the repeated subject. For soil C and N pools (MBC, MBN, PMN, K₂SO₄-C, inorganic N) a repeated measures analysis was used, with plots serving as the repeated subject. Several covariance structures were modeled (autoregressive, compound symmetry, unstructured, and spatial powered), and the covariance structure with the best model fit (AIC, AICC, and BICC) was used for analysis. Differences were deemed significant at $P < 0.05$.

3.4 Results

3.4.1 Effect of farming system and field N inputs on gas emissions and soil C and N dynamics

3.4.1.1 Nitrous oxide emissions rate from 70% WFPS soil

Differences emerged between sampling dates as well as farming systems on individual sampling dates (Table 3.2). For most farming systems, N₂O emissions from soil collected at each of the four sampling dates peaked between 12-36h and fell afterwards. However, for CCT soil collected in June after a split UAN application was made, gas production rates peaked around 60h without abating (Figure 3.3). The rate of N₂O emissions for individual farming systems depended on both sampling date and the hour of incubation (Hour X date X system interaction $P = 0.0005$). From soil sampled in April before cover crop termination, corn planting, and fertilization, OLR emissions were greater than OCT, CLR, and CNT at 12 and 24h (Table 3.3). In May, immediately after corn planting and fertilizer application, ORT

emissions were higher any other system at 12, 36, and 60h. In addition, CCT emitted N₂O at a rate 462-525% higher than CNT at 24 and 60h. After the split UAN fertilizer application, N₂O emissions rates were higher in CCT than CNT soil for most sampling intervals after the first 24h, with CLR falling between the other two conventional soils. Initial OCT and OLR emissions rates were also high, with both organic systems rates surpassing those of CNT from 12-36h. In September when soil samples were taken at corn harvest, there were clear differences only among farming systems in the initial 12-24h period. Organic long rotation exceeded all others at these points, while CNT emissions were lower than those of both OLR and ORT at 12h and lower than OLR and OCT at 24h. Contrast statements revealed that organic soil overall emitted higher rates of N₂O than conventional (P=0.009).

There were no consistent differences between emissions rates for the April, May, and June dates. However, for all farming systems except for OLR, soils collected in September emitted N₂O at a rate less than or equal to other dates.

3.4.1.2 Cumulative nitrous oxide emissions from 70% WFPS soil

Regardless of system, total N₂O production was highest when soil was incubated following the June sampling date, with April emissions levels following those in May and September falling between these two early dates. Across dates, more total N₂O was released during incubations from CCT soil than CLR, OCT, or CNT with CCT emitting 271% more N₂O than the next highest system, CLR (Table 3.7). Levels in CCT were similar to those of OLR and ORT.

3.4.1.3 Nitrous oxide emissions rate from 60% WFPS soil

Unlike with N₂O measured from 70% WFPS soil, differences in gas emissions among systems when exposed to 60% WFPS did not depend on the sampling date (Table 3.2).

Furthermore, there were no differences detected among systems after the initial 12-36h flush of N₂O. At 12h, all three organic systems emitted more N₂O per hour than any conventional system's soil. At 24h, however, only OLR emissions were higher than all conventional soils, and by 36h OLR and ORT emissions exceeded those of CLR and CNT with the two conventional tillage systems having similar rates (Table 3.5). Production of N₂O from these drier soil incubations was higher in organic systems than conventional (contrast P = 0.0008). The time of the year soils were collected for incubation also played a role in determining N₂O emissions. September soils' emissions rates were higher than those of July but typically similar to those of soil collected even earlier in the year.

3.4.1.4 Cumulative nitrous oxide emissions rate from 60% WFPS soil

Regardless of farming system, the highest cumulative N₂O production was observed in May, when levels were similar to June but 299% larger than September and 137% larger than April. Across the four sampling dates, ORT emissions exceeded those of OCT, CLR, and CNT (Table 3.7).

3.4.1.5 Carbon dioxide emissions rate from 70% WFPS soil

Carbon dioxide production from incubated soil peaked between 12 and 36h for all systems and gradually fell thereafter. Similar with 60% wfps N₂O, any differences among farming systems were consistent across soil sampling dates (Table 3.2). One-way contrasts showed greater emissions from organic than conventional soils overall. OLR and ORT always emitted more CO₂ per hour than CLR and CNT (Table 3.5). The two conventional tillage systems' soils were similar, however, from 0-204h of the incubation. Emissions of CO₂ consistently declined from April to September across rotations and times during the incubation.

3.4.1.6 Cumulative carbon dioxide emissions from 70% WFPS soil

Cumulative CO₂ in the wetter soil incubations fell during the growing season in the order April = May > June > September. In 16 day incubations, April sampled soil emitted 110% more CO₂ from the same systems in June and 242% more than in September. All organic systems' emissions were higher than conventional (contrast P <0.0001), with no differences detected within tillage systems of either organic or conventional (Table 3.6).

3.4.1.7 Carbon dioxide emissions rate from 60% WFPS soil

Carbon dioxide was emitted at different rates from soil collected on different sampling dates, as well as from the various farming systems on each date (Table 3.2). At 12, 36, and 60h incubation sampling points of pre-corn-planting collected soil, CO₂ was produced at a higher rate from ORT soil than all conventional soils, while OCT soil was similar to conventional ones before 108h (Table 3.4). Emissions rates from conventional long rotation soil fell below those of CCT at 12, 60, and 108h. In May, after planting and the first fertilizer or only manure application, ORT once again produced the most N₂O per hour than any other system's soil except OLR until 204h. Organic conventional till once again had emissions rates similar to CCT for all hours sampled until 396h. One-way contrast statements showed organic soil to have higher overall CO₂ emissions than conventional in May.

June CO₂ emission rates from incubated soil were unusual among the four sampling dates in that ORT soils fell between the two other organic systems' soils and conventional soil for all hours that gas was sampled. Organic conventional till still fell below OLR at several time points (12, 36h) but had emissions greater than CCT at 12 and 24h. In common with CO₂ emissions from 70% WFPS soil incubations, the rate of gas production fell from each sampling date to the next.

In September, after harvest, CO₂ emissions only differed among the treatments during the initial 60h. Organic reduced till rates surpassed those of all conventional systems during that time, while OCT and CCT emissions weren't different at 12, 36 and 60h.

3.4.1.8 Cumulative carbon dioxide emissions rate from 60% WFPS soil

Averaged across systems, carbon dioxide emissions over the course of the incubation in 60% WFPS soil declined every sampling date from a high of 10.9 in April to a low of 3.13 mg CO₂-C kg⁻¹ soil in September. Carbon dioxide-C was the only cumulative gas flux measurement to show the effect of farming system as dependent upon sampling date (system X date interaction P = 0.0012). Both OLR and ORT systems soils produced more CO₂-C all year compared to either reduced-till conventional systems, CLR and CNT (Table 3.8).

Organic long rotation and ORT were similar to one another except for OLR exceeding ORT by 62% in June, while no differences emerged between CLR and CNT all year. Organic conventional tillage soil emitted more CO₂-C relative to CCT in May and June, but the two were similar in April and September. Conventional long rotation produced less CO₂-C than any system except for CNT in April and May, while all three conventional systems' CO₂-C production were surpassed by the organic systems in June and September.

3.4.1.9 Microbial biomass C and Microbial biomass N

For neither MBC nor MBN were there detectable differences between the six individual farming systems (Table 3.9). However, contrasts grouping the three organic and three conventional systems together showed the organic systems to have higher MBN than the conventional (Figure 3.9), and both MBC and MBN changed during the year. Microbial biomass C in May was 23-34% higher than in soils collected in April, June, or September, which were similar to one another. Microbial biomass N levels were lower after May, in the

order April = May > June = September. The ratio of MBC:MBN was not significantly different among either farming systems or dates.

3.4.1.10 K₂SO₄ –extractable C

Farming systems differed in terms of K₂SO₄ – C only in May and June (Table 3.9). In May, ORT levels of this extractable C pool rose above those of any conventional system, but by June ORT had decreased to a level similar to the three conventional systems and less than either OLR or OCT (Figure 3.7). Conventional no tillage soil contained less K₂SO₄ – C than either OLR or OCT in both May and June. There was no difference detected among the four sampling dates consistent across systems.

3.4.1.11 Extractable inorganic N (NH₄⁺+NO₃⁻N)

Plots fertilized with a synthetic N fertilizer showed that soil N differed among sampling dates in the order > June>May>Sept>April across the three conventional farming systems. On the other hand, for organic systems plots, the only consistent temporal trends in soil N was that May extractable inorganic N was higher than any other date. Ammonium and nitrate N differed among treatments (Table 3.9), but only in the latter part of the year. In the case of June, CCT mineral N was 44.6% (48.6) higher than CLR (CNT), both of which further exceeded all organic systems' concentrations (Figure 3.5). In September, both CCT and OLR had soil mineral N greater than all of the other systems.

3.4.1.12 Potentially mineralizable N in 60% WFPS soil

The effect of farming system on potentially mineralizable N (PMN) depended on the sampling period soil was collected (Table 3.9). In April and June, PMN was similar among all systems, but in May each organic system's soil contained more PMN than any conventional systems (Figure 3.6). Also, in September ORT, OCT, and OLR exceeded CCT

and CNT PMN, with CLR being similar to all other systems but ORT. Organic soils overall had higher PMN than conventional according to a one-way contrast ($P < 0.0001$).

3.4.1.13 Net N flux in 70% WFPS soil

In soil used for incubations to determine N_2O emissions potential, there appeared to be a significant loss of mineral N through immobilization and nitrification and/ or denitrification from some jars, with values ranging from -30.1 to 54.0 mg N kg^{-1} soil over 4 weeks of incubation. No differences were observed between farming systems (Table 3.9), but the flux for each date did depend upon the farming systems (System X Date $P = 0.0348$). Only in CCT, June N flux was markedly lower than other dates, at -22.9 mg N kg^{-1} soil compared to 7.23 mg N kg^{-1} soil in May.

3.4.2 *Gas emissions and soil C and N dynamics of soil amended with synthetic or organic N*

3.4.2.1 Nitrous oxide emissions rate from amended 70% WFPS soil

Over the course of the incubations, cumulative N_2O emitted was in proportion to about 1.4-3.8% of added UN (Urea Nitrogen) and 0.08-1.1% of added LN (Litter Nitrogen). Conventional fertility with conventional tillage had a 777% higher emissions rate relatively late during the incubation at 204 hours in the non-amended soil, compared to the other systems. Additionally, there were differences in the instantaneous rate of N_2O emissions from soils receiving either amendment at different times throughout the incubation. In the urea-amended soil, OLR emissions rates were 212-265% of those in conventional and 314% of ORT but similar to OCT at 108h. Most strikingly, CCT emission rate in litter-amended soil were higher than other systems at 24h and higher than all systems but OLR at 36h.

Conventional no-till soils amended with litter also produced N₂O at a higher rate relative to CLR at 24h, while OLR rates exceeded ORT, OCT, and CLR at 36h.

3.4.2.2 CO₂ emissions rate from 60% and 70% WFPS soil

Cumulative CO₂ emissions in 60% WFPS soil were no different from one another among the different systems. However, at 70% WFPS, CNT and CLR produced greater CO₂ than either ORT or OCT. Organic systems' soil overall emitted more total CO₂ from 70% WFPS incubations than conventional 70% WFPS soils (organic vs. conventional P = 0.0076).

3.4.2.3 Potentially mineralizable N in 60% WFPS soil and net N flux in 70% soil

Potentially mineralizable N measured in the drier soil incubations was greater in CLR soil than any other system, and 198% higher than CNT, the next highest system. No differences were detected among farming systems' N flux in either LN, UN, or control 70% WFPS soil.

3.5 Discussion

3.5.1 *Effect of farming system and field N inputs on gas emissions and soil C and N dynamics*

This study detected differences in the response of soil N₂O emissions to either mineral or organic N inputs, as well as to field management such as fertilizer application, cover crop selection, and cultivation. At some soil sampling dates and gas sampling intervals within incubations, combinations of UAN with tillage or reduced tillage with litter produced the highest emission rates. On other dates and or intervals, there were no differences.

Among conventionally fertilized systems, the CCT emissions rate rose above that of CNT for several time points of the incubations from May and June soil samplings. These soil sampling dates occurred immediately after one of two split applications of UAN. The CCT

emissions rate was equal to CNT in April before fertilizer application, however. Differences between the two systems were likely driven in part by two soil parameters: elevated $\text{NH}_4^+ + \text{NO}_3^-$ -N and K_2SO_4 -C. Soil mineral N in CCT was nearly double that of CNT in both June and September, while extractable C was also higher in June in CCT than CNT. Both elevated mineral N and K_2SO_4 -C likely combined to encourage denitrification. Nitrate N levels have long been known to determine N_2O emissions under the conditions that C limitations are alleviated, such as by plant residues (R. Bowden et al., 1991; Nugroho and Kuwatsuka, 1992) or worm casts (Elliott et al., 1991). The loss was so great that 76% of the mineral N available in June was lost during 28d incubations, presumably as N_2 and N_2O . This loss exceeds by 18% the total 182.28 kg UAN-N applied in two applications in the field. It is important to note that this is a measure of potential loss, under optimum conditions for denitrification of soil N. Furthermore, CCT cumulative N_2O emission was greater than either the other conventional systems or OCT. This suggests that CCT emissions are likely greater due to not only tilling in residues but also synthetic fertilizers.

Labile C availability in CCT as indicated by elevated extractable C also likely encouraged high N_2O emissions in CCT. This may have been due to tilled in cover crop biomass in this system. It has been shown that conditions of low soluble C can limit N_2O production (Loro et al., 1997). Despite receiving the same N and cover crop inputs and supporting a similar N sink in corn biomass and grain as the other conventional systems (Table 3.10), the tilled system saw increased availability of C and N. This likely explains the enhanced N_2O emissions potential in this system.

Conventional with tillage and CNT differ principally in the cultivation that occurs in CCT to prepare for planting and control weeds. Tillage is a disruptive process that can

fundamentally alter C content and nutrient availability within the soil. It is known to decrease CEC (Kitur et al., 1994) and increasing mineral N levels (Gomez-Rey et al., 2012; Sainju et al., 2012) while also shifting N proportionally away from organic pools (Sainju et al., 2012). This occurs in part because soil disturbance speeds the loss of recent C additions both in favor of mineralization (Bossuyt et al., 2002) and release of K_2SO_4 extractable C (Mueller et al., 2012) Although over the long term tillage is expected to decrease levels of water soluble C (Neogi et al., 2014), CCT soil yielded more extractable C when sampled after tillage events, possibly contributing to greater cumulative CO_2 emissions from 70% WFPS soil (Table 3.7). This could be explained by the CCT system's soils receiving additional C in the form of aboveground biomass tilled into the soil, which stimulates N_2O emissions by enhancing extractable C (Jahangir et al., 2014). Both CCT and OCT systems would have received tilled in cover crop residues, but low MBN and different cover crop biomass productivity may have caused higher N_2O emissions in CCT. These findings offer some support to our hypothesis that greater mineralizable C levels may stimulate N_2O -N losses. However, the difference in mineralizable C is not as evident in 60% WFPS soil, and K_2SO_4 – C may be a more reliable indicator of C availability to denitrifiers. Both the incorporation of cover crop residues (Haque et al., 2014) and soil disturbance itself has previously been linked to elevated N_2O emissions (Liu and Qiu, 2016). What is most striking is that these differences in N_2O and CO_2 -C emissions persist even several weeks after the cultivation event, and that soil disturbance via sieving does not erase differences between tilled and non-tilled soil.

On the other hand, there is evidence that less frequent tillage in an organic system increased N_2O emissions. Reduced till organic soils usually had higher N_2O emissions than

the two tilled organic systems according to rate measurements in May. This is in addition to the fact that organic tilled plots were disked or cultivated a total of four times during the season compared to two times in CCT. Furthermore, a higher calculated cumulative N_2O flux was observed from CCT 60% WFPS soil than CLR, CNT, and OCT. The organic reduced till system in this study is dramatically different from the no-till conventional setting in that a much larger cover crop biomass is present on the soil surface, and also that UAN is sprayed onto the soil in CNT whereas poultry litter is applied to the soil surface and remains there in ORT. Poultry litter is both a source of crop N and a much more substantial source of C for soil microbes, and manure application is known to drive N_2O emissions (Liu and Qiu, 2016). While a recent meta-analysis showed that surface application by itself increases gaseous N loss as NH_3 (Huang et al., 2016), injecting manure decrease N_2O production (Hou et al., 2015). The emissions rate in organic reduced till only surpassed all other systems in May, immediately after litter application and cover crop rolling. This suggests that when both litter and rolled legume cover crop aboveground biomass N are concentrated to a narrow layer at the soil surface, large flushes of N_2O are possible. It is unlikely that the surface cover crop biomass mulch alone as opposed to residue incorporation would've created this spike (Nett et al., 2015). Potential soil N mineralization was similar among organic systems in May, and the relatively undecomposed rye-legume cover crop mulch likely could not have accounted for the large mineral N pool observed in ORT after termination and litter application (Ibewiro et al., 2000). Although stratification of organic matter has led to an increase in N_2O emissions in certain situations (Ball et al., 2008), denitrification makes up a larger proportion of N_2O following cover crop incorporation (Li et al., 2016), meaning nitrification of N from cover crop biomass was an unlikely N_2O source. These results show that a concentrated layer

of broadcast litter can increase N losses as N_2O . Since it is already known that runoff losses of N (Kibet et al., 2016) NH_3 emissions (Huang et al., 2016; Mannheim et al., 1995) increase with surface application, this work further supports the notion that injection practices should be developed to avoid N losses.

Despite the role of surface application of litter in high N_2O emissions in May, OLR emissions were higher than OCT in April and again in September. These correspond to dates before and several months after litter application, respectively. Despite the cover crop's relatively large aboveground biomass and lack of incorporation, ORT had higher calculated cumulative mineralizable C than OCT in April and May. In contrast with a previous study (Angers et al., 2006), we found tilling in manure to actually increase K_2SO_4 -C relative to ORT.

Mineralizable N was higher in ORT than all conventional soils in May and September, in addition to being higher than OCT in September. While grass-legume cover crop residues are slower to release mineral N to the soil than synthetic fertilizer, organic systems are subject to mineralization of increased organic inputs applied over multiple years. Furthermore, the grass legume mixture biomass itself in ORT likely experienced less net N and C mineralization as a function of total biomass due to surface placement (Curtin et al., 2008) and higher C:N ratios (Lynch et al., 2016; O'Connell et al., 2015b). Mineralizable C declined from April to May or June in the three conventional soils, but large residue inputs from cover crops and then manure resulted in similar cumulative CO_2 -C emissions among the first 2-3 months for organic systems.

Additionally, there are fundamental differences between the organic and conventional soils that may contribute to how similar systems within each group may respond differently

to incoming N and C. In support of our hypothesis, larger MBN overall in organic systems regardless of tillage suggests that these soils have more of an ability to immobilize N, tying up potential substrate in microbial biomass that would otherwise be available for denitrification. Indeed, for the two sampling dates after application of poultry litter, there were no differences observed among organic systems in extractable inorganic N ($\text{NH}_4^+ + \text{NO}_3^-$ -N).

However, high MBN did not always occur with low N_2O emissions. In September for example, the high MBN OLR system also had greater $\text{NH}_4^+ + \text{NO}_3^-$ -N than any system except CCT. This in turn likely resulted in its greater N_2O emissions at that point. At this time of presumably attenuated crop N demand, $\text{NH}_4^+ + \text{NO}_3^-$ -N concentrations were still similar to those in June. Additionally, N mineralization potential in OLR was higher than either CCT or CNT in September, suggesting that the soil may be able to mineralize considerable N even after harvest. These findings suggest asynchrony between plant N demand and mineralization from soil organic matter and organic inputs. This would be an ideal situation to interseed a cover crop or deploy a catch crop to immobilize N (Baributsa et al., 2008; Rasse et al., 2000). Nevertheless, it is difficult to perfectly time organic input N mineralization due to variability in factors such as temperature (Quemada and Cabrera, 1997; Cook et al., 2010), meaning that the excess N observed may not remain in wetter or warmer years. Furthermore, despite the enhanced ability of the three organic systems to mineralize N, organic rotations have been shown to accrue total N over time when conventional experience net loss (Sommer et al., 2015), suggesting that despite N_2O -N losses they are relatively efficient at cycling N.

3.5.2 Gas emissions and soil C and N dynamics of soil amended with synthetic or organic N

Although no differences were observed between farming systems in terms of MBC, MBN, MBC:MBN ratio, K_2SO_4 -C, or inorganic N in June 2014-collected soil, there were differences between systems in terms of gas emissions depending on fertilizer N source (Table 3.10). In CLR, cumulative calculated N_2O from litter amended soil was lower than in any other system, while cumulative N_2O was similar among systems in both the control and UAN amended soil. Low emissions in CLR amended with litter may be a result of a combination of low cover crop productivity and low mineralizable C limiting denitrification of litter N. The above ground cover crop biomass produced in CLR would have been relatively unavailable to decomposer microorganisms due to limited surface area in contact with soil (Kuang et al., 2014), although it is unclear why this would not have prevented high N_2O emissions from the CNT soil as well. While rye residues can cause temporary immobilization of N (Sarkodie-Addo et al., 2003), CLR soil actually experienced the highest net N mineralization of any soil, indicating that reduced emissions were likely not attributable to lack of N.

3.6 Conclusions

Nitrogen inputs can directly influence soil mineral N concentrations, thereby indirectly controlling N_2O emissions shortly after fertilization. Tillage plays a key role in determining C available to denitrifiers by increasing K_2SO_4 extractable C in the week following cultivation. These two elements together made CCT soils highly susceptible to potential N loss as N_2O . On the other hand, reducing tillage in organic systems receiving considerable C inputs and cover crop biomass can also result in elevated $NO_3^-+NH_4^+-N$

shortly after manure additions. Asynchrony between crop N demand and soil N mineralization could be responsible for potentially high N₂O emissions from OLR soil after corn harvest. In laboratory incubations, CLR soil produced less N₂O than other systems when fertilized with poultry litter, possibly due to a lack of mineralizable C. This study provides an argument for avoiding tillage in systems receiving synthetic N, especially soon after N application, and for developing methods of delivering manure belowground on organic reduced tillage farms. In addition, this work suggests that incorporated cover crop residues can serve as a C source for denitrifiers in systems also receiving synthetic N application.

Acknowledgements

This work was supported by USDA's National Institute of Food and Agriculture [Grant 2012-02978-230561]. Thanks to Cherry Research Farm staff for field maintenance.

3.7 Tables and figures

3.7.1 Tables

Table 3.1 Field operations and sampling dates

| Date | Activity | Plots |
|------------|---|-----------------------------|
| 6/4/2014 | Soil sampling for incubations with N amendments | All |
| 11/4/2014 | Cover crops planted | OCT ¹ , OLR, ORT |
| 12/18/2014 | Wheat cover crop planted | CCT, CLR, CNT |
| 4/1/2015 | Soil and cover crop biomass sampling | All |
| 4/6/2015 | Cover crop termination by disking | CCT, OCT, OLR |
| 4/23/2015 | Second disking | CCT, OCT, OLR |
| 4/23/2015 | Application of poultry litter | OCT, OLR, ORT |
| 4/23/2015 | Biomass sampling and termination of cover crops | ORT |
| 4/24/2015 | Cover crop termination CNT and CLR | CNT, CLR |
| 4/24/2015 | Corn planting | All |
| 4/27/2015 | UAN applied | CCT, CLR, CNT |
| 5/4/2015 | Soil sampling | All |
| 5/8/2015 | Cultivation | OCT, OLR |
| 5/18/2015 | Second rolling of cover crops between rows | ORT |
| 5/22/2015 | Cultivation | OCT, OLR |
| 6/4/2015 | Split UAN applied | CCT, CLR, CNT |
| 8/17/2015 | Corn biomass and yield sampling | All |
| 9/8/2015 | Soil sampling | All |

¹CCT: Conventional Conventional Tillage; CLR: Conventional Long Rotation; CNT: Conventional No Tillage; OCT: Organic Conventional Tillage; OLR: Organic Long Rotation; ORT: Organic Reduced Tillage

Table 3.2 Summary of laboratory incubation gas emissions rate ANOVA results. day⁻¹

| Effect | 70% WFPS | | 60% WFPS | |
|------------|---------------------|-----------------|------------------|-----------------|
| | N ₂ O | CO ₂ | N ₂ O | CO ₂ |
| System (S) | ¹ 0.0018 | <0.001 | 0.0104 | <0.001 |
| Date (D) | <0.001 | <0.001 | <0.001 | <0.001 |
| S*D | 0.3414 | 0.1268 | 0.0978 | 0.0303 |
| Hour (H) | <0.001 | <0.001 | <0.001 | <0.001 |
| H*S | <0.001 | 0.0186 | <0.001 | <0.001 |
| H*D | <0.001 | <0.001 | <0.001 | <0.001 |
| H*S*D | <0.001 | 0.1449 | 0.0688 | 0.0270 |

¹Rate of gas production P > F. N₂O: mg N₂O kg⁻¹ soil day⁻¹; CO₂: mg CO₂-C kg⁻¹ soil day⁻¹

Table 3.3 2015 field soil incubations N₂O emissions, mg N₂O kg⁻¹ soil day⁻¹

| Hrs. | Sys | April | | | May | | | June | | | September | | | | | | |
|------|------------------|-------|---|-----------|------|-------|-----------|-------|-----|-----------|-----------|-------|-----------|-------|---|-------|----|
| | | Mean | ± | Std. Err. | Mean | ± | Std. Err. | Mean | ± | Std. Err. | Mean | ± | Std. Err. | | | | |
| 12 | CCT ¹ | 0.611 | ± | 0.259 | B | 0.342 | ± | 0.189 | B | 0.102 | ± | 0.034 | BC | 0.018 | ± | 0.017 | C |
| | CLR | 0.345 | ± | 0.251 | B | 0.042 | ± | 0.013 | C | 0.022 | ± | 0.019 | C | 0.046 | ± | 0.032 | C |
| | CNT | 0.302 | ± | 0.191 | B | 0.068 | ± | 0.030 | BC | 0.019 | ± | 0.006 | C | 0.013 | ± | 0.012 | C |
| | OCT | 0.541 | ± | 0.250 | B | 0.323 | ± | 0.102 | BC | 1.471 | ± | 1.090 | AB | 0.233 | ± | 0.173 | BC |
| | OLR | 1.518 | ± | 0.525 | A | 0.302 | ± | 0.064 | BC | 4.416 | ± | 0.333 | A | 1.879 | ± | 1.001 | A |
| | ORT | 1.444 | ± | 0.847 | A | 1.650 | ± | 0.457 | A | 0.742 | ± | 0.546 | BC | 0.498 | ± | 0.414 | B |
| 24 | CCT | 0.608 | ± | 0.116 | AB | 0.610 | ± | 0.321 | A | 0.166 | ± | 0.031 | BC | 0.069 | ± | 0.085 | BC |
| | CLR | 0.146 | ± | 0.096 | C | 0.117 | ± | 0.070 | BC | 0.488 | ± | 0.342 | ABC | 0.152 | ± | 0.103 | BC |
| | CNT | 0.204 | ± | 0.141 | BC | 0.098 | ± | 0.038 | C | 0.029 | ± | 0.028 | C | 0.011 | ± | 0.018 | C |
| | OCT | 0.122 | ± | 0.054 | C | 0.450 | ± | 0.188 | ABC | 2.001 | ± | 1.884 | A | 0.443 | ± | 0.321 | B |
| | OLR | 0.932 | ± | 0.722 | A | 0.799 | ± | 0.266 | A | 2.653 | ± | 2.007 | A | 1.287 | ± | 0.500 | A |
| | ORT | 0.307 | ± | 0.124 | BC | 0.499 | ± | 0.274 | AB | 1.688 | ± | 1.740 | AB | 0.260 | ± | 0.262 | BC |
| 36 | CCT | 0.251 | ± | 0.215 | | 0.387 | ± | 0.422 | B | 1.495 | ± | 0.773 | A | 0.120 | ± | 0.142 | |
| | CLR | 0.130 | ± | 0.067 | | 0.129 | ± | 0.162 | B | 0.464 | ± | 0.348 | AB | 0.231 | ± | 0.157 | |
| | CNT | 0.169 | ± | 0.139 | NS | 0.239 | ± | 0.234 | B | 0.023 | ± | 0.014 | B | 0.025 | ± | 0.011 | NS |
| | OCT | 0.055 | ± | 0.023 | | 0.404 | ± | 0.215 | B | 1.903 | ± | 2.134 | A | 0.249 | ± | 0.180 | |
| | OLR | 0.248 | ± | 0.227 | | 0.530 | ± | 0.047 | B | 2.482 | ± | 0.776 | A | 0.770 | ± | 0.267 | |
| | ORT | 0.169 | ± | 0.113 | | 1.876 | ± | 0.422 | A | 1.860 | ± | 1.117 | A | 0.271 | ± | 0.178 | |

continued on following page

Table 3.3 (continued)

| Hrs. | Sys | April | | May | | June | | September | |
|------|------------------|---------------|-----------|---------------|-----------|---------------|-----------|---------------|-----------|
| | | Mean | Std. Err. | Mean | Std. Err. | Mean | Std. Err. | Mean | Std. Err. |
| 60 | CCT ¹ | 0.030 ± 0.018 | | 0.523 ± 0.259 | B | 3.863 ± 2.685 | A | 0.334 ± 0.618 | |
| | CLR | 0.020 ± 0.007 | | 0.169 ± 0.098 | BC | 0.224 ± 0.167 | BC | 0.228 ± 0.194 | |
| | CNT | 0.052 ± 0.008 | NS | 0.093 ± 0.030 | C | 0.020 ± 0.006 | C | 0.020 ± 0.012 | NS |
| | OCT | 0.024 ± 0.010 | | 0.441 ± 0.427 | BC | 1.047 ± 0.835 | ABC | 0.320 ± 0.205 | |
| | OLR | 0.062 ± 0.020 | | 0.353 ± 0.051 | BC | 1.678 ± 0.552 | AB | 0.470 ± 0.266 | |
| | ORT | 0.065 ± 0.016 | | 1.287 ± 0.293 | A | 1.672 ± 0.767 | AB | 0.110 ± 0.047 | |
| 108 | CCT | 0.017 ± 0.003 | | 0.478 ± 0.231 | | 3.368 ± 1.883 | A | 0.195 ± 0.400 | |
| | CLR | 0.014 ± 0.006 | | 0.086 ± 0.078 | | 0.126 ± 0.238 | B | 0.120 ± 0.100 | |
| | CNT | 0.025 ± 0.006 | NS | 0.077 ± 0.048 | NS | 0.079 ± 0.040 | B | 0.013 ± 0.005 | NS |
| | OCT | 0.007 ± 0.005 | | 0.221 ± 0.201 | | 0.064 ± 0.041 | B | 0.012 ± 0.005 | |
| | OLR | 0.043 ± 0.006 | | 0.221 ± 0.042 | | 0.446 ± 0.172 | B | 0.035 ± 0.004 | |
| | ORT | 0.023 ± 0.004 | | 0.365 ± 0.201 | | 0.527 ± 0.747 | B | 0.033 ± 0.016 | |
| 204 | CCT | 0.012 ± 0.008 | | 0.151 ± 0.063 | | 1.980 ± 1.149 | | 0.257 ± 0.619 | |
| | CLR | 0.005 ± 0.004 | | 0.033 ± 0.029 | | 0.836 ± 0.612 | | 0.020 ± 0.018 | |
| | CNT | 0.017 ± 0.015 | NS | 0.029 ± 0.028 | NS | 0.145 ± 0.104 | NS | 0.018 ± 0.012 | NS |
| | OCT | 0.012 ± 0.010 | | 0.084 ± 0.089 | | 0.017 ± 0.008 | | 0.004 ± 0.002 | |
| | OLR | 0.015 ± 0.004 | | 0.111 ± 0.027 | | 0.051 ± 0.035 | | 0.015 ± 0.008 | |
| | ORT | 0.013 ± 0.002 | | 0.096 ± 0.072 | | 0.081 ± 0.062 | | 0.003 ± 0.001 | |
| 396 | CCT | 0.008 ± 0.008 | | 0.221 ± 0.162 | | 3.378 ± 1.488 | A | 0.036 ± 0.086 | |
| | CLR | 0.005 ± 0.005 | | 0.029 ± 0.029 | | 1.310 ± 1.011 | AB | 0.002 ± 0.001 | |
| | CNT | 0.010 ± 0.010 | NS | 0.050 ± 0.075 | NS | 0.527 ± 0.455 | B | 0.043 ± 0.024 | NS |
| | OCT | 0.010 ± 0.006 | | 0.200 ± 0.168 | | 0.076 ± 0.077 | B | 0.004 ± 0.003 | |
| | OLR | 0.011 ± 0.006 | | 0.381 ± 0.203 | | 0.052 ± 0.046 | B | 0.005 ± 0.003 | |
| | ORT | 0.010 ± 0.005 | | 0.252 ± 0.087 | | 0.063 ± 0.044 | B | 0.008 ± 0.002 | |

¹ CCT: Conventional Conventional Tillage; CLR: Conventional Long Rotation; CNT Conventional No Tillage; OCT: Organic Conventional Tillage; OLR: Organic Long Rotation; ORT: Organic Reduced Tillage. Means within a column for each date and hour are deemed significantly different if not followed by a shared letter according to Fisher's protected LSD ($p < 0.05$)

Table 3.4 2015 field soil incubations CO₂ emissions, mg CO₂-C kg⁻¹ soil day⁻¹ from 60% WFPS soil.

| Date | System | Hours of incubation | | | | | | | |
|-----------|--------|---------------------|----|-----|-----|-----|-----|-----|--|
| | | 12 | 24 | 36 | 60 | 108 | 204 | 396 | |
| April | CCT | BC | A | BCD | B | A | NS | A | |
| | CLR | D | AB | D | C | B | NS | A | |
| | CNT | CD | B | CD | C | B | NS | A | |
| | OCT | BC | A | BC | AB | A | NS | A | |
| | OLR | AB | A | AB | AB | A | NS | A | |
| | ORT | A | A | A | A | A | NS | A | |
| May | CCT | C | BC | B | CD | C | BC | BC | |
| | CLR | C | C | C | D | C | C | C | |
| | CNT | C | C | BC | D | C | C | C | |
| | OCT | B | BC | B | BC | BC | AB | A | |
| | OLR | B | AB | B | AB | AB | AB | A | |
| | ORT | A | A | A | A | A | A | AB | |
| June | CCT | C | BC | BC | BC | BC | BC | B | |
| | CLR | D | D | C | C | C | C | B | |
| | CNT | CD | CD | C | C | C | C | B | |
| | OCT | B | A | B | AB | AB | AB | A | |
| | OLR | A | AB | A | A | A | A | A | |
| | ORT | C | AB | B | AB | ABC | ABC | B | |
| September | CCT | BC | B | BC | BC | NS | NS | NS | |
| | CLR | BC | B | C | BC | NS | NS | NS | |
| | CNT | C | B | BC | C | NS | NS | NS | |
| | OCT | AB | A | AB | ABC | NS | NS | NS | |
| | OLR | A | A | A | AB | NS | NS | NS | |
| | ORT | A | A | A | A | NS | NS | NS | |

CCT: Conventional Conventional Tillage; CLR: Conventional Long Rotation; CNT Conventional No Tillage; OCT: Organic Conventional Tillage; OLR: Organic Long Rotation; ORT: Organic Reduced Tillage. Means within a column for each date and hour are deemed significantly different if not followed by a shared letter according to Fisher's protected LSD ($p < 0.05$)

Table 3.5 Means of farming systems' mg N₂O kg⁻¹ soil day⁻¹ emitted from soil wetted to 60% WFPS across four sampling dates

| System | Hours of incubation | | | | | | |
|--------|---------------------|----|----|----|-----|-----|-----|
| | 12 | 24 | 36 | 60 | 108 | 204 | 396 |
| CCT | B | BC | AB | NS | NS | NS | NS |
| CLR | B | C | B | NS | NS | NS | NS |
| CNT | B | BC | B | NS | NS | NS | NS |
| OCT | A | AB | AB | NS | NS | NS | NS |
| OLR | A | A | A | NS | NS | NS | NS |
| ORT | A | AB | A | NS | NS | NS | NS |

CCT: Conventional Conventional Tillage; CLR: Conventional Long Rotation; CNT Conventional No Tillage; OCT: Organic Conventional Tillage; OLR: Organic Long Rotation; ORT: Organic Reduced Tillage. Means within a column for each hour deemed significantly different if not followed by a shared letter according to Fisher's protected LSD ($p < 0.05$)

Table 3.6 Means of farming systems' mg CO₂ kg⁻¹ soil day⁻¹ emitted from soil wetted to 70% WFPS across four sampling dates.

| System | Hours of incubation | | | | | | |
|--------|---------------------|----|----|----|-----|-----|-----|
| | 12 | 24 | 36 | 60 | 108 | 204 | 396 |
| CCT | BC | B | CD | BC | C | BC | B |
| CLR | D | C | D | C | C | BC | B |
| CNT | CD | C | D | C | C | C | B |
| OCT | B | AB | BC | AB | BC | AB | A |
| OLR | A | AB | A | A | AB | A | A |
| ORT | A | A | AB | A | A | A | A |

CCT: Conventional Conventional Tillage; CLR: Conventional Long Rotation; CNT Conventional No Tillage; OCT: Organic Conventional Tillage; OLR: Organic Long Rotation; ORT: Organic Reduced Tillage. Means within a column for each hour deemed significantly different if not followed by a shared letter according to Fisher's protected LSD ($p < 0.05$)

Table 3.7 Means of farming systems' cumulative gas emissions over four sampling dates.

| System | 70% WFPS | | 60% WFPS | |
|--------|------------------|-----------------|------------------|-----------------|
| | N ₂ O | CO ₂ | N ₂ O | CO ₂ |
| CCT | 8.334192 A | 6.0567 B | 0.747973 AB | |
| CLR | 2.30645 B | 4.8455 B | 0.37074 BC | |
| CNT | 1.326413 B | 4.5034 B | 0.182701 C | |
| OCT | 2.178281 B | 9.386 A | 0.425197 BC | |
| OLR | 4.081612 AB | 10.9599 A | 1.094554 AB | |
| ORT | 3.644281 AB | 11.059 A | 1.304109 A | |

N₂O: mg N₂O kg⁻¹ over 16 days; CO₂: mg CO₂-C kg⁻¹ over 16 days; CCT: Conventional Conventional Tillage; CLR: Conventional Long Rotation; CNT Conventional No Tillage; OCT: Organic Conventional Tillage; OLR: Organic Long Rotation; ORT: Organic Reduced Tillage. Means within a column with different letters indicate differences among systems within a month according to Fisher's protected LSD (p<0.05).

Table 3.8 Means of farming systems' cumulative mg CO₂-C kg⁻¹ dry soil from 60% WFPS incubations.

| System | Month of Sampling | | | |
|--------|-------------------|-----------|-----------|-----------|
| | April | May | June | September |
| CCT | 12.0715 B | 7.4754 C | 4.7835 BC | 1.5054 BC |
| CLR | 6.8774 C | 4.514 D | 3.4956 C | 1.3257 C |
| CNT | 7.3086 C | 4.7327 CD | 3.3391 C | 1.0064 C |
| OCT | 11.2264 B | 12.0087 B | 10.4945 A | 4.2996 AB |
| OLR | 12.761 AB | 14.102 AB | 11.6745 A | 4.7388 A |
| ORT | 15.1308 A | 15.1441 A | 7.1987 B | 5.8989 A |

CCT: Conventional Conventional Tillage; CLR: Conventional Long Rotation; CNT Conventional No Tillage; OCT: Organic Conventional Tillage; OLR: Organic Long Rotation; ORT: Organic Reduced Tillage. Means within a column with different letters indicate differences among systems within a month according to Fisher's protected LSD (p<0.05). Cumulative CO₂-C calculated over 16 days.

Table 3.9 Summary of soil extraction parameters ANOVA

| | $\text{NH}_4^+ + \text{NO}_3^-$ -N | $^1\text{K}_2\text{SO}_4$ - C | MBN | MBC | MBC: MBN | PMN | N flux - 70% WFPS |
|------------------------|---------------------------------------|----------------------------------|--------|--------|-------------|--------|-------------------------|
| System | ² 0.0024 | 0.035 | 0.1544 | 0.5239 | 0.7001 | 0.0024 | 0.1873 |
| Date | <.0001 | 0.1103 | 0.0003 | <.0001 | 0.1912 | 0.0009 | 0.0388 |
| System*Date | <.0001 | 0.0023 | 0.3675 | 0.0736 | 0.3136 | 0.0439 | 0.0348 |
| Contrasts | | | | | | | |
| Org. v. Con. | 0.0027 | 0.0046 | 0.0132 | 0.2635 | 0.9937 | <.0001 | 0.0888 |
| ¹ CT v. RT. | 0.0175 | 0.1312 | 0.2674 | 0.5509 | 0.6522 | 0.3227 | 0.6215 |

¹ K_2SO_4 – C: C extractable with K_2SO_4 solution; MBN: Microbial biomass N; MBC: Microbial biomass C; PMN: Potentially mineralizable N at 60% WFPS; N flux -70% WFPS: Net change in NO_3^- -N over 4 weeks.

$\text{NH}_4^+ + \text{NO}_3^-$ -N, K_2SO_4 – C, MBN, MBC, PMN, N flux -70% WFPS

Table 3.10 Soil parameter and gas emissions summary of ANOVA results ($P>F$) of lab incubations with added manure and UAN.

| | System | Org. v. Con. | CT v. RT. |
|---|---------|--------------|-----------|
| ¹ UAN N ₂ O | 0.9446 | 0.7883 | 0.7390 |
| Litter N ₂ O | 0.0074 | 0.3357 | 0.7629 |
| 0N Ctrl. N ₂ O | 0.1244 | 0.3838 | 0.1185 |
| NH ₄ ⁺ +NO ₃ ⁻ -N | 0.0501 | 0.2342 | 0.0765 |
| ² K ₂ SO ₄ - C | 0.4403 | 0.1633 | 0.1233 |
| MBN | 0.1293 | 0.7133 | 0.9824 |
| MBC | 0.6479 | 0.3757 | 0.989 |
| MBC:MBN | 0.4129 | 0.1187 | 0.8984 |
| PMN | 0.0134 | 0.0252 | 0.6224 |
| SOC | 0.3213 | 0.0508 | 0.9126 |
| TN | 0.5142 | 0.1688 | 0.8721 |
| Soy biomass | 0.1299 | 0.1620 | 0.0512 |
| Soy yield | 0.3363 | 0.4045 | 0.2803 |
| Cover crop biomass | <0.0001 | <0.0001 | 0.0006 |
| Corn biomass 2013 | 0.0284 | 0.1458 | 0.0165 |

¹UAN, litter, and 0N Ctrl N₂O: Calculated cumulative N₂O emissions over 16d. with UAN, poultry litter, or no N added.
²K₂SO₄ – C: C extractable with K₂SO₄ solution; MBN: Microbial biomass N; MBC: Microbial biomass C; PMN: Potentially mineralizable N at 60% WFPS; N flux -70% WFPS: Net change in NO₃⁻-N over 4 weeks.
 NH₄⁺+NO₃⁻-N, K₂SO₄ – C, MBN, MBC, PMN, N flux -70% WFPS

Table 3.11 Means of farming systems' biomass inputs and total soil organic C and total nitrogen.

| System | ¹ Cover crop biomass | | Soil % C | Soil % N | Corn biomass | Corn yield | 2014 Soy biomass | 2014 soy yield | |
|--------|---------------------------------|---|----------|----------|--------------|------------|------------------|----------------|------|
| CCT | 0.7933 | B | 0.993 | 0.0733 | 10.4 | A | 79.4 | 9.62 | 47.9 |
| CLR | 0.4737 | B | 0.913 | 0.0700 | 12.9 | A | 94.3 | 9.13 | 61.9 |
| CNT | 0.6217 | B | 1.037 | 0.0733 | 9.90 | A | 84.3 | 7.19 | 33.8 |
| OCT | 2.6743 | A | 1.15 | 0.0767 | 11.8 | A | 74.1 | 7.52 | 36.6 |
| OLR | 2.958 | A | 1.30 | 0.0967 | 13.0 | A | 71.3 | 8.63 | 54.5 |
| ORT | 3.265 | A | 1.133 | 0.0800 | 1.26 | B | 20.0 | 6.71 | 29.1 |

CCT: Conventional Conventional Tillage; CLR: Conventional Long Rotation; CNT Conventional No Tillage; OCT: Organic Conventional Tillage; OLR: Organic Long Rotation; ORT: Organic Reduced Tillage.

¹Cover crop biomass: Dry matter t ha⁻¹; Maize Biomass: t ha⁻¹; Maize yield: Bu. Ac.⁻¹; Soy Biomass: t ha⁻¹; Soy Yield: Bu. Ac.⁻¹

¹. Means within a column followed by a different letter indicate significant differences between farming systems according to Fisher's protected LSD (P < 0.05)

3.7.2 Figures

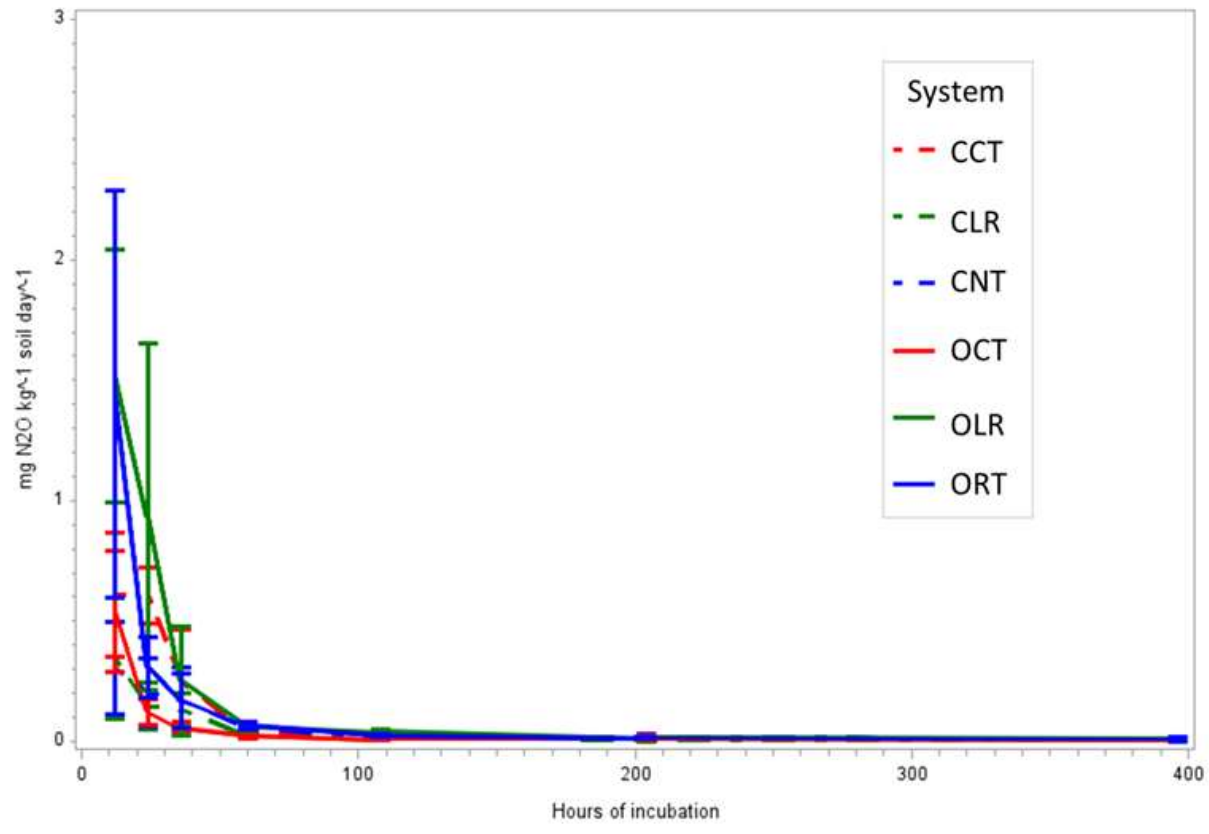


Figure 3.1 N₂O emissions by farming system over time during lab incubations of soil collected in April, before cover crop termination, fertilizer application, and tillage.

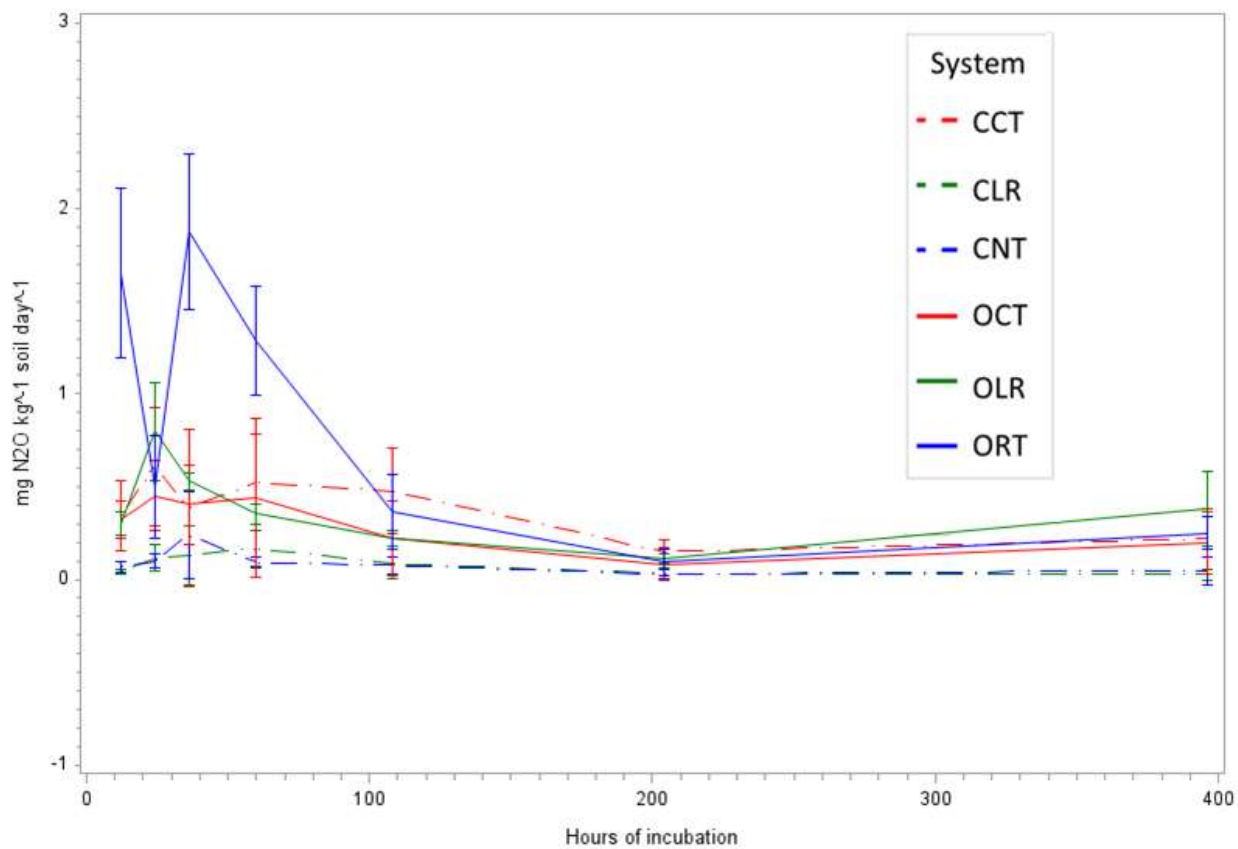


Figure 3.2 N₂O emissions by farming system over time during lab incubations in May, after cover crop termination, fertilizer application, and tillage (OCT, CCT, OLR).

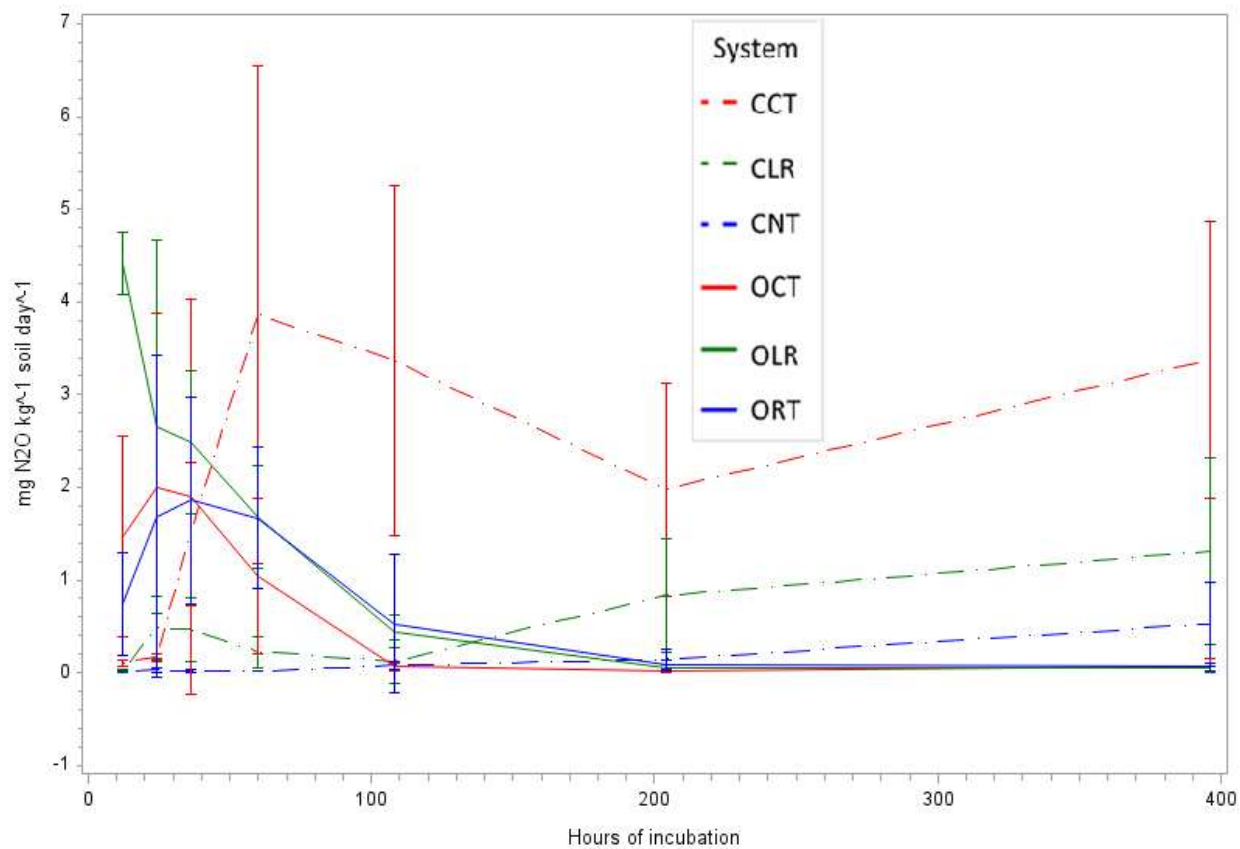


Figure 3.3 N₂O emissions by farming system over time during lab incubations of soil collected in June, after split UAN application (CCT, CLR, CNT).

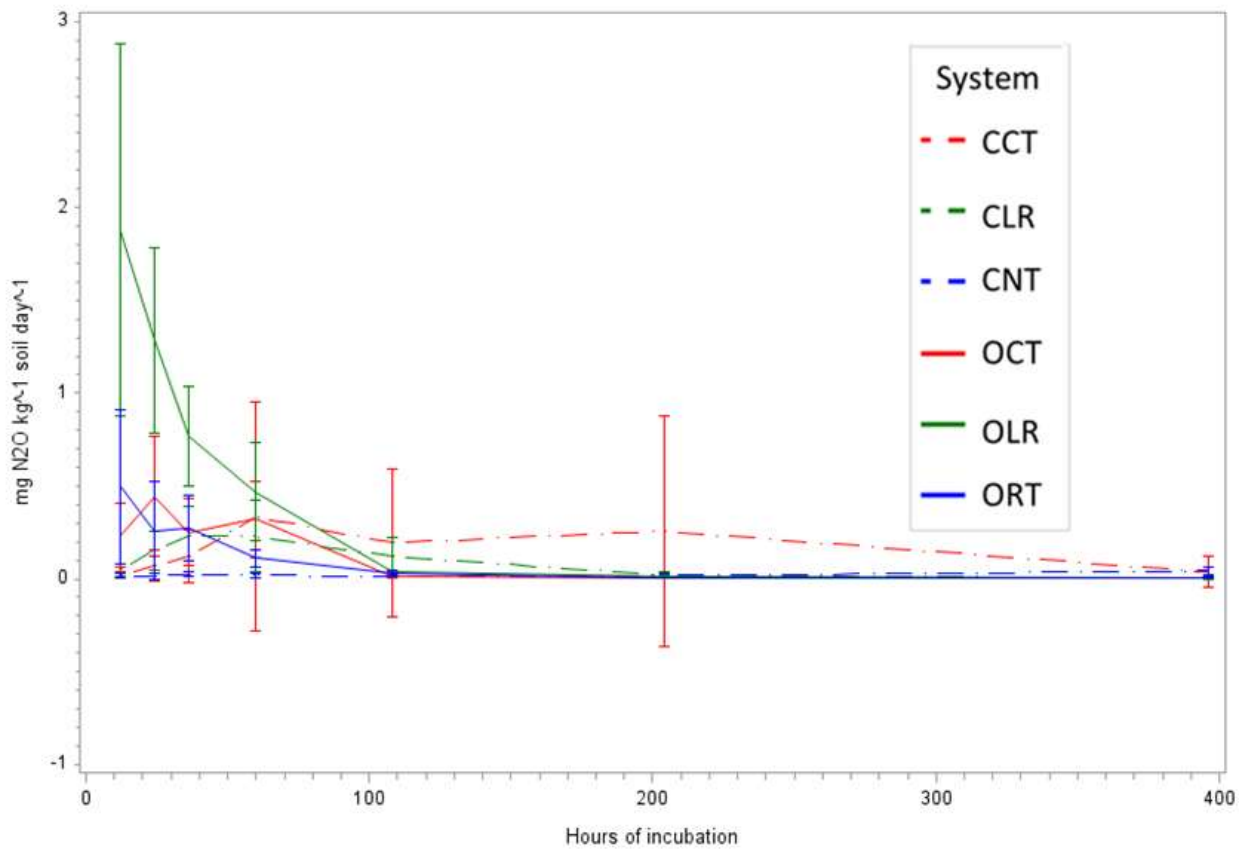


Figure 3.4 N₂O emissions by farming system over time during lab incubations of soil collected in September, following harvest.

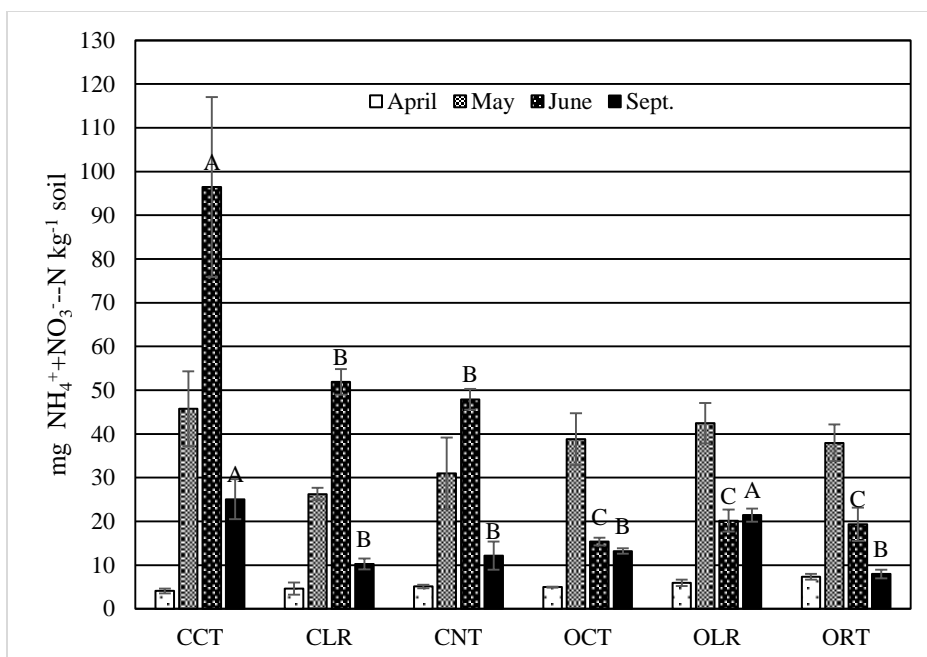


Figure 3.5 Effect of farming system on extractable N. Farming systems sharing the same letter are not different for a given date according to Fisher's protected LSD ($p < 0.05$).

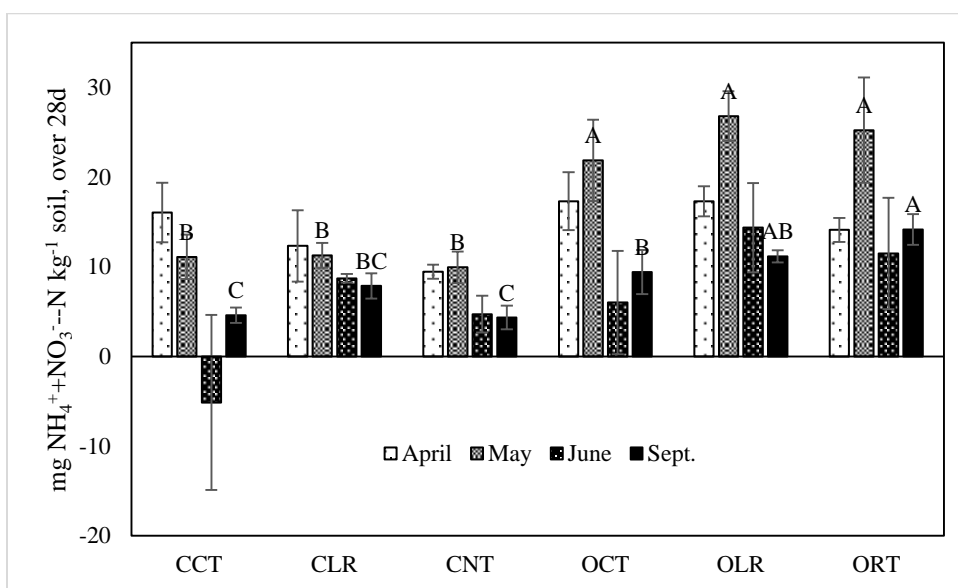


Figure 3.6 Effect of farming systems on potentially mineralizable N. Farming systems sharing the same letter are not different for a given date according to Fisher's protected LSD ($p < 0.05$).

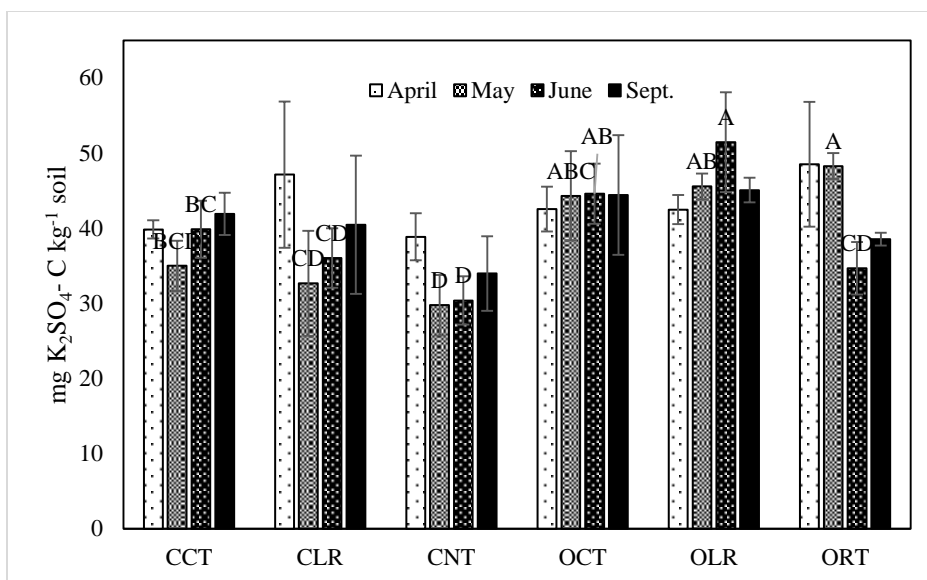


Figure 3.7 Effect of farming systems on K₂SO₄—extractable C. Farming systems sharing the same letter are not different for a given date according to Fisher's protected LSD ($p < 0.05$).

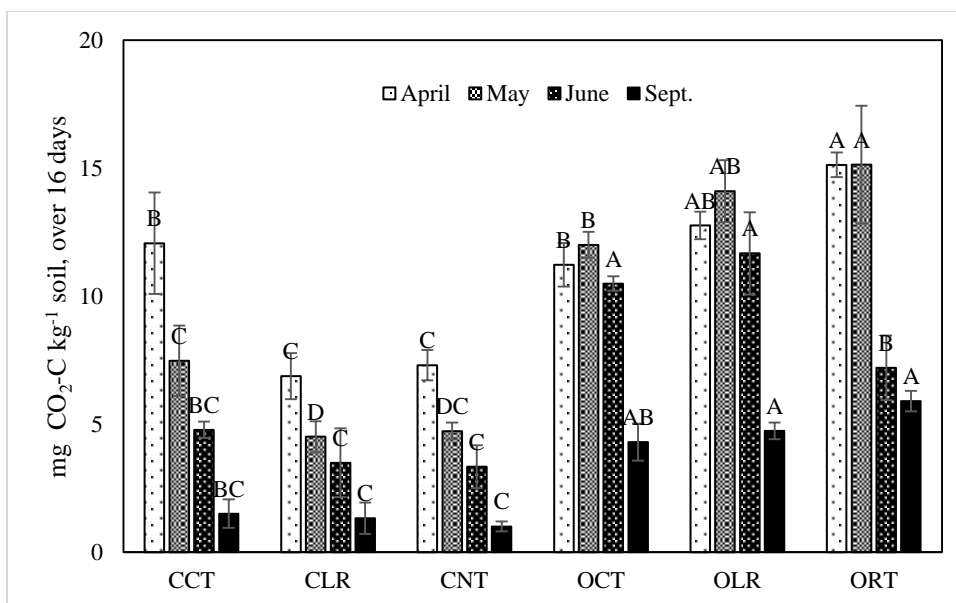


Figure 2.7 Effect of farming systems on cumulative mineralizable C. Farming systems sharing the same letter are not different for a given date according to Fisher's protected LSD ($p < 0.05$).

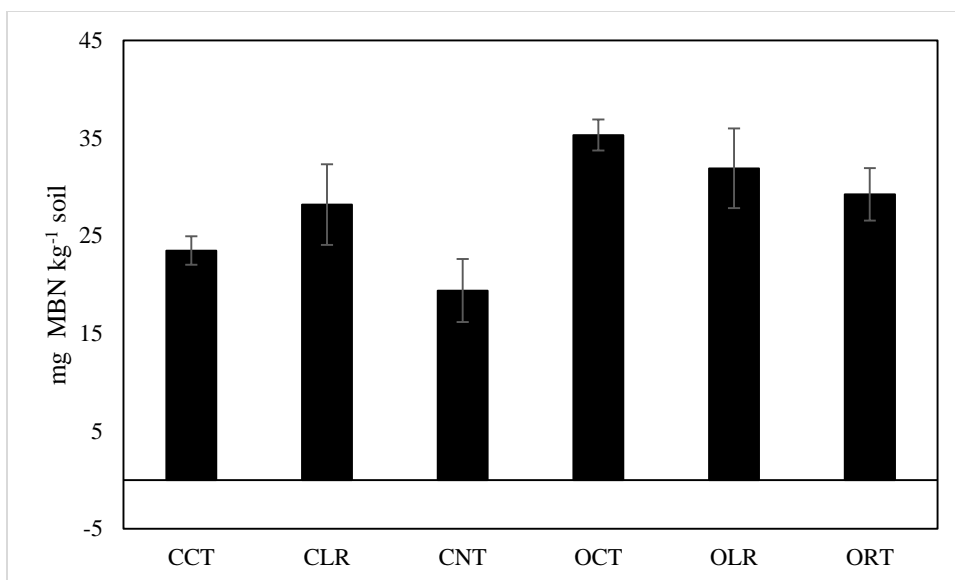


Figure 3.9 Effect of farming systems on microbial biomass N. Farming systems sharing the same letter are not different for a given date according to Fisher's protected LSD ($p < 0.05$).

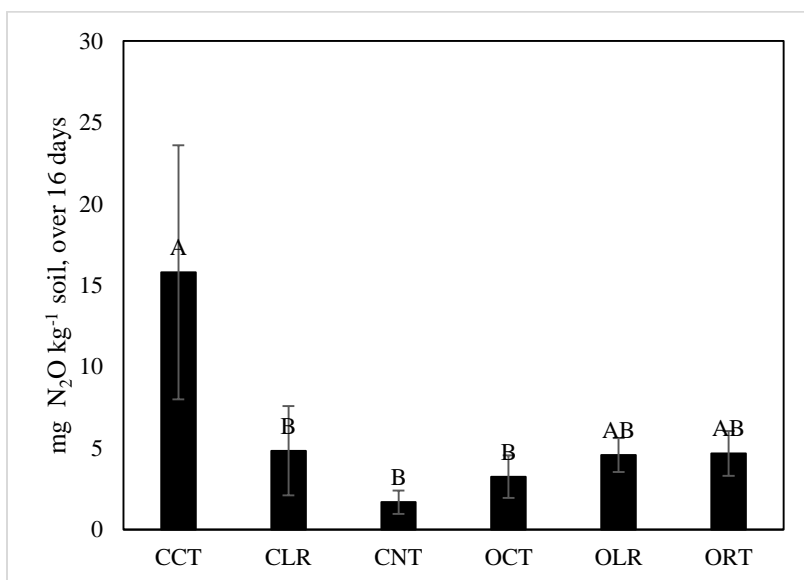


Figure 3.10 Effect of farming systems on cumulative mean N₂O emissions over the full season. Farming systems sharing the same letter are not different for a given system according to Fisher's protected LSD ($p < 0.05$).

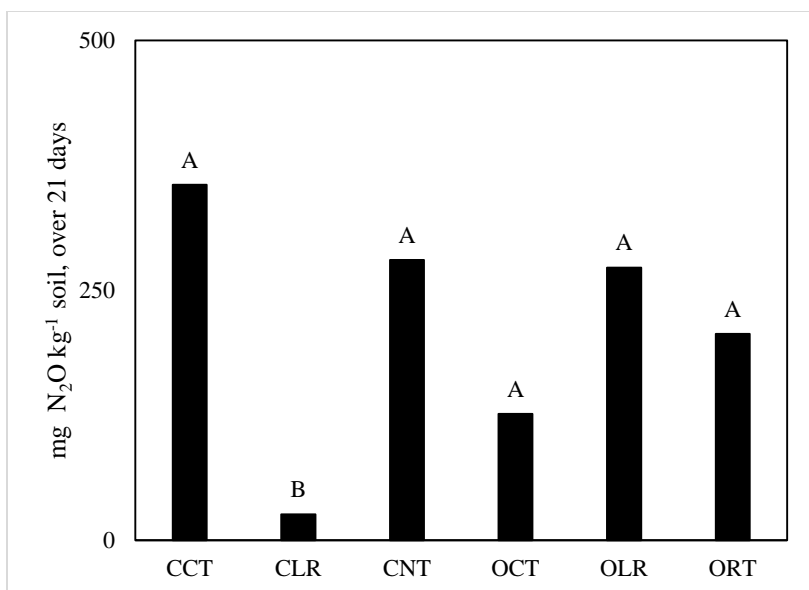


Figure 3.11 Effect of farming systems on calculated cumulative N₂O in poultry litter N amended soil across dates. Farming systems sharing the same letter are not different according to Fisher's protected LSD ($p < 0.05$).

CHAPTER 4

SOIL N₂O EMISSIONS POTENTIAL AMONG ORGANIC AND CONVENTIONAL FARMING SYSTEMS AND ASSOCIATED SOIL C AND N DYNAMICS

4.1 Abstract

A majority of the world's anthropogenic nitrous oxide emissions come from agricultural soils. Because emissions levels depend on a combination of factors including soil organic matter (SOM) levels and mineral N availability, it is not clear how farm management systems, as opposed to individual practices, may affect production of this potent greenhouse gas. However, it is vital to understand how different SOM pools affect N₂O emissions to build a framework of mechanisms linking emissions across farming systems. This research examines both organic and conventional farming methods as well as different rotations within each for their impact on soil N₂O emissions and soil labile C and N. Both organic and conventional best management practice (BMP) annual cropping systems have been managed since 1999 in the Coastal Plain of North Carolina using reduced-till, clean till, or a 3 year long rotation with pasture in a long-term replicated field experiment. A total of six farming systems were used: BMP with conventional tillage (CCT), BMP with reduced tillage (CRT), BMP with no tillage (CNT), organic with conventional tillage (OCT), organic with long rotation (OLR), and organic with reduced tillage (ORT). Measurements determined from field soil (0-15cm) include microbial biomass C (MBC) and nitrogen (MBN), K₂SO₄-extractable C, inorganic N (NH₄⁺+NO₃⁻N), potentially mineralizable N (PMN), and total C

and N. Laboratory incubations were also conducted to quantify N₂O emissions and mineralizable C from the same systems' soils. Results indicate that the farming systems differ in the rate of N₂O production that occurs shortly following rewetting soils (70% WFPS) in soils sampled at soybean harvest, and across sampling dates that cumulative N₂O emissions over the course of incubations were higher in CCT soils. Conventional CT soil N₂O emissions rate exceeded CLR, OCT, and CNT a several points during the June and October soil incubations, while ORT emissions rates were also higher than CNT, OCT, and OLR for much of the October incubations. Microbial biomass N was greater in OCT and OLR soil than CCT in early spring, while organic RT MBN was higher than that of conventional NT at harvest.

4.2 Introduction

Soils under agricultural management contribute a large proportion of global anthropogenic nitrous oxide (N₂O) emissions. Nitrous oxide (N₂O) is a powerful greenhouse gas, with up to 296 times the amount of heat trapping potential as carbon dioxide (CO₂) (Ramaswamy et al., 2001). In the US, farm soils account for 74% of nationwide N₂O emissions (EPA, 2010), but soils under some management systems emit considerably less N₂O than others (Asgedom and Kebreab, 2011). Certain practices are already widely acknowledged to reduce emissions (Rees et al., 2013). For example, cultivar selection and water management (Hussain et al., 2014), using non-leguminous cover crops (Basche et al., 2014), and conservation tillage instead of conventional tillage (Abdalla et al., 2013; Gregorich et al., 2006) all are methods shown to be capable of reducing N₂O losses from arable land.

Certain alternative management strategies have been touted for their potential to mitigate the rise in global CO₂ emissions by sequestering soil C, namely cover cropping and

reduced tillage (Halvorson et al., 2002; Bowman et al., 1990; Maughan et al., 2009; Sainju et al., 2003; Torbert et al., 1999; Venkateswarlu et al., 2007). However, cover crop use can increase N₂O emissions (Basche et al., 2014), as can reduced tillage (Novak and Fiorelli, 2011; Powlson et al., 2012), suggesting that the higher rates of nitrous oxide production may partially offset the CO₂ mitigation effects of these practices. It's important, therefore, to understand how the soils of systems with not only different crops and cover crops, but also different weed control strategies compare for their potential to emit N₂O. The interaction of cover crop presence and how its biomass is managed is a remaining unknown in assessing their GHG mitigation potential (Eagle and Olander, 2012b).

Microbial transformation of soil NH₄⁺ and NO₃⁻ under limited oxygen availability are responsible for the majority of N₂O produced by nitrification and denitrification. Broadly, because the denitrification process occurs when an electron acceptor such as NO₃⁻ is reduced to N₂O in the presence of an electron donor, labile C availability and NO₃⁻ concentrations determine the proportion of N that is reduced to N₂O (Del Grosso et al., 2000). Crops, plant residues, manures, and tillage all can contribute to labile C levels used by denitrifying microorganisms. This can occur through their direct contribution of soluble C to the soil, or by increasing its concentration through turnover of microbial biomass and soil organic matter. Also, different soil organic matter pools either can influence N availability, either by serving as a source of mineralization or encouraging microbial immobilization. Thus, while extractable organic C in particular may drive an increase in N₂O (Huang et al., 2004), inorganic N supply can either co-limit its effect on N₂O (Qiu et al., 2015) or not (Huang et al., 2004) depending on quality and quantity of SOC.

Because soil C and N largely cycle together, and because farm soil management can affect soil C and N contents, this experiment was conducted to examine N₂O emissions from soils differing in legacy of inputs and soil management. The objectives were to 1) compare the N₂O emission potential of soils under different farming systems for over a decade, and 2) to build linkages among N₂O emissions, microbial activity, and C and N pools. We hypothesized that N₂O would be high in systems with high mineralizable C and mineral N, and that systems with lower microbial biomass would emit higher rates of N₂O due to reduced immobilization of N.

4.3 Materials and Methods

4.3.1 Site description, soil sampling and preparation

The fields used for soil sampling and biomass collection in this study were located on the North Carolina Department of Agriculture and Consumer Services Cherry Research Farm, located in Goldsboro, NC (35°23'N, 78°02'W, elevation 35 m above sea level). The plots, as part of the Farming Systems Research Unit (FSRU), have been under the same management regime since establishment of the long-term study in 1999. The plots are also relatively large, varying from 1.2-3.6ha. The FSRU is divided into three replicate blocks according to intensive soil mapping and consists of several organic and conventional best management practice (BMP) systems. Predominant diagnostic soil series were Tarboro loamy sands (Typic Udipsamments) in block A, Wickham sandy loams (Typic Hapludults) in block B, and and Tarboro loamy sands in block C. The 6 systems selected for use in this study were as follows: BMP with conventional synthetic N fertilizer and conventional tillage (CCT), BMP with conventional synthetic N fertilizer in a long rotation of three years of annual crops > three years of perennial grass hay (CLR), BMP with conventional synthetic N fertilizer and

no tillage using herbicides (CNT), organic poultry litter fertilizer with conventional tillage (OCT), organic long rotation (OLR), and organic reduced tillage using cover crops and fallow for weed control (ORT). More information about the FSRU long term study has been reported by Mueller et al. (2002). To compare soil C and N pools and greenhouse gas emissions among farming systems, soil samples were collected over the course of a year in which winter rye (*Secale cereale* L., var. 'Wrens Abruzzi'; WR) was grown from October 2013 until spring in all plots, and soybean [*Glycine max* L. Merr., var. 'Hutchinson' (organic) and 'Pioneer 96M' (BMP)] was grown from May to October 2014 in all plots. A total of thirty 2.5cm x 15cm deep cores were taken per plot and mixed completely to form a composite sample. Sampling dates were selected to coincide with key soil and crop management events (Table 4.1). Soils were sampled April 4, before rye termination; May 8, after termination; June 4, after planting and ORT termination; and October 21, at soy harvest. (Table 4.1). Soils were not sampled in ORT on May 8 because cover crops were still growing. Legume hairy vetch (*Vicia villosa* Roth, var. 'AU Early Cover'), crimson clover (*Trifolium incarnatum* L., var. 'Dixie'; CC), and rye were grown in organic plots from fall 2014 until spring 2015, while BMP plots were planted with wheat (*Triticum aestivum* L.) in December 2014. Samples were collected from plots that had received either poultry litter before the May sampling date (organic systems), or split applications of urea ammonium nitrate before the May (82 kg N ha⁻¹) and June (98) sampling dates (BMP systems).

4.3.2 Cover crop biomass

No more than one week before cover crop termination, aboveground rye biomass was collected within two representative 0.5m² quadrats per plot. Tissues were harvested with shears and dried to a constant weight at 40°C to calculate biomass production in Mg ha⁻¹ dry biomass.

4.3.3 Incubations and determination of N₂O and CO₂ emissions rate

After collection on each of the dates listed above, soils were sieved to pass a 2mm mesh, and moisture content determined by mass loss upon drying at 105°C. One hundred grams of soil (dry weight equivalent) was placed into each of four 237mL glass canning jars with screw-top lids and packed to a bulk density of 1.1g cm⁻³ (Chen et al., 2014). Three of these jars were wetted to 70% water-filled pore space (WFPS) with DI water, while the remaining jar was wetted to 60% WFPS. These moisture contents have previously been shown to be ideal for measuring N₂O produced by denitrification (70% WFPS) and C and N mineralization as well as N₂O produced by nitrification (60% WFPS) (Baral et al., 2016; Franzluebbers, 1999). Jar lids were perforated with rubber stoppers to allow gas sampling with a needle and syringe. Soils were sampled for N₂O and CO₂ at 12, 24, 36, 60, 108, 204, and 396 hours after adding water. Gases were stored in 12mL glass vials with crimp-top lids until analyzing on a Shimadzu GC-2014 gas chromatograph equipped with an electron capture device and a flame ionization device and autosampler (Shimadzu Corporation, Kyoto, Japan). Lids were capped tightly for 90 minute intervals at each sampling point, and then uncapped and loosely covered to allow gas exchange in the interim periods. Soils were monitored for moisture loss and water was added as necessary to maintain original moisture content. Nitrous oxide emissions rates are expressed on a dry weight equivalent basis and calculated as follows:

$$\text{mg N}_2\text{O kg soil}^{-1} \text{ d}^{-1} = \frac{\left[\left(\frac{3.2 \times N_2O_b}{h-5mL} - 3.2 \times N_2O_a \right) \times a \times m \right]}{1000 \times R \times {}^\circ K \times t \times g \text{ soil}}$$

where N_2O_a and N_2O_b represent concentrations of N₂O in the jar headspace at the time of sealing the jar and of gas sampling, respectively; m = molecular mass of N₂O (44.013 g/mol); a = atmospheric pressure in the lab in Raleigh, NC = 0.965 atm; t = time in days jar was

closed; h = jar headspace in mL; R = the gas constant, $0.08206 \text{ L atm mol}^{-1} \text{ }^\circ\text{K}^{-1}$; $^\circ\text{K} = 273^\circ + 22^\circ$ (Laboratory temperature, $^\circ\text{C}$); and $g \text{ soil}$ = dry weight equivalent soil in the jar.

Carbon dioxide production rates, as a measurement of mineralizable C, are expressed on a $\text{CO}_2\text{-C}$ per soil dry weight equivalent basis and calculated similarly to N_2O , as follows:

$$\text{mg CO}_2\text{-C kg soil}^{-1} \text{ d}^{-1} = \frac{\left[\left(\frac{3.2 \times \text{CO}_{2b}}{h-5\text{mL}} - 3.2 \times \text{CO}_{2a}\right) \times a \times m_1\right]}{m_2 \times 1000 \times R \times ^\circ\text{K} \times t \times g \text{ soil}}$$

where CO_{2a} and CO_{2b} represent concentrations of CO_2 in the jar headspace at the time of sealing the jar and of gas sampling, respectively; m_1 = molecular mass of C (12.011g /mol); m_2 = molecular mass of CO_2 (44.01g /mol); and a , t , h , R , $^\circ\text{K}$, and $g \text{ soil}$ are as described in the paragraph above.

4.3.4 Calculation of cumulative N_2O and CO_2 flux

Cumulative N_2O and CO_2 flux were estimated using an area under the curve approach based on the hours of incubation X rate of $\text{mg N}_2\text{O kg}^{-1} \text{ soil day}^{-1}$ or $\text{mg CO}_2\text{-C kg}^{-1} \text{ soil day}^{-1}$.

$$\text{mg N}_2\text{O kg}^{-1} = \sum \left[\left((d_2 - d_1) \times \frac{\text{N}_2\text{O}_{d1} + \text{N}_2\text{O}_{d2}}{2} \right) + \dots + \left((d_k - d_j) \times \frac{\text{N}_2\text{O}_{dj} + \text{N}_2\text{O}_{dk}}{2} \right) \right]$$

$$\text{mg CO}_2\text{-C kg}^{-1} = \sum \left[\left((d_2 - d_1) \times \frac{\text{CO}_2\text{-C}_{d1} + \text{CO}_2\text{-C}_{d2}}{2} \right) + \dots + \left((d_k - d_j) \times \frac{\text{CO}_2\text{-C}_{dj} + \text{CO}_2\text{-C}_{dk}}{2} \right) \right]$$

4.3.5 Microbial Biomass C and Nitrogen and K_2SO_4 -extractable C

Microbial biomass C (MBC) and microbial biomass N (MBN) were determined using the chloroform-fumigation–extraction method (Ross, 1992; Vance et al., 1987) where 20.0 g of field moist soil was fumigated with ethanol–free chloroform for 48 h. Both fumigated and non-fumigated soils were extracted with 50 mL of $0.5 \text{ mol L}^{-1} \text{ K}_2\text{SO}_4$ by shaking for 30 min on an

orbital shaker. A Shimadzu TOC-5050 analyzer was used to determine the organic C concentration (C_{org}) in the extractants. MBC was calculated as follows: $[(C_{org} \text{ in fumigated soil}) - (C_{org} \text{ in nonfumigated soil})] / k_{ec}$, where $k_{ec} = 0.33$ and serves to convert the extracted organic C to MBC (Sparling and West, 1988). The concentration of N in the extractant was determined on a Lachat flow injection analyzer after digestion with alkaline persulfate oxidation (Cabrera and Beare, 1993), and calculated using the equation: $(total\ N\ extracted\ from\ fumigated\ soil - total\ N\ extracted\ from\ nonfumigated\ soil) / k_{en}$, where $k_{en} = 0.45$ and is used to convert the extracted organic N to MBN (Jenkinson, 1988). K_2SO_4 -extractable C was determined from the organic C concentration in extractant from non-fumigated soil. NH_4^+ - NO_3^- -N was determined from the total inorganic N extracted from non-fumigated soil. Both MBC and MBN are expressed on a soil dry weight basis.

4.3.6 Potentially mineralizable N in 60% WFPS soil, net N flux in 70% WFPS soil

Potentially mineralizable N (PMN) was determined as the difference between extractable N determined immediately after soil sampling and $mg\ NH_4^+ + NO_3^- - N\ kg^{-1}$ soil as determined from 20g subsampled from the 60% WFPS soil samples at the end of 28 days.

Net N flux was determined as the difference between extractable inorganic N and $mg\ NH_4^+ + NO_3^- - N\ kg^{-1}$ soil as determined from 20g subsampled from the 70% WFPS soil samples at the end of 28 days.

4.3.7 Total C and N

Total C and N concentrations in the June-sampled soil only were determined at the NCSU Environmental and Agricultural Testing Services using a Perkin-Elmer PE 2400 CHN Elemental Analyzer (Norwalk, CT, USA). Results are presented as percent C and N of total soil mass.

4.3.8 Statistical analyses

The six farming systems were randomly assigned to one of three replicates for a total of 18 plots arranged into blocks based on soil type. Analysis of variance statistical analysis was carried out using the MIXED procedure in SAS 9.3 (Cary, NC), and multiple comparisons between dates and farming systems were also completed using Fisher's Protected LSD. For ANOVA among farming systems' gas emissions data for soil sampled on a particular date, a repeated measures analysis was used, with incubation jars serving as the repeated subject. For soil C and N pools (MBC, MBN, PMN, K₂SO₄-C, inorganic N) a repeated measures analysis was used, with plots serving as the repeated subject. Several covariance structures were modeled (autoregressive, compound symmetry, unstructured, and spatial powered), and the covariance structure with the best model fit (AIC, AICC, and BICC) was used for analysis. Differences were deemed significant at $P < 0.05$.

4.4 Results

4.4.1 Cover crop biomass

Cover crop biomass production in ORT and OLR plots exceeded that of all of the conventionally managed fields (Table 4.8). The later-terminated ORT biomass was 149% to 249% higher than the conventional plots' biomass production, while OLR accrued to 74.9% to 107% higher biomass than conventional.

4.4.2 N₂O and CO₂ cumulative emissions and rate over time

Cumulative N₂O emissions were highest in June from sampled soil for all systems but ORT and CLR. An initial peak of N₂O emissions occurred by 36 hours in most systems' soils. Afterwards, rates fell to less than 0.01 mg N₂O g soil⁻¹ hr⁻¹ by 4.5 days in pre-termination and post-plant soil and by 7 days in harvest and mid-season soil. In soil sampled

while cover crops were still growing, CCT and CLR emissions rates were higher than the other four systems. Emissions from post-plant soil were similar among all treatments (Table 4.3). Conventionally tilled BMP soil displayed higher emissions at 60h during incubations of June soil. Differences among farming systems N₂O emissions rate were detected in soil collected in October. In these soils, CCT emissions rate was higher than all but ORT from 36-108h, and ORT soil emissions were greater than in CLR from 60-108h (Table 4.4, figure 4.1). In addition, cumulative CCT emissions were greater 57.1%-187% than in any other system, regardless of sampling date, over the 7 day incubation window (Table 4.2).

Mineralizable C, as measured by cumulative CO₂ emissions, was highest in October and lowest in April in all systems. Values were similar before termination and mid-season, and lowest in harvest soil. Cmineralization rates over the course of each incubation experienced a gradual decline from initially high values. Cumulative emissions in OLR soil were 27%, 31%, and 69% greater than in CCT, CNT, and CLR soil, regardless of sampling date (Table 4.2). One-way contrasts showed a difference between organic and conventional soils at each sampling date in terms of rate of CO₂ emissions. Individual treatments differences mostly reflected this trend, with individual organic systems' soils in general producing more CO₂ than conventional systems. In addition, at several times during incubations of June, CCT soil CO₂ emissions rates were higher than the other two conventional systems (Table 4.5). Emissions from CCT and OCT were similar to one another during most of the periods gas was sampled from incubations in October and June. Also, ORT emissions rate exceeded that of the other two organic systems at several points during October incubations. No differences were detectable in CO₂ emissions rate after 108h except in May soils, when OLR and OCT soils rate still surpassed that of CLR and CNT at 204h.

4.4.3 Microbial biomass C and N, NH_4^+ - NO_3^- -N, K_2SO_4 -C

After cover crop termination and soybean planting, CCT and CNT MBN was lower than that of OLR or OCT (Figure 4.3). No differences were detected among systems in terms of MBC (Table 4.7). However, there was a one-way contrast difference between organic and conventional plots at the pre-termination sampling date in April ($P < 0.025$), with organic treatments having higher MBC than conventional.

Inorganic N (NH_4^+ + NO_3^-) rose from pre-termination to post-plant to mid-season sampling dates, before falling at harvest. No differences were detected between rotation treatments with the exception of at mid-season in June, when OLR was found to have higher N concentrations than either CLR, CNT, or ORT (Figure 4.2). Soils from organically managed plots had higher inorganic N (NH_4^+ + NO_3^-) than conventional at harvest (Contrast $P < 0.05$). Concentrations of organic C extractable with K_2SO_4 solution were similar between treatments and between dates.

4.4.4 Potentially mineralizable N in 60%WFPS soil, net N flux in 70% WFPS soil and, total C and N

Potentially mineralizable N was similar among farming systems. Only in October, the net N flux, at 52 mg N kg^{-1} soil over three weeks of incubation, was greater in soil from all systems except OLR, ranging from $30\text{-}43 \text{ mg N kg}^{-1}$ soil. Total organic C and total N concentrations were similar among all systems, ranging from $0.9\text{-}1.3\%$ and $0.070\text{-}0.097\%$, respectively.

4.5 Discussion

A complex array of factors determine N_2O production in addition to soil inorganic N concentrations (King and Ball, 1992). This study was particularly interested in factors

attributable to microbial activity and labile C, because soil biotic processes are important in determining N₂O emissions responses to soil N (R. D. Bowden et al., 1991). Rye cover crop termination represents a large influx of labile C for soil microbes, as evidenced by higher mineralizable C in soil following termination in every system. In addition, N₂O was highest in June in four of six systems. Cover crop residues have been shown to be an important source of input C in explaining N₂O emissions (Pramanik et al., 2014). Mitchell et al. (2013) concluded that rye residues are able to spur emissions because they are a source of labile C, and that soil NO₃⁻ may not limit N₂O in an annual cropping system receiving inorganic fertilizer N. In our study, harvest soil inorganic N (NH₄⁺ +NO₃⁻) was actually no higher in CCT, despite having higher N₂O emissions rates at harvest than four of the treatments. It may be that the current season's cover crop root and shoot residues served to either provide energy for denitrifiers, or served to cause anoxic conditions in the incubated soil. The organic reduced till system, which is managed for maximum cover crop biomass production, accumulated 68% more rye biomass than the next highest. This may explain its soil's considerable CO₂ emissions and high N₂O emissions occurring at harvest. On the other hand, Negassa et al. (2015) found high C:N rye biomass to have no effect on emissions, and cover crops termination was only one of several events occurring before June soil sampling in this study. Furthermore, under these circumstances the farming systems with the highest rates of C mineralization did not have the highest rates of N₂O emission. It is possible that higher amounts of substrate in the form of rye biomass which grew in the organic treatments might have spurred more complete denitrification to N₂ (Iqbal et al., 2015). Alternatively, another pool of soil C besides what was readily mineralizable at 60% WFPS may have been more important to activity of N₂O producers, though it is not clear what that was. Extractable

organic C, which is typically viewed as the predominant immediate C source for N₂O producers, was no different among systems. It also does not appear that the same pool of organic matter serving as a substrate for microbial biomass was also driving N₂O emissions. For example, at harvest, when microbial biomass C was highest, possibly due to root inputs (Yang and Cai, 2006; Zhu and Cheng, 2012), N₂O emissions were 60.9% lower than post-termination and 55.8% lower than mid-season. Others have found high respiration and N₂O to positively correlate with microbial biomass (Qiu et al., 2015), which would not have predicted these results. Microbial biomass N followed a trend similar to that of MBC, rising from June to October in CCT, CNT, and OLR. This, together with decreased emissions in October and lower inorganic N in the same systems, lends some support to the hypothesis that immobilization may have a negative impact on denitrification.

Dynamics of N₂O emissions rates over the season also highlight the impacts of soil management for gas emissions. Although CCT and CNT had similar, high N₂O emission rates before cover crop termination, at the end of the season CNT N₂O emissions were among the lowest while CCT remained high. The two tillage events in CCT between April and October may have increased its soil's potential to produce N₂O. Somewhat contrary to these results, it has been shown in a meta-analysis by Rochette (2008) that reducing tillage can result in an increase in N₂O emissions. This conclusion was based on emissions observed in the field, however. Others have concluded that a higher soil gas diffusivity and lower total C in tilled soil are responsible for the commonly reported greater emissions in reduced tillage than conventionally tilled soils (Palma et al., 1997; Skiba et al., 2002). The fact that soil was mixed and sieved thoroughly in the present experiment offers context for these contrasting results. Also, total C did not differ between these two systems. Furthermore, experiments

measuring emissions over short periods have also led to the conclusion that tilled soils emit more than no-till soils (Chatskikh and Olesen, 2007). García-Marco et al. (2014) suggest that enhanced supplies of organic C may favor more complete denitrification to N_2 , but there is no difference between these two treatments in terms of SOC, MBC, or mineralizable C. Also, net increase in inorganic N was 27% higher in incubations of CCT soil at 70% WFPS than in CNT, indicating that our N_2O measurements did not obscure even larger losses of N as N_2 . A possible explanation for the higher N_2O emissions rate in CCT pre-termination and in June is a lack of microbial immobilization. Microbial biomass N was lower in CCT than OCT and OLR in April, and OCT and OLR in July. This would lend support to the hypothesis that immobilization can play a role in reducing N_2O emissions. Higher microbial biomass N in these systems may be a result of higher C and N contributions from a history of cover crops and organic inputs in the rotation (Fonseca et al., 2007). While immobilization of NO_3^- into microbial biomass can be important decreasing N_2O emissions (S. Qiu et al., 2015), others have found that with even small amounts of NH_4^+ in the soil, NO_3^- will not be immobilized in significant quantities (Rice and Tiedje, 1989). Typically immobilization is associated with initial decomposition of high C:N substrates, and it is not clear that rye biomass would have still immobilized N as late as October. Therefore, additional factors besides soil N availability and transformations and the measured SOM pools likely contributed to these differences in N_2O emissions among systems.

4.6 Conclusions

This study found that soils under different farming systems differ in their potential to produce the potent greenhouse gas N_2O , even in a year of the rotation when management practices were similar among them. This shows there is a legacy of management inherent in

the systems. With the exceptions of cover crop biomass production and tillage, these differences largely stem from legacy effects of organic and conventional inputs in years past. There was also evidence that organic and conventional systems' microbial sequestration of N may explain some of the difference between their N₂O production rates. Lastly, cover crop C may serve as a substrate for N₂O producers, as high N₂O emissions were observed in the high cover crop biomass ORT system.

Acknowledgements

This work was supported by USDA's National Institute of Food and Agriculture [Grant 2012-02978-230561]. Thanks to Cherry Research Farm staff for field maintenance.

4.7 Tables and Figures

4.7.1. Tables

Table 4.1 Field activities, sampling, and dates

| Date | Activity | Systems |
|---------------------------|---------------------------------------|---------------|
| 10/23/2013- 10/30/2013 | Rye cover crop planted | All |
| 4/4/2014 | Pre-termination soil sampling | All |
| 4/10/2014 | Cover crop biomass sampling | All but ORT |
| 4/10/2014 | Cover crops flail mown | OCT, OLR |
| 4/10/2014 | Disking | OCT, OLR |
| 4/14/2014- 4/21/2014 | Cover crops flail mown, disking | OCT, OLR, CCT |
| 4/24/2014 | Cover crop glyphosate termination | CLR, CNT |
| 5/7/2014 | Cover crop biomass sampling | ORT |
| 5/8/14 AM | Post-termination soil sampling | All but ORT |
| 5/8/14 PM | Second disking | CCT, OCT, OLR |
| 5/20/2014 | Cover crop roller-crimper termination | ORT |
| 5/21/2014 | Third disking | CCT, OCT, OLR |
| 5/27/2014 | Soybean planting | All |
| 6/4/14 | Post-planting soil sampling | All |
| 6/5/14 | Cultivation | OCT, OLR |
| 6/13/14 | Cultivation | OCT, OLR |
| 7/1/14 | Cultivation | OCT, OLR |
| 7/15/14 | Cultivation | OCT, OLR |
| 10/21/2014 | Harvest soil sampling | All |
| 10/22/2014 | Soybean harvest and sampling | All |

Table 4.2 Means of cumulative gas emissions over four sampling dates

| System | ¹ N ₂ O | | | CO ₂ | | | |
|------------------|-------------------------------|----------|----------------|-----------------|----------|----|--|
| | Mean | Std. Err | | Mean | Std. Err | | |
| ² CCT | 0.66 | ± 0.18 | ³ A | 3.56 | ± 0.72 | B | |
| CLR | 0.29 | ± 0.10 | B | 2.67 | ± 0.56 | B | |
| CNT | 0.27 | ± 0.09 | B | 3.45 | ± 0.87 | C | |
| OCT | 0.23 | ± 0.10 | B | 3.97 | ± 0.75 | AB | |
| OLR | 0.34 | ± 0.11 | B | 4.52 | ± 0.90 | A | |
| ORT | 0.42 | ± 0.13 | - | 4.22 | ± 1.31 | - | |

¹N₂O: mg N₂O kg⁻¹ over 16 days; CO₂: mg CO₂-C kg⁻¹ over 16 days

²CCT: Conventional Conventional Tillage; CLR: Conventional Long Rotation; CNT Conventional No Tillage; OCT: Organic Conventional Tillage; OLR: Organic Long Rotation; ORT: Organic Reduced Tillage.

³Means within a column with different letters indicate differences among systems according to Fisher's protected LSD (p<0.05).

Table 4.3 Summary of gas emissions rate ANOVA results

| | Sample collection date | | | | | | | |
|------------|------------------------------|------------------|-----------------|------------------|-----------------|------------------|-----------------|------------------|
| | April | | May | | June | | Oct. | |
| | ¹ CO ₂ | N ₂ O | CO ₂ | N ₂ O | CO ₂ | N ₂ O | CO ₂ | N ₂ O |
| System (S) | ** | ² NS | ** | NS | ** | NS | *** | ** |
| Hour (H) | *** | *** | *** | ** | *** | NS | *** | *** |
| S x H | * | NS | *** | NS | *** | ** | ** | *** |

¹CO₂: mg CO₂-C kg⁻¹ soil day⁻¹; N₂O: mg N₂O kg⁻¹ soil day⁻¹

²NS: not significant at P=0.05; * significant at P=0.05; ** significant at P=0.01; *** significant at P=0.001

Table 4.4 Means separation among farming systems' incubation N₂O emissions, mg N₂O kg⁻¹ soil day⁻¹ in October.

| System | 24 | Hours of incubation | | | 204 |
|------------------|-----------------|---------------------|----|-----|-----|
| | | 36 | 60 | 108 | |
| ¹ CCT | ² AB | A | A | A | NS |
| CLR | ABC | C | BC | BC | |
| CNT | C | C | C | BC | |
| OCT | AB | C | C | C | |
| OLR | BC | C | C | BC | |
| ORT | A | B | AB | AB | |

¹CCT: Conventional Conventional Tillage; CLR: Conventional Long Rotation; CNT Conventional No Tillage; OCT: Organic Conventional Tillage; OLR: Organic Long Rotation; ORT: Organic Reduced Tillage. Means within a column for a sampling hour are deemed significantly different if not followed by a shared letter according to Fisher's protected LSD ($p < 0.05$)

²Means within a column with different letters indicate differences among systems according to Fisher's protected LSD ($p < 0.05$). NS: not significant at $P = 0.05$

Table 4.5 Soil incubations CO₂ emissions, mg CO₂-C kg⁻¹ soil day⁻¹.

| Hrs | Sys | April | | May | | June | | October | | | | |
|-----|------------------|-------|-----------|------|-----------|----------------|-----------|---------|-----------|------|--------|----|
| | | Mean | Std. Err. | Mean | Std. Err. | Mean | Std. Err. | Mean | Std. Err. | | | |
| 24 | ¹ CCT | | | 0.64 | ± 0.11 | ² B | 0.22 | ± 0.03 | BC | 0.60 | ± 0.09 | B |
| | CLR | | | 0.45 | ± 0.03 | B | 0.17 | ± 0.03 | CD | 0.34 | ± 0.03 | C |
| | CNT | | | 0.51 | ± 0.01 | B | 0.15 | ± 0.02 | D | 0.68 | ± 0.10 | B |
| | OCT | | | 0.84 | ± 0.05 | A | 0.26 | ± 0.02 | AB | 0.70 | ± 0.09 | B |
| | OLR | | | 0.87 | ± 0.08 | A | 0.31 | ± 0.03 | A | 0.75 | ± 0.09 | B |
| | ORT | | | - | ± - | - | 0.27 | ± 0.01 | AB | 1.02 | ± 0.11 | A |
| 36 | CCT | 0.18 | ± 0.02 | 0.80 | ± 0.05 | A | 0.21 | ± 0.01 | | 0.72 | ± 0.08 | BC |
| | CLR | 0.15 | ± 0.01 | 0.41 | ± 0.03 | B | 0.20 | ± 0.04 | | 0.52 | ± 0.03 | C |
| | CNT | 0.20 | ± 0.02 | 0.48 | ± 0.03 | B | 0.28 | ± 0.07 | NS | 0.63 | ± 0.08 | BC |
| | OCT | 0.25 | ± 0.02 | 0.74 | ± 0.05 | A | 0.31 | ± 0.04 | | 0.82 | ± 0.08 | B |
| | OLR | 0.29 | ± 0.02 | 0.89 | ± 0.12 | A | 0.27 | ± 0.03 | | 0.82 | ± 0.05 | B |
| | ORT | 0.30 | ± 0.05 | - | ± - | - | 0.29 | ± 0.03 | | 1.10 | ± 0.10 | A |
| 60 | CCT | 0.19 | ± 0.02 | 0.50 | ± 0.04 | B | 0.16 | ± 0.02 | BC | 0.49 | ± 0.03 | BC |
| | CLR | 0.12 | ± 0.01 | 0.46 | ± 0.02 | B | 0.13 | ± 0.04 | C | 0.36 | ± 0.05 | C |
| | CNT | 0.24 | ± 0.05 | 0.51 | ± 0.01 | B | 0.11 | ± 0.00 | C | 0.50 | ± 0.05 | BC |
| | OCT | 0.25 | ± 0.02 | 0.70 | ± 0.06 | A | 0.23 | ± 0.03 | A | 0.60 | ± 0.06 | B |
| | OLR | 0.29 | ± 0.04 | 0.63 | ± 0.03 | A | 0.21 | ± 0.02 | AB | 0.60 | ± 0.08 | B |
| | ORT | 0.27 | ± 0.04 | - | ± - | - | 0.23 | ± 0.02 | AB | 0.92 | ± 0.11 | A |

Continued on following page

Table 4.5 (continued)

| | | | | | | | | | | | | | | | | | |
|-----|-----|------|---|------|------|------|------|----|------|------|------|------|------|------|------|------|----|
| 108 | CCT | 0.11 | ± | 0.01 | 0.62 | ± | 0.03 | | 0.26 | ± | 0.02 | A | 0.49 | ± | 0.06 | BC | |
| | CLR | 0.09 | ± | 0.01 | 0.38 | ± | 0.08 | | 0.15 | ± | 0.03 | B | 0.36 | ± | 0.07 | C | |
| | CNT | 0.15 | ± | 0.03 | 0.45 | ± | 0.03 | NS | 0.15 | ± | 0.03 | B | 0.51 | ± | 0.03 | BC | |
| | OCT | 0.17 | ± | 0.02 | 0.63 | ± | 0.07 | | A | 0.22 | ± | 0.02 | A | 0.51 | ± | 0.01 | BC |
| | OLR | 0.19 | ± | 0.02 | 0.70 | ± | 0.15 | | A | 0.22 | ± | 0.00 | A | 0.54 | ± | 0.06 | B |
| | ORT | 0.17 | ± | 0.03 | - | ± | - | | A | 0.21 | ± | 0.01 | A | 0.75 | ± | 0.06 | A |
| CCT | | | | 0.39 | ± | 0.08 | AB | | 0.27 | ± | 0.06 | | 0.44 | ± | 0.04 | | |
| CLR | | | | 0.29 | ± | 0.01 | B | | 0.18 | ± | 0.07 | | 0.32 | ± | 0.03 | | |
| 204 | CNT | | | | 0.33 | ± | 0.03 | B | 0.16 | ± | 0.07 | NS | 0.63 | ± | 0.23 | NS | |
| | OCT | | | | 0.50 | ± | 0.02 | A | 0.22 | ± | 0.07 | | 0.49 | ± | 0.04 | | |
| | OLR | | | | 0.51 | ± | 0.07 | A | 0.31 | ± | 0.03 | | 0.60 | ± | 0.09 | | |
| | ORT | | | | - | ± | - | - | 0.17 | ± | 0.03 | | 0.34 | ± | 0.03 | | |

¹CCT: Conventional with conventional Tillage; CLR: Conventional Long Rotation; CNT Conventional No Tillage; OCT: Organic Conventional Tillage; OLR: Organic Long Rotation; ORT: Organic Reduced Tillage.

²Means within a column for each date and hour are deemed significantly different if not followed by a shared letter according to Fisher's protected LSD ($p < 0.05$). NS: not significant at $P = 0.05$

Table 4.6 Mean CO₂ emissions rate from soils incubated at 60% WFPS, ug CO₂ kg⁻¹ soil d⁻¹

| System | April | | | May | | | June | | | October | | | |
|--------|-------|---|-----------|------|-----|-----------|------|-----|-----------|---------|-----|-----------|----|
| | Mean | ± | Std. err. | Mean | ± | Std. err. | Mean | ± | Std. err. | Mean | ± | Std. err. | |
| CCT | 161 | ± | 15 | BC | 571 | ± | 48 | 200 | ± | 18 | 515 | ± | 36 |
| CLR | 120 | ± | 10 | C | 399 | ± | 23 | 154 | ± | 16 | 366 | ± | 22 |
| CNT | 197 | ± | 22 | AB | 457 | ± | 20 | 151 | ± | 22 | 540 | ± | 50 |
| OCT | 224 | ± | 16 | A | 680 | ± | 37 | 226 | ± | 19 | 572 | ± | 46 |
| OLR | 257 | ± | 22 | A | 721 | ± | 53 | 242 | ± | 19 | 622 | ± | 47 |
| ORT | 246 | ± | 28 | A | - | ± | - | 212 | ± | 16 | 764 | ± | 82 |

¹CCT: Conventional with conventional Tillage; CLR: Conventional Long Rotation; CNT Conventional No Tillage; OCT: Organic Conventional Tillage; OLR: Organic Long Rotation; ORT: Organic Reduced Tillage.

²Means within a column with different letters indicate differences among systems within a date according to Fisher's protected LSD (p<0.05).

Table 4.7 Summary of soil extraction ANOVA

| | $\text{NH}_4^+ + \text{NO}_3^- - \text{N}$ | $\text{K}_2\text{SO}_4 - \text{C}$ | MBN | MBC | PMN | N flux -70% WFPS |
|------------|--|------------------------------------|-----|-----|-----|------------------|
| System (S) | NS | NS | * | NS | NS | ** |
| Date (D) | *** | NS | * | * | * | *** |
| S x D | ** | NS | *** | NS | NS | * |

¹ $\text{K}_2\text{SO}_4 - \text{C}$: C extractable with K_2SO_4 solution; MBN: Microbial biomass N; MBC: Microbial biomass C; PMN: Potentially mineralizable N at 60% WFPS; N flux -70% WFPS: Net change in $\text{NO}_3^- - \text{N}$ over 4 weeks.

$\text{NH}_4^+ + \text{NO}_3^- - \text{N}$, $\text{K}_2\text{SO}_4 - \text{C}$, MBN, MBC, PMN, N flux -70% WFPS all expressed on mg kg^{-1} soil basis.

²NS: not significant at $P=0.05$; * significant at $P=0.05$; ** significant at $P=0.01$; *** significant at $P=0.001$

Table 4.8 Summary of plant biomass and soil C and N means separation

| System | Cover crop biomass | Soil % C | Soil % N | Soy Biomass | Soy Yield |
|------------------|--------------------|--------------------|-----------|-------------|-----------|
| ¹ CCT | 2.5 | ² C 0.9 | 0.0733 | 9.62 | 47.9 |
| CLR | 2.4 | C 0.9 | 0.0700 | 9.13 | 61.9 |
| CNT | 2.1 | C 1.0 NS | 0.0733 NS | 7.19 NS | 33.8 NS |
| OCT | 3.4 | BC 1.1 | 0.0767 | 7.52 | 36.6 |
| OLR | 4.4 | B 1.3 | 0.0967 | 8.63 | 54.5 |
| ORT | 7.4 | A 1.1 | 0.0800 | 6.71 | 29.1 |

¹CCT: Conventional with conventional Tillage; CLR: Conventional Long Rotation; CNT Conventional No Tillage; OCT: Organic Conventional Tillage; OLR: Organic Long Rotation; ORT: Organic Reduced Tillage.

²Means within a column with different letters indicate differences among systems according to Fisher's protected LSD ($p < 0.05$).

NS: not significant at $P=0.05$; * significant at $P=0.05$; ** significant at $P=0.01$; *** significant at $P=0.001$

4.7.1. Figures

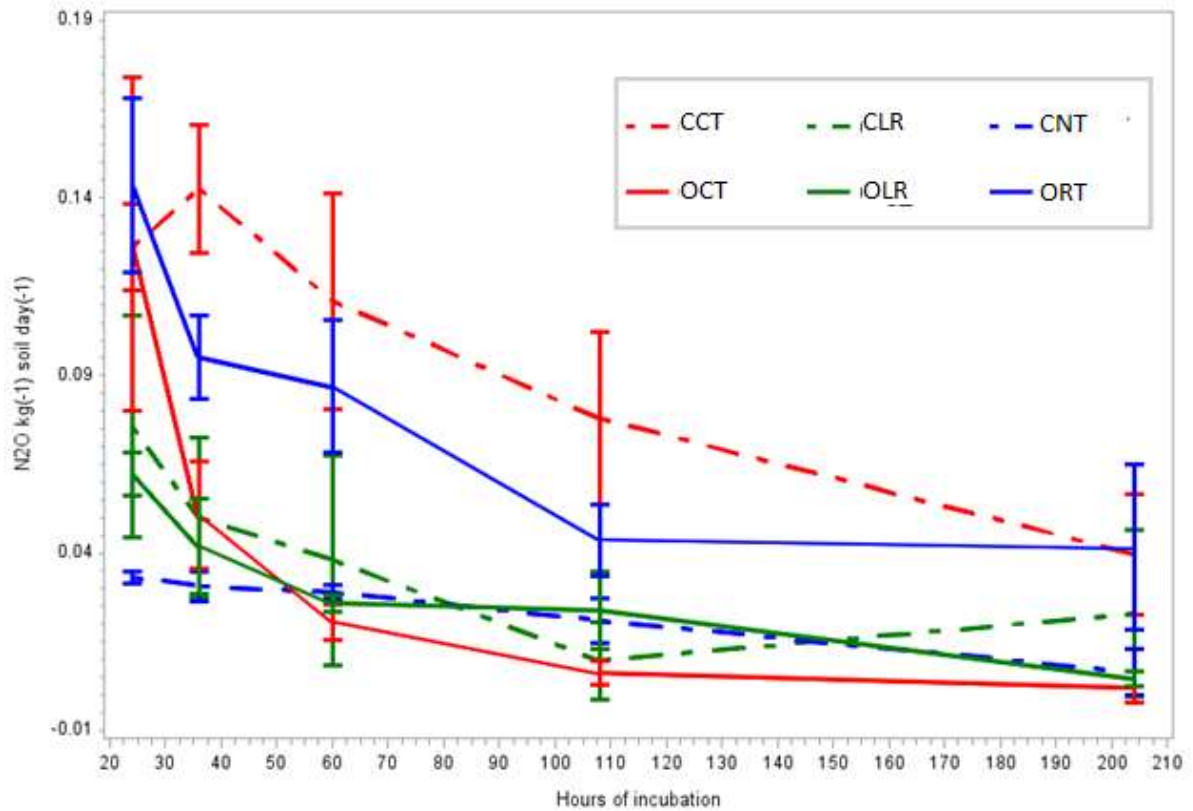


Figure 4.1 N₂O emissions by farming system over time during lab incubations of soil collected in October at soy harvest. Con_CT: Conventional with conventional Tillage; Con_LR: Conventional Long Rotation; Con_NT Conventional No Tillage; Org_CT: Organic Conventional Tillage; Org_LR: Organic Long Rotation; Org_RT: Organic Reduced Tillage.

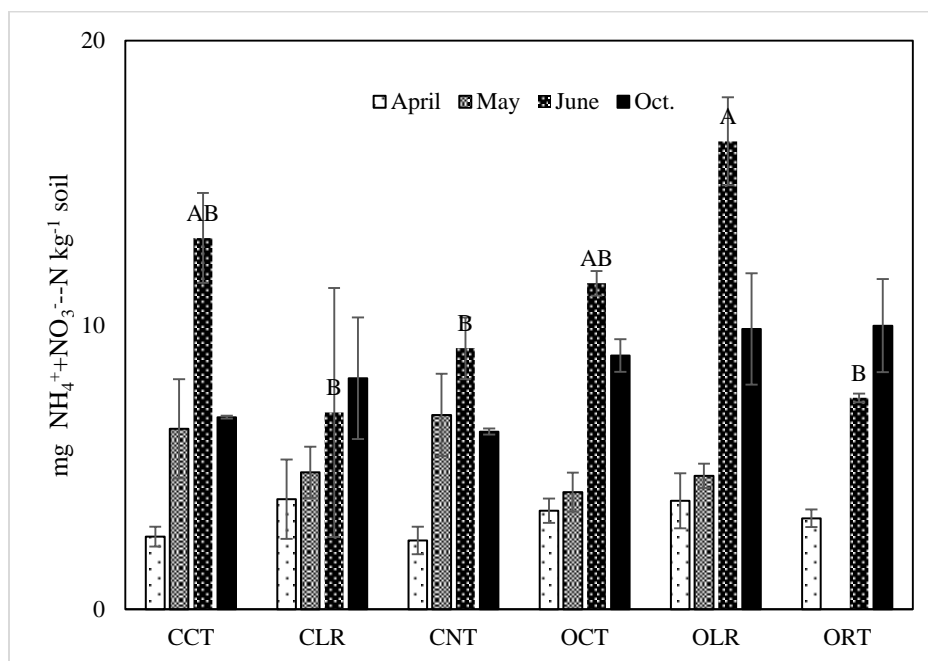


Figure 4.2 Effect of farming system on extractable inorganic N. Farming systems' bars topped with the same letter are not different for a given date according to Fisher's protected LSD ($p < 0.05$).

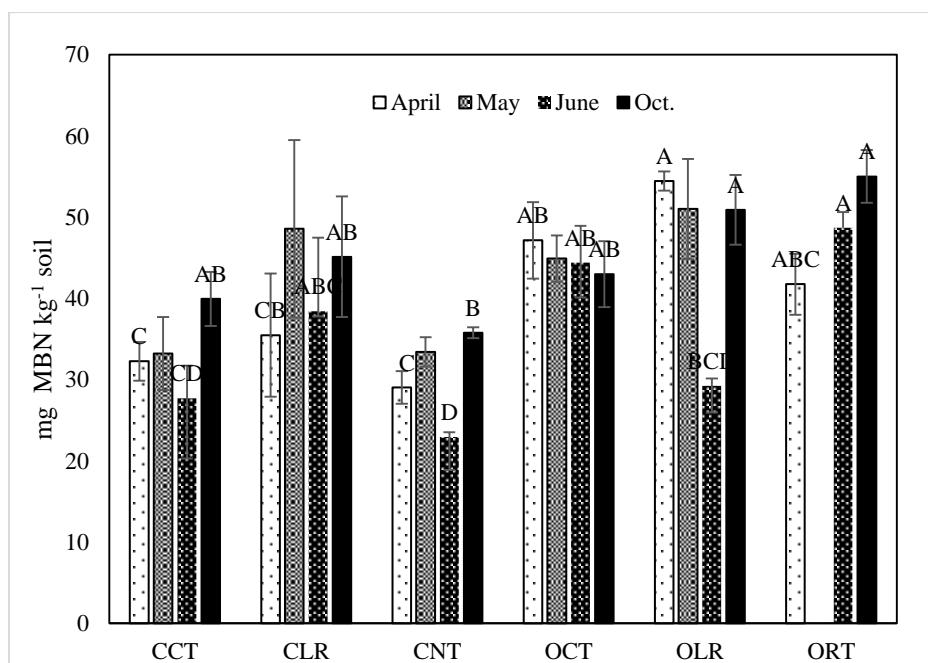


Figure 4.3 Effect of farming systems on microbial biomass N. Farming systems' bars topped with the same letter are not different for a given date according to Fisher's protected LSD ($p < 0.05$).

CHAPTER 5

ROOT AND ARBUSCULAR MYCORRHIZAL FUNGAL REGULATION OF DECOMPOSITION: EFFECTS OF N AVAILABILITY AND SOURCE

5.1 Abstract

Increasing evidence has recently shown that arbuscular mycorrhizal fungi (AMF) can enhance the N acquisition of host plant from decomposing organic materials. There is a need to better understand how N availability affects both root and arbuscular mycorrhizal fungal (AMF) controls over decomposition of recent organic additions to soil. Four independent but complementary experiments were designed to test the extent to which roots or AMF may increase or decrease C loss from organic residues and to determine the effect of N availability and plant growth on these changes. Soil microcosms or litterbags accessible to roots + AMF, AMF hyphae only, or neither were deployed in field row crop and perennial grass settings and pot-grown corn. This allowed observation of the impacts of these belowground C inputs in both field and greenhouse settings. A greenhouse experiment was conducted with N rates of 0, 30, or 60mg urea-N kg⁻¹ applied to corn growing in pots with microcosms accessible to roots + AMF (RIC), AMF hyphae only (MIC), or neither (NIC). Shoot residues labelled with ¹⁵N were buried within the cores to allow tracking of residue into corn biomass. Corn uptake of residue N, and core soil microbial biomass C (MBC) and microbial biomass N (MBN), light fraction organic matter (LFOM), and inorganic N were measured. In turfgrass field plots, litterbags were used to measure C loss from litterbags accessible to AMF or roots + AMF under

varying levels of inorganic N application. In the final experiment, RIC, MIC, and NIC soils were analyzed for changes in residue C and N after burial in plots with control switchgrass or plants infected with *Burkholderia phytofirmans* strain PsJN, a beneficial bacterial endophyte. Microbial biomass C and N, K₂SO₄-extractable C, potentially mineralizable N (PMN), and inorganic N were also measured. Greenhouse corn biomass residue N concentrations (residue N g⁻¹ biomass) in plants with root access (RIC) to these organic residues were 43% and 154% higher than those of plants with MIC or NIC, respectively. Furthermore, an even larger proportion of RIC plants' N originated in residue at very low N levels compared with MIC or NIC plants. In turfgrass, root access litterbags contained less C and N at retrieval than AMF bags at every date bags were sampled ($P < 0.0001$), with 60% less C and 66% less N on the last sampling date. There was no effect of the switchgrass endophyte on MBC, MBN, inorganic N, or PMN, but infected plant soil contained lower amounts of water extractable C. Switchgrass root ingrowth cores had higher levels of the original residue C, total C, and total N relative to cores accessible by AMF hyphae (MIC) only (by 12%, 16%, and 13%, respectively). These results lead to the conclusions that both roots and AMF may prime the mineralization of residue N or C in corn and turfgrass, while switchgrass roots may slow C and N loss from buried litter relative to AMF.

5.2 Introduction

Arbuscular mycorrhizal fungi (AMF) form mutualistic relationships with the majority of land plant species, offering valuable services to their host in the form of enhanced nutrient delivery (Clark and Zeto, 2000; Smith and Read, 2008) drought tolerance (Wu and Xia, 2006), and protection from pathogens (Srivastava et al., 2010). They substantially expand plants' belowground network, and because of this, their growth and metabolism represent a large

Csink for their host—up to 20% of plant photosynthate (Jakobsen and Rosendahl, 1990). As such, they represent a major regulator over ecosystem C cycling as both a source of new soil C and by potentially altering turnover of extant soil organic C (SOC). Historically, they have been recognized for their contribution of relatively stable organic compounds like glomalin (Rillig et al., 2001) and chitin and for their role in protecting or building soil aggregates capable of storing soil organic matter (Guggenberger et al., 1999; Hu et al., 1995). However, their effect on soil's ability to sequester C is uncertain, as most studies have focused on individual compounds or been correlative in nature (Wilson et al., 2009). A conceptual framework of how and in what instances AMF affect SOC needs to take into account their indirect effects on soil saprobes.

The extension of the rhizosphere into soil is known to alter C and N cycling via a number of factors (Cheng and Kuzyakov, 2002), most importantly through biological activity (Dormaar, 1990). The rhizosphere is not only a hotspot of microbial activity due to the large C influx from roots and AMF, but also is a sink for soil nutrients. Roots can thus either positively (Kuzyakov and Cheng, 2001) or negatively (Bader and Cheng, 2007) prime decomposition of organic matter. It has also been discovered that AMF play a similar roll, by either stimulating (Hodge et al., 2001; Tu et al., 2006) or hindering (Chen et al., 2015) decomposition. In addition, they augment plants' nutrient acquisition when they are able to deliver nutrients, particularly N, to their host (Cheng et al., 2012). An understanding that plants may effectively compete with soil microorganism demand for N has emerged and grown over the last two decades (Schimel and Bennet 2004). Under conditions of strong competition for nutrients between plants, AMF can increase N uptake from an organic patch (Hodge, 2003).

While pot experiments strongly suggest a role in stimulating decomposition, both the occurrence and extent of this phenomenon have seldom been evaluated in the field.

Specifically, there is a need to better understand how N availability affects both root and AMF controls over decomposition (Hodge et al., 2010). We hypothesized that moderate N availability would increase plant growth and delivery of photosynthate belowground, serving as a source of C priming decomposition of plant residues or soil C. Additionally, we hypothesized that roots and AMF would differ in their relative importance in regulating C cycling and N uptake from decomposing litter. Four independent but complementary experiments were designed to 1) test the extent to which roots or AMF may stimulate or hinder C loss from organic residues and 2) determine the effect of N availability on the regulation of C turnover and N translocation by either roots or AMF. Three of these experiments focused on the effect of N availability on root and AMF regulation of decomposition and N transformations, while a fourth centered on root- and AMF-mediated C and N cycling under the effects of plant infection with a bacterial endophyte.

5.3 Materials and Methods

5.3.1 Effects of N input source and rate on AMF activity and decomposition of buried litter in a field corn setting

In order to meet the second objective of describing how N availability affects soil C turnover in the presence of AMF and roots, an experiment was carried out to measure residue decomposition in microcosms accessible by roots + AMF or AMF alone in a field setting with varying N inputs and cover crop species. Both AMF colonization (Azcon et al., 1982; Hepper, 1983; Liu et al., 2000) and root growth (Karrou and Maranville, 1993; Voisin et al., 2002) respond to soil N, making it important to understand how N-driven changes in nutrient demand

and belowground allocation of photosynthate affect C dynamics. Also, it is unclear how organic sources of N fertility like legume biomass might differ from inorganic N in their impact on root and AMF regulation of organic matter turnover.

5.3.1.1 Experimental site and field operations

In the fall of 2011 a field experiment was initiated at Caswell Research Farm in Kinston, NC, part of the North Carolina Department of Agriculture and Consumer Services system of research stations. The soil in the field selected for the study was a Johns sandy loam (35°16'23"N 77°36'50"W, fine-loamy over sandy or sandy-skeletal, siliceous, semiactive, thermic Aquic Hapludult) with a 0-2% slope. In the fall of 2011, lime, phosphorus, and potassium were applied to meet soil test recommendations and the field was disked and cultivated. Four treatment plots were established in each of four randomized complete blocks, for a total of 16 plots measuring 3m wide (four corn rows) by 15m long each. Two cover crops varieties that would either add fixed atmospheric N to the soil or rely on soil N were planted. These were winter rye (*Secale cereale* L., var. 'Wrens Abruzzi'; WR) planted at 154 kg live seed ha⁻¹ and the legume hairy vetch (*Vicia villosa* Roth, var. 'AU Early Cover'; HV), planted at 30 kg ha⁻¹. Both were planted on 15 cm centers September 15, 2011 and allowed to grow all winter until soft dough stage in rye (Feekes 11.2) and early pod set in vetch (Hoffman et al., 1993; Mischler et al., 2010). Crop roll-kill termination was performed using a 3.1m roller crimper (I and J Mfg, Gap, PA). Certified organic corn (*Zea mays* var. Doeblers N631, 110d, Jersey Shore, PA) was planted at a density of 81,502 seeds ha⁻¹ on 76cm rows on May 4, 2012 with a 13cm row spacing using a no-till planter (John Deere 7200, Moline, IL) parallel to the direction of cover crop rolling. Both non-planted low N (LN) and high N (HN) and cover crop

(HV, WR) plots received fertilizer applications of UAN once at 89.6 kg N ha⁻¹ on May 7, 2012 and again at 30.2 kg N ha⁻¹ on June 30, 2012. Non-planted HN plots also received another split application of UAN at 78.4 kg ha⁻¹ on June 11, 2012. Total application rates were 119.8 kg ha⁻¹ in the low N and cover crop plots and 198.2 kg ha⁻¹ in the high N plots. All plots were maintained weed free using a combination of glyphosate (Honcho® Plus, Monsanto Company, St. Louis, MO) on February 7 in HN and LN; atrazine plus S-metolachlor (Medal® II AT, Syngenta Crop Protection, Greensboro, NC) on May 7 in all 4 treatments; and nicosulfuron on July 2 in all treatments. Corn yield data were collected using a small plot combine for 12.2m of the two center rows of each plot on September 7.

5.3.1.2 Root and mycorrhizal access core construction and burial

Microcosms were constructed to observe buried residue C changes and isolate the effects of roots vs. AMF. These consisted of PVC cores 10cm wide, 25cm long, with a 5 x 10cm opening on opposite sides, and sealed bottoms and a top open to rainfall but covered with 1.6mm mesh to exclude organic debris (Figure 5.1). Root ingrowth cores (RIC) were covered with 1.6mm diameter mesh over the two openings while mycorrhizal ingrowth cores (MIC) were covered with 20µm mesh. Cores were filled with a mixture of 175g of long term C₃-crop field soil ($\delta^{13}\text{C}$ of -24.8‰, 1.04% C, 0.08%N) and 2.19g rye residue collected from the WR plots on February 27 ($\delta^{13}\text{C}$ of -29.6‰, 39.6% C, 1.3%N). Rye residue was oven dried at 40° C and cut into 1cm sections before mixing with the core soil. Cores were deployed in the field for a single growing season. These were installed in the field June 5, 2012 by creating a 22cm deep cylindrical hole of the same diameter as the core and gently firming the soil around the core. Two cores each of both the RIC and MIC mesh variety for a total of four cores

were installed in a random order within a single corn row per plot, approximately 3m apart from each other. Cores were removed on August 1, 2012 by carefully cutting roots growing into RIC's before gently removing the microcosm from the soil profile. After removing the contents of each core in the lab, soil was air dried at room temperature. A representative subsample of less than 100mg was removed by repeatedly mixing the soil and removing two quadrants of soil spread out on a dish until a small enough portion remained. This was ball ground (Spex CertiPrep 2000 Geno/Grinder, Metuchen, NJ) at 1,100 strokes minute⁻¹ for four minutes before 50-60mg of the homogenous subsample was weighed into 9 x 5mm tin capsules to be sent to University of California, Davis to be analyzed for C isotope ratios using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK).

The proportion of SOC originating in either the rye residue or the core soil as opposed to corn roots was able to be determined due to the difference in $\delta^{13}\text{C}$ between the two C pools. Carbon originating in corn, a C₄ plant, is differentiated by its -12.5 $\delta^{13}\text{C}\%$ compared to 26.3 $\delta^{13}\text{C}\%$ for the C₃ rye residue/ C₃ crop legacy soil mixture. As an indicator of the core soil's proportion of the original C₃ value, results are presented as $\delta^{13}\text{C}$.

Five whole corn plants per plot were dug from locations at least 3m from cores 54 and 71 days after planting (June 26 and July 12, 2012). These were transported immediately back to the lab, where soil was thoroughly washed from the roots and young roots were clipped from the root ball. Roots were stained with trypan blue (Phillips and Hayman, 1970) to enable quantification of colonization by mycorrhizal fungi, using the gridline-intersect method (Giovannetti and Mosse, 1980). Washed root samples (further cut into 1 cm lengths) were

cleared in 5% (w/v) KOH, acidified in 1% (v/v) HCl, and then stained with acidic glycerol-trypan blue solution. These stained roots were then spread on a plastic Petri dish with gridlines and examined for infection using a dissecting microscope at $\times 40$ magnification. Results obtained are expressed as percentage root length colonized (PRLC) by AM fungi.

5.3.1.3 Statistical analyses

Isotopic measurements made on microcosm soil and PRLC were analyzed as a split plot, randomized complete block, 2 access levels \times 4 cover/N treatment factorial, with four replications. Analysis of variance was performed in SAS 9.4 using PROC MIXED.

5.3.2 *Effect of N application rate and root and mycorrhizally mediated N acquisition from decomposing residues*

A greenhouse corn study was designed to fulfill both objectives of describing how AMF and roots affect residue decomposition and N uptake and how the magnitude of these effects changes with changing soil N. To assess the ability of AMF and plant roots to translocate N from decomposing residues, ^{15}N -enriched plant material (plants grown with ^{15}N fertilizer) was buried in cores accessible by roots + AMF, AMF alone, or neither. By containing root growth to a small soil volume, this system offered the advantage over a field study of making it possible to observe residue ^{15}N translocation into plant biomass.

5.3.2.1 Greenhouse setup, fertilization, watering, and harvest

The growing media used in this experiment was a mixture of sand, field soil, and AMF inoculum soil. Sand, which was added to improve drainage, was obtained from the NCSU

Method Road Greenhouse Facility. Soil was the same as that used to fill the cores in the field corn experiment ($\delta^{13}\text{C}$ of -24.8‰, $\delta^{15}\text{N}$ of 8.78‰, 1.04% C, 0.08%N). AMF inoculum soil consisted of a sandy soil in which several AMF species had been pot cultured on corn plants, and spore counts were performed to determine the mass of inoculum soil to add to each pot. Pots (25cm diameter) were filled with a mixture of 2,100g soil, 2,100g sand, and 75g inoculum soil.

To isolate the effects of either roots + AMF hyphae, AMF alone, or the absence of both on N removal and C dynamics, microcosms of different levels of belowground access were created. These consisted of PVC RIC and MIC microcosms identical to those used in the field corn study. In addition, no root / no hyphae ingrowth cores (NIC) covered with 0.45 μm membrane (Millipore Corporation, Darmstadt, Germany) was used.

Core soil consisted of 180g of a 1:1 mixture of the same sand and soil as the pot soil, with the addition of 1.8g of green, dried, ^{15}N labeled wild oat (*Avena fatua* L.) leaf and stem residue grown in the greenhouse and fertilized with ^{15}N -(NH_4) $_2\text{SO}_4$ ($\delta^{13}\text{C}$ of -28.7‰, $\delta^{15}\text{N}$ of 27,075‰, 39.9% C, 0.8%N). Cores were buried 22cm deep within the pots, so that the soil surfaces within the cores and in the pots were parallel.

The soil outside the core was sown directly with 4 corn (*Zea mays* var. Doeblers N631, 110d, Jersey Shore, PA) seeds per pot, adjacent to the core's two mesh-covered openings, on October 7, 2013. After germination around 4-8 days after sowing, plants were thinned to two per pot. All pots received two applications of N (for low N and high N treatments) or an addition of water (for the no N treatment) on November 14, 2013 and again on January 6, 2014 as 15 (low N) or 30 (high N) mg kg $^{-1}$ urea-N in 50 mL water for a total rate

of 0, 30 or 60 mg kg⁻¹ N. These correspond to approximately 0, 67, and 134 kg N ha⁻¹ in the field assuming a 15cm depth. Additionally, 50 mg kg⁻¹ P as K₂HPO₄ in 33 mL water and 12 mg kg⁻¹ Mg and 12 mg kg⁻¹ S as MgSO₄-7(H₂O) per pot were applied on November 21, 2013 and again on December 18, 2013 to all pots. Soil was watered with tap water to 60% of water holding capacity of the pots. Pots were spaced on one foot centers on benches in a heated greenhouse with individual pots representing an access x N x harvest group x replication combination placed in completely randomized order. Pots were shifted slightly at every watering to minimize the effects of spatial variables such as shading and airflow on plant growth. Pot soil, core soil, root biomass, and shoot biomass were destructively harvested twice (December 13, 2013, and again on January 17, 2014). After removing the contents of each core in the lab, a subsample was removed on the January 17 date only for determination of light fraction organic matter (LFOM), microbial biomass C (MBC), microbial biomass N (MBN), and inorganic N. The remainder of the core soil on both dates was air dried at room temperature. Corn root and shoot biomass was oven-dried at 40°C and weighed to obtain biomass data (g dry root or shoot biomass pot⁻¹). Shoot tissues were then ground once using a Wiley mill, and ground again using a ball grinder at 1,100 strokes minute⁻¹ for four minutes. Five to six mg was weighed into 9 x 5mm tin capsules to be sent to University of California, Davis to be analyzed for C and N isotope ratios using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK).

5.3.2.2 N content and isotopic signature of corn biomass N

Differences among treatments in the proportion of corn N originating in wild oat residue as opposed to soil organic matter or inorganic N were able to be determined due to the

difference in $\delta^{15}\text{N}$ between the residue (2,7075‰) and soil or fertilizer N (8.7818‰). Total uptake of residue ^{15}N for each pot was calculated using the following equation: *corn mg residue $^{15}\text{N pot}^{-1} = \text{mg } ^{15}\text{N g}^{-1}\text{corn biomass} \times \text{g corn shoot biomass pot}^{-1}$* . The proportion of plant N originating in residue (mg residue N g^{-1} corn biomass, f_{R}) was calculated using the single isotope, two-source mixing model (Phillips and Gregg, 2001) as $f_{\text{R}} = (\delta_{\text{T}} - \delta_{\text{S}}) / (\delta_{\text{R}} - \delta_{\text{S}})$, where δ_{R} represents the $\delta^{15}\text{N}$ value for the labeled wild oat residue, δ_{S} refers to the mean $\delta^{15}\text{N}$ value for the initial soil, and δ_{T} is the mean $\delta^{15}\text{N}$ value for the corn biomass at harvest.

5.3.2.3 Light fraction organic matter

Light fraction organic matter (LFOM) in core soil was extracted by density fractionation. This served as an indicator of the remaining mass of recent organic matter inputs at pot harvest. A sequential fractionation procedure slightly modified from that of Baisden et al. (2002) was used. Briefly, 25.00g of soil and 50mL of deionized water were shaken together in flasks on an orbital shaker at 150rpm for 30 minutes. Afterwards, the soil and solution were allowed to settle undisturbed for 24-36h. Taking care not to disturb the sediment, supernatant and floating organic matter was then aspirated with a wheel pump pipette into vacuum filter funnels with pre-weighed micro filter disks. Solution was removed from this first fraction (F1) by vacuum suction, and filter disks containing organic matter were dried at 80°C and weighed to determine F1 mass. Fifty mL of 1.6g cm^{-3} KI solution were then added to the same sediment remaining in the flasks after the F1 extraction. Samples and KI solution were shaken again for 30 minutes at 150rpm and allowed to settle for 24-36 hours. The same procedures were used to aspirate floating organic matter (F2) in the KI onto pre-weighed filter disks. Organic matter was rinsed with 0.5M CaCl_2 and then water to remove heavy KI from the filter and any solution was removed by vacuum filtration. Filters plus F2 were dried at 80°C and weighed to determine

F2 mass. Light fraction organic matter (LFOM) was calculated by adding F1 and F2 and is expressed as mg LFOM kg⁻¹ dry soil.

5.3.2.4 Microbial biomass carbon, microbial biomass nitrogen, NH₄⁺-N and NO₃⁻-N

Microbial biomass C (MBC) and microbial biomass N (MBN) were determined using the chloroform-fumigation–extraction method (Ross, 1992; Vance et al., 1987) where 20.0 g of field moist soil was fumigated with ethanol–free chloroform for 48 h. Both fumigated and non-fumigated soils were extracted with 50 mL of 0.5 mol L⁻¹ K₂SO₄ by shaking for 30 min on an orbital shaker. A Shimadzu TOC-5050 analyzer was used to determine the organic C concentration (C_{org}) in the extractants. MBC was calculated as follows: $[(C_{org} \text{ in fumigated soil}) - (C_{org} \text{ in nonfumigated soil})] / k_{ec}$, where $k_{ec} = 0.33$ and serves to convert the extracted organic C to MBC (Sparling and West, 1988). The concentration of N in the extractant was determined on a Lachat flow injection analyzer after digestion with alkaline persulfate oxidation (Cabrera and Beare, 1993), and calculated using the equation: $(total N \text{ extracted from fumigated soil} - total N \text{ extracted from nonfumigated soil}) / k_{en}$, where $k_{en} = 0.45$ and is used to convert the extracted organic N to MBN (Jenkinson, 1988). Concentrations of NH₄⁺ and NO₃⁻ in non-oxidized extractants were also determined using a Lachat flow-injection analyzer. Microbial biomass C, MBN, NH₄⁺, and NO₃⁻ are expressed on a soil dry weight basis.

5.3.2.4 Statistical analyses

Isotopic and total N measurements made on corn plant biomass were analyzed as a completely randomized design, 3 access levels x 3 N levels x 2 harvest groups factorial, with three replications. Core soil measurements, which were made once at the second harvest only,

were analyzed as a completely randomized design, 3 x 3 factorial, with three replications. Analysis of variance was performed in SAS 9.4 using PROC MIXED. Interactions were probed by either performing means separation of simple effects following indication of a statistically significant simple main effect by the SLICE option and by using contrast statements.

5.3.3 Effect of N application rate on root and AMF controls over decomposition in a turfgrass setting

A field turfgrass experiment was conducted to examine root and AMF regulation of C turnover and N translocation in a high input, high SOC, perennial plant environment. This study was designed to address both objectives of determining roots' and mycorrhizal fungal controls over of recent residue additions, and how that control changes with N availability. The site was located in a residential *Zoysia matrella* (L.) lawn established more than 5 years before sample collection in Raleigh, North Carolina (35° 35' 37" N, 78° 39' 16"W). The soil was an Appling gravelly sandy loam (Fine, kaolinitic, thermic Typic Kanhapludult). *Zoysia* grass was fertilized at one of three N application rates: 0, 50, or 100 kg N ha⁻¹ month⁻¹ beginning in May 2012. In 2013, 50 kg N ha⁻¹ month⁻¹ plots received N as granular NH₄NO₃ on June 7, July 9, and August 11. The same year, 100 kg N ha⁻¹ month⁻¹ plots received N on May 27, June 7 and 23, July 9 and 24, and August 11 and 27. Four replicate plots per N level were arranged in a completely randomized design. A total of 12 plots were aligned in a 3 x 4 grid, and each plot measured 75cm x 75cm, for a total area of 6.75m. Litter bags (10 cm x 5 cm) were made entirely out of mesh material that would either allow roots and AMF (1.6mm opening) or AMF alone (20µm nylon mesh (Tetko/Sefar mesh, Sefar America, NY) to penetrate. These were filled with 0.50g of the same rye litter used in the field corn experiment (39.63% C, 1.29% N).

Three of each of the root+AMF and AMF bags were buried at a 45° angle to that the top of the bag was flush with the soil surface, with the bottom approximately 7cm deep. Three root+AMF and 3 AMF bags per plot were buried on May 17, 2013, for a total of 72 bags. One pair per plot of the two mesh size bags were retrieved on June 20, August 1, and September 5. Litter bags and their contents were dried at 40°C before weighing the contents. The residue contents remaining were finely milled and analyzed for total C and N content using a Perkin-Elmer 2400 CHNS/O elemental analyzer (Norwalk, CT, USA).

Total C and N measurements made bag contents were analyzed as a repeated measures, split-plot, completely randomized design, with N level serving as the main plot and bag type serving as the subplot. The repeated measures subject was the main plot, with measurements repeated over the three sampling dates. Analysis of variance was performed in SAS 9.4 using PROC MIXED.

5.3.4 Effect of infection with the beneficial bacterial endophyte Burkholderia phytofirmans PsJN on switchgrass soil C and N cycling

The objective of this fourth experiment was to determine the impact of endophyte infection on N mineralization and removal from soil as well as how infected plants alter soil C turnover through enhanced belowground C inputs. Because switchgrass growth is higher with the endophyte (Kim et al., 2012; Lowman et al., 2014), its root growth and AMF may serve to either enhance soil C sequestration or potentially act to prime decomposition of native organic matter. This study was conducted to address the first overall objective of quantifying AMF and roots effects on soil C and N cycling.

5.3.4.1 Experimental site and field operations

The field activities of the experiment were carried out in Danville, Virginia, at Walden Farm (36° 36' 42" N, 79° 19' 32" W) on a soil in the Cecil sandy loam series (Fine, kaolinitic, thermic Typic Kanhapludults). This on-farm site was previously managed as a tobacco farm before converting to pasture five years before planting switchgrass in 2012. Extant vegetation was killed 30 days before transplanting using one application of glyphosate. Sites were cultivated mechanically and hand-weeded and then 144 plots were planted on August 20, 2012 on 76.2cm centers with switchgrass (*Panicum virgatum* L. var. 'Alamo') either infected with *Burkholderia phytofirmans* strain PsJN (Infected) or left uninfected (Control). Additional material about the seedling and inoculum material sources has been published by Lowman et al (2014). A randomly selected subset of 24 plots were included in the present study for all soil and microcosm analyses—12 infected and 12 control plants.

5.3.4.2 Soil and core sampling activities

Twenty-eight cores similar to those used in the corn field and greenhouse experiments were created by adding soil and ¹⁵N-labelled plant material to PVC cylinders with two mesh-covered openings. Mesh sizes over the openings were as follows: 0.45µm for control no AMF hyphae / no root ingrowth cores (NIC); 20µm for mycorrhizal hyphal ingrowth cores (MIC); and 1.6mm for root + AMF ingrowth cores (RIC). All cores were filled with 180g of field soil (δ¹³C of - 26.3‰, δ¹⁵N of 8.8 ‰ 1.04‰ C, 0.08‰N, additional information on the soil used can be found in Cheng et al., 2012) and 1.8g dried wild oat leaf and stem residue grown in the greenhouse and fertilized with ¹⁵N-(NH₄)₂SO₄ (δ¹³C of -28.7‰, δ¹⁵N of 27,075‰, 39.9‰ C, 0.8‰N). Cores were installed in the field June 5, 2015 by creating a 22cm deep cylindrical

hole of the same diameter as the core and gently firming the soil around the core. One core each of both the RIC and MIC mesh variety were buried within a plot in 10 of each of the 12 infected and 12 control plots, approximately 5cm apart from each other. In the remaining 2 infected and 2 control plots, two NIC microcosms were also installed in the same manner. Cores were removed on September 17, 2015 by carefully loosening the soil around NIC's and MIC's or cutting roots growing into RIC's with a knife before gently removing the microcosm from the soil. After removing the contents of each core in the lab, soil was air dried at room temperature and visible roots were removed. A representative subsample of less than 100mg taken by mixing the soil and removing two quadrants of soil spread out on a dish until a small enough portion remained. This was ball ground (Spex CertiPrep 2000 Geno/Grinder, Metuchen, NJ) at 1,100 strokes minute⁻¹ for four minutes before 50-60mg of the homogenous subsample was weighed into 9 x 5mm tin capsules to be sent to University of California, Davis to be analyzed for C and N isotope ratios using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). On both the core installation and retrieval dates, plots were also sampled for soil to a depth of 15cm using a 2.5cm probe to obtain five subsamples. These soil subsamples were thoroughly mixed and transported back to the lab, where they were kept refrigerated at 4°C until laboratory analyses (potentially mineralizable N, NO₃⁺-N, NH₄⁺-N, microbial biomass C, and microbial biomass N) were conducted.

5.3.4.3 Potentially mineralizable N, NO₃⁺-N, NH₄⁺-N, microbial biomass C and microbial biomass N

All plot soil samples were sieved to pass a 2mm mesh screen and thoroughly mixed. A 20g subsample was used for immediate N extraction to determine NO₃⁺-N, NH₄⁺-N, and serve as a baseline for potentially mineralizable N (PMN). A separate 20g subsample was incubated for 28 days at 22±1°C. At the end of 28 days, N was extracted using 50 mL of 0.5 mol L⁻¹ K₂SO₄ in order to determine potentially mineralizable N (Hart et al., 1994). Extractant N concentrations of NH₄⁺ and NO₃⁻ were determined using a Lachat flow-injection analyzer. Potentially mineralizable N was calculated as the difference between incubated and non-incubated (immediate extraction) soil samples. Microbial biomass C and MBN were determined as detailed in the section describing the greenhouse corn experiment.

5.3.4.4 Proportions and concentrations of residue C and N in soil microcosms

The concentration of C and N originating in either the oat residue or the core soil as opposed to switchgrass roots or litter was able to be determined because of the difference in isotopic signature between the two C and N pools. C originating in switchgrass, a C₄ plant, is differentiated by its -12.7 ‰ $\delta^{13}\text{C}$ compared to -25.9‰ for the C₃ wild oat residue/ C₃ crop legacy soil mixture. The mean proportion (f_R) of the residue C in the whole soil or soil fractions of the f_R was calculated using the single isotope, two-source mixing model (Phillips and Gregg, 2001) as $f_R = (\delta_T - \delta_S) / (\delta_R - \delta_S)$, where δ_R represents the mean $\delta^{13}\text{C}$ value for the residue/soil mixture, δ_S refers to the mean $\delta^{13}\text{C}$ value for the initial soil, δ_T is the mean $\delta^{13}\text{C}$ value for the treated microcosm soil retrieved at the end of the season. Using this proportion together with

the known SOC content of the soil sample, the concentration of C3 plant C (C_{C3} , g C3-C kg⁻¹ soil) was determined as $C_{C3} = C_t \times f_R$, where C_t is the core SOC concentration (g C kg⁻¹ soil).

The concentration of N originating specifically in the wild oat residue as opposed to core soil N or switchgrass roots or litter was able to be determined by the relative difference in $\delta^{15}\text{N}$ between the two N pools. Nitrogen originating in soil is differentiated by its 8.8 ‰ $\delta^{15}\text{N}$ compared to 27,075‰ for the ¹⁵N-enriched wild oat residue mixed within. The mean proportion (f_R) of the residue N in the whole soil or soil fractions of the f_R was calculated using the single isotope, two-source mixing model (Phillips and Gregg, 2001) as $f_R = (\delta_T - \delta_S) / (\delta_R - \delta_S)$, where δ_R represents the mean $\delta^{15}\text{N}$ value for the residue, δ_S refers to the mean $\delta^{15}\text{N}$ value for the initial soil, and δ_T is the mean $\delta^{15}\text{N}$ value for the treated microcosm soil retrieved at the end of the season. Using this proportion together with the known TN content of the soil sample, the concentration of residue N (N_R , mg residue N kg⁻¹ soil) was determined as $N_R = N_t \times f_R$, where N_t is the core's total N concentration (mg N kg⁻¹ soil).

5.3.4.4 Statistical analyses

Measurements made on bulk soil within the plots were analyzed as either a repeated measures or split plot completely randomized design, with individual plots serving as the repeated measures subject or whole plot factor. Data were analyzed using a repeated measures analysis in SAS 9.4 using PROC MIXED. An unstructured (UN) mixed model was compared to a mixed model with date treated as a split-plot factor for their model goodness of fit based on Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC), and Akaike's Information Criterion Corrected (AICC). For MBN, $\text{NH}_4^+ + \text{NO}_3^-$ -N, and PMN a better fit was

allowed with an unstructured covariance structure, while for K_2SO_4 -C and MBC a better model fit was found using the split-plot approach.

Measurements made on soil removed from cores at the end of the season were analyzed as a split plot completely randomized design using PROC MIXED, with individual plots serving as the whole plot factor and core access level serving as the subplot factor.

5.4 Results and discussion

5.4.1 Effects of N input source and rate on AMF activity and decomposition of buried litter in a field corn setting

Establishing field treatments contrasting both in terms of bare ground versus cover crop mulch and high versus low N allowed for comparing the effects of N source and C and N availability on AMF, soil C signature, and crop yield. Of foremost interest was the effect of N supply and source on turnover of fresh residue input C in the presence of either roots and AMF or AMF alone. As legume cover crops typically fix large quantities of N, it can be expected that the vetch added a larger amount of N to the corn crop than either the LN or WR treatments (Parr et al., 2014). There was a difference in soil C isotopic signature between the treatments with the LN and WR contrasting with HN and HV ($P=0.02$, figure 5.2) regardless of core mesh size. The LN and WR treatments, with presumably lower N input, produced cores with 0.4‰ lower $\delta^{13}C$ values. The mean isotopic signature of all four core types ranged from -25.7 ‰ to -26.1 ‰ $\delta^{13}C$, compared with the starting signature of -26.3‰. The difference between high- and low-N treatments may be due to either more prevalent root or hyphal growth into the cores or a difference in decomposition rates. Although all visible root residues were removed from cores, rhizodeposition makes up 10-40% of plant C assimilation (Grayston et al., 1997) and

would have been a substantial C input, possibly resulting in a signature more like that of C4 corn if root and AMF growth was higher. Furthermore, yield and therefore likely corn biomass (Mousavi et al., 2013) followed the same trend, with lower yielding LN and WR contrasting with high yielding HN and HV ($P=0.03$). Larger corn rhizodeposition, whether in the form of exudates or sloughed cells, or more prevalent AMF hyphae, therefore could've contributed to the lower $\delta^{13}\text{C}$ reading. If any of these inputs were significantly large and of appropriate quality to initiate a priming of decomposition, however, part of the change could've also been due to a loss of C3 C from either the soil or residues.

In a greenhouse experiment Tu et al. (2006) found that adding mineral N to plants colonized by AMF resulted in loss of light fraction soil carbon, which they attributed to enhanced mycorrhizal growth and associated microbial activity. However, unlike this greenhouse study, there were no detectable differences in percent root length colonization (PRLC) by AMF among different N inputs. Colonization of plant roots correlates well with growth of hyphae externally into the soil (Abbott and Robson, 1985; Schweiger et al., 1999), making it unlikely that the similar levels of colonization resulted in divergent amounts of hyphal exudate or turnover to cause the observed dissimilar C signatures.

Over the two times corn plants were sampled for PRLC, not planting a cover crop resulted in higher colonization ($P = 0.007$, figure 5.3). The most likely reason is a difference in temperature between plots that were planted with cover crops and those left bare (Gavito et al., 2005, 2003), since surface cover crop biomass serves to keep soil cooler (Stipesevic and Kladvko, 2005). Biomass from the two cover crops covering the soil surface was considerable, ranging from 7.2 to 8.9 Mg ha^{-1} in rye and 8.5 to 12.4 Mg ha^{-1} in vetch. Other factors known to discourage AMF colonization include excessive amounts of P or N in soil, both all of which

were unlikely in this scenario. Although AMF colonization is responsive to soil N levels and application rates (Azcon et al., 1982; Hepper, 1983; Liu et al., 2000), the lack of a difference either between the two levels of inorganic N or the legume and grass cover crop may reflect that range in soil N was too narrow for this to dominate the effect of soil temperature.

This study offered some evidence of a shift toward a smaller proportion of residue or old C in core soil from plots receiving more N. However, AMF colonization did not follow the same trend, suggesting that mycorrhizal inputs alone were not driving this contrast. There is not enough evidence that specifically roots or AMF are determining the change in the proportion of residue and new C.

5.4.2 Effect of N application rate and root and mycorrhizally mediated N acquisition from decomposing residues in a greenhouse setting

Decomposing plant residues are known to contribute directly to plant N (Bremer and Vankessel, 1992; Thomsen, 1993; Xu et al., 1993). However, how much litter N is delivered by AMF or roots under different levels of soil N availability is less clear. The highest corn aboveground uptake of residue ^{15}N ($\mu\text{g } ^{15}\text{N pot}^{-1}$) occurred in high N pots among N levels ($60 > 30 > 0 \text{ mg N kg}^{-1}$) and in root-access plants among residue access treatments (RIC > MIC > NIC). This adds strong evidence that N availability plays a role in determining plant ability to exploit patches of organic matter in soil for N (Hgaza et al., 2012). Moreover, both root + AMF presence and AMF hyphal presence alone also increased uptake. This did not depend on N rate, indicating both AMF and root delivery were equally stimulated as N went from severely limiting to low to adequate. These levels correspond to field applications of approximately 0, 67, and 134 kg N ha⁻¹ assuming a 15cm depth. Root biomass per pot was increased by N at

each level ($P < 0.0001$), from 2.1g at no N, to 3.5g at low N, to 4.7g at high N, signifying that root exploration of RIC soil may have been higher with elevated N, resulting in higher residue N uptake (Cheng, 2009; Robinson et al., 1999).

In addition to total residue ^{15}N uptake per pot, corn concentrations of residue N (residue N g^{-1} biomass) in plants with root access (RIC) to these organic residues were 43% and 154% higher than those of plants with only AMF access (MIC) or no access (NIC), respectively, regardless of N application rate or harvest date. There were no detectable differences in proportion of residue N between fertilizer application rates, which is understandable as corn root and shoot biomass rose as fertilizer N increased ($P < 0.0001$ each). There was no impact of belowground access to residues on root or shoot biomass production total N plant concentrations, showing that the slightly increased soil volume available in RIC and MIC did not affect plant growth or total N uptake.

Mycorrhizal fungi are known for their role in phosphorus and inorganic N delivery to plants (Frey and Schüepp, 1993). Over the last two decades, evidence has accumulated showing that recently mineralized N from organic residues may also directly serve as N sources for hyphal uptake (Hawkins et al., 2009; Hodge et al., 2001; Kähkölä et al., 2011). However, whether added N suppresses or stimulates this ability has been unclear. There is some indication that adding N, especially from low to adequate levels as in the case of this study, can increase root colonization and extraradical hyphal lengths (Treseder and Allen, 2002). This suggests that cores in pots receiving the highest N level may have been more fully explored by AMF growth. Another possibility is that adding N to the pot soil stimulated decomposition of residues in the core soil, by stimulating mycorrhizal growth and associated microbial activity

(Tu et al., 2006). There was a weak indication of a difference among belowground access ($P = 0.051$) but not N application ($P = 0.72$) in terms of LFOM remaining at the second pot harvest (Table 5.1). It may be that decomposition of the wild oat residues was increased by root or AMF presence. However, a substantial increase in decomposition would likely result in increased microbial biomass as well (Gagnon et al., 2001). No differences were detected for any effect on MBC, MBN, or $\text{NH}_4^+\text{-N}$.

The higher rates of residue N uptake even in NIC's at the highest N level serves as evidence that urea application may have increased mineralization of ^{15}N from the wild oat biomass. If urea was converted to NO_3^- and diffused into the core, this could represent a case of pool substitution or immobilization followed by re-mineralization (Cookson et al., 2000; Jenkinson, 1988). With a C:N ratio of nearly 50:1, the grass leaves and stems likely would've been decomposed by microbes that immobilized soil N, and for which added N would then hasten mineralization (Vitti et al., 2008). Immobilization of applied N into the biomass of residue decomposers may be evidenced by the lack of an increase in nitrate levels within NIC's between 0 and 30 mg kg^{-1} applied N. No-AMF ingrowth cores did have higher concentrations of NO_3^- than RIC's and MIC's regardless of fertilizer rate, showing that roots and AMF were either better able to take up N from the core soil, or prevent it from diffusing into the microcosms. These findings indicate that applying urea-N to corn may enhance plant uptake of residue N, either by direct N-primed decomposition of residues, or by increased root and AMF exploration of decomposing residues.

Plants receiving less urea-N not only took up less residue N, but also a larger proportion of their N originated in residues - 96% more than the low N treatment and 123% more than

high N ($\text{mg residue N g}^{-1}$ corn biomass N, Table 5.2). Similar to plant biomass residue N concentrations, this value can be expected to increase as total N uptake decreases (Rowlings et al., 2016). On the other hand, as a general trend disregarding harvest date and N availability, increasing belowground access to residues allowed plants to obtain a larger proportion of their N from the organic source ($\text{RIC} > \text{MIC} > \text{NIC}$), likely due simply to growth into the labelled-residue soil.

Central to this study was the question of a possible interaction between N rate and either root or AMF uptake of organic matter N. The above N and access main effects are qualified by a three-way interaction between harvest date, access, and N level. Larger differences between RIC versus MIC and NIC plants' proportion of residue N were found when N fertility was either 30 mg kg^{-1} on the first date or not added for the second date (Figure 5.4, figure 5.5). In other words, while plants obtained more of their N from residue in RIC's than MIC's and NIC's, there is an even more substantial difference at very low levels of N. There was no similar interaction for mg total N g^{-1} corn biomass, indicating that this difference was not due simply to lower total N uptake in root cores at these times. Instead, it appears that plants rely more heavily on root uptake of litter N at low levels of N availability. Nevertheless, values were very low, ranging from 0.059 to $1.6 \text{ mg residue N g}^{-1}$ corn biomass N, which is line with those of Kähkölä et al. (2011). This suggests several possibilities. First, N additions are known to directly control native inorganic N influx into roots (Leon et al., 1995). Rye deprived of N is also known to allocate more of its root growth to and increase N uptake in areas of NO_3^- supply (Laine et al., 1998). However, the design of the present study represents a test of N enriched versus non-enriched plants rather than a comparison of single plants with temporally varying N application, and if N was taken directly from residue decomposition hotspots it

probably would have been as NH_4^+ . Furthermore, this doesn't explain the much stronger separation between delivery from root+AMF and AMF alone at low N.

One interesting possible mechanism behind this distinction between roots and AMF or lack of either is that priming has been suggested to depend not just on total C and N inputs, but the relative proportions of both (Diochon et al., 2015). The greatest C priming losses in a study by Guenet et al. occurred when C additions were largest, but C:N ratios were lowest (2010). They attributed this to change in microbial metabolism or community C:N ratio, but there were no differences detected in our case in terms of MBC, MBN, or MBC:MBN. It is possible, therefore, that an increase in microbial "mining," or foraging for N under limiting conditions (Moorhead and Sinsabaugh, 2006), coupled with increased MBN turnover could have allowed corn roots to access this mineralized N. Accordingly, the lowest nitrate levels were seen in root cores in pots receiving no N.

This study adds to the literature reporting that both AMF and roots improve plant uptake of N from organic sources, and that lessening plant N limitation may enhance this effect. In addition, it provides new evidence that the magnitude of priming of N removal from litter is codependent on both rhizosphere and AMF C and N inputs.

5.4.3 Effect of N application rate on root and AMF controls over decomposition in a turfgrass setting

Root bags contained less C and N at retrieval than AMF bags at every date bags were sampled ($P < 0.0001$), with 60% less C and 66% less N on the last sampling date, in September. In addition, both mesh size bags continued to lose C throughout the study from each date to

the next ($P < 0.0001$). Rhizosphere and root C inputs can contribute strongly to priming of soil organic matter (Fu and Cheng, 2002; Kuzyakov and Cheng, 2001), and perennial grasses are known to allocate large proportions of photosynthate belowground (Carbone and Trumbore, 2007). It is possible that zoysia root exudates, fine root turnover, in addition to AMF inputs served as such a source of priming C in this case. On the other hand, the difference observed between root + AMF and AMF litter bags may represent an overrepresentation of the effects of roots and AMF inputs alone on decomposition, as litter bags can result in overestimation of decomposition (Fahey and Arthur, 1994). Smaller mesh openings in AMF bags may have prevented access by arthropod decomposers, which are a key group governing litter mass loss (Heneghan et al., 1999). This factor is especially important in the case of this study because no soil was added to the bags, in contrast with the experiments using soil-filled cores. The fine mesh of the AMF bags lessened soil contact with residues relative to the root+AMF bags. More soil was able to enter the 1.6mm mesh, evidenced by the 166% higher ($P < 0.0001$) total mass in root + AMF bags relative to AMF, as well as visual observation of bag contents. Intimate soil contact is responsible for speeding mineralization in topsoil (Henriksen and Breland, 2002), and these fine mesh bags likely prevented this somewhat.

Lack of a significant effect of N application rate led to the rejection of the hypothesis in this experiment that N availability would affect either root or AMF controls over decomposition. As opposed to intensely managed agricultural soil in the case of the field and greenhouse corn experiments in this chapter, the soil in this zoysia turf system had a relatively high SOC of 1.94%, after years of little soil disturbance and the root, stolon, and leaf litter inputs of a productive perennial plant (Dhital et al., 2010). Soils rich in C and N are more susceptible to priming losses (Hart et al., 1994; Zhang et al., 2013), and soil organic

matter quality may affect priming of decomposition (Hamer and Marschner, 2005). These findings suggest more work needs to be done to understand how soil C quality and content regulate priming losses of recently added C. In addition, they point out the shortcomings of soilless litterbag studies as opposed to those using soil microcosms.

5.4.4 Effect of infection with the beneficial bacterial endophyte Burkholderia phytofirmans PsJN on switchgrass soil C and N cycling.

Overall, there were few detectable differences between soils collected within plots of switchgrass either infected with *B. phytofirmans* or not. Also, there was no discernible influence of the endophyte on soil C and N cycling within core microcosms that either excluded roots and AMF hyphae, AMF hyphae, or allowed root and AMF access. Bacterial inoculation only seemed to have a small negative effect on K₂SO₄ extractable C (Table 5.4) over two sampling dates, amounting to a 9.4% lower level. Plant and mycorrhizal exudate can comprise up to half of dissolved soil C (Hogberg and Hogberg, 2002). *Burkholderia phytofirmans* strain PsJN is known to degrade oxalate, a major component of plant exudation (Dessureault-Rompere et al., 2007). It is likely that *B. phytofirmans* PsJN also colonizes the rhizosphere (Compant et al., 2008) in addition being an endophyte, where it is possible that the bacterium is degrading plant exudates. Nevertheless, it is unlikely this activity could explain most of this difference in total extractable C. Research has found that another plant growth promoting bacterium, *Azospirillum lipoferum* 4B, can qualitatively change the suite of secondary metabolites produced by plant roots (Chamam et al., 2015). Plant physiology likely is altered by endophyte

presence, possibly resulting in tradeoff between root exudation in favor of structural C or enhanced utilization of photosynthate within plant tissues by bacteria.

Regardless of switchgrass endophyte presence, there were significant differences between the soil sampled at core burial and retrieval in terms of extractable C, $\text{NH}_4^+ + \text{NO}_3^- \text{N}$, and MBC. Extractable C declined 6.4%, inorganic N declined 56%, and MBC rose 41%. Extractable C is subject to numerous influences, including temperature and moisture (Campbell et al., 1999) as well as availability of soil organic matter and residues (Embacher et al., 2007). In the case of this study, no substantial C inputs were made available during the growing season besides switchgrass root growth and exudation, meaning that the decline extractable C may be attributable to either differences in weather, decomposition of organic matter and previous seasons' residues, and plant inputs. Inorganic N likely declined substantially simply due to switchgrass plants serving as a N sink, while MBC, which typically largely is composed of C from recent plant additions rather than old SOC (Novara et al., 2014), may have risen along with growing switchgrass root and AMF inputs during the season.

Despite the lack of other detectable effects of infection on soil C and N pools, this study served to further elucidate the impact of roots and AMF on soil C cycling. Cores allowing the ingrowth of roots and AMF hyphae (RIC) had elevated levels of the original C₃-plant-derived C, total C, and total N relative to cores accessible by AMF hyphae (MIC) only (by 12%, 16%, and 13%, respectively, table 5.3). Total C simply would have risen with the ingrowth of root C from C₄ switchgrass, but there was no C₃-C source to increase C₃-C in the root cores. Therefore, roots must have had an inhibitory effect on decomposition relative to AMF hyphae alone. Typically, root priming of decomposition is thought of as the loss of soil C due to increased labile C availability to decomposer microbes (Kuzyakov et al., 2000; Wang et al.,

2016). However, in contrast with positive priming effects, there are examples of negative priming effects by live plant roots that support the conclusion that roots inhibited decomposition (Coq et al., 2011; Saar et al., 2016; Van Der Krift et al., 2002). A drier soil microcosm due to water removal by roots would have prevented RIC's residue from being decomposed, as C mineralization is strongly influenced by soil moisture and wetting-drying cycles (Park et al., 2007; Tucker et al., 2016). The possibility that soil moisture limits C mineralization is an important distinction between the greenhouse corn experiment of this chapter as well as other comparisons of root vs. AMF priming effects where moisture is tightly regulated.

Additionally, preferential substrate utilization could help explain some of the increased loss of C3-C in cores without root C inputs (Cheng and Kuzyakov 2005). Under this hypothesis, microbes preferentially metabolize substrates that are less energy intensive to degrade, such as root exudates. With a C:N ratio of 50:1, the wild oat residues used in this were close to those of grass and forb residues used in another study that found negative plant root priming effects of litter decomposition. They also attributed their findings to preferential substrate utilization, whereby microbes use root C and N rather than residue for their metabolism.

This experiment lends some evidence to support that AMF and roots differ in their regulation over mineralization of recent C additions. However, the direction of root growth's impact was the opposite of what was clearly shown in the corn pot experiment and suggested by the turf grass experiment, with negative priming of mineralization occurring with root + AMF access under switchgrass. This highlights that root and AMF controls over C stability

vary with factors such as soil moisture, native SOC content, plant species, and nutrient availability.

5.5 Conclusions

This series of experiments shows that plant litter in soil can respond in diverse ways to either AMF or root growth and C inputs. In the field, higher corn N inputs in either an inorganic form or from legume residues decreased soil's proportion of residue C, regardless of root or AMF access. Similarly, higher inorganic N can increase mineralization of residue N and translocation to corn plants in the greenhouse. The proportion of corn biomass N delivered by roots from decomposing plant material, however, can actually increase under low N relative to AMF and without both. Roots may have an impact on C loss from decomposing residues in a high SOC soil under turfgrass, but there was no interacting effect of inorganic N application rate. Finally, switchgrass AMF and roots have differing effects on residue C and total N removal from microcosm soil, regardless of plant infection with a beneficial endophytic bacteria.

Acknowledgements

This work was supported by USDA's National Institute of Food and Agriculture [Grant 2012-02978-230561]. Thanks to USDA ARS Plant Science Research Unit for C₃ plant history soil, and to collaborators Dr. Chuansheng Mei and Scott Lowman at Virginia Tech's Institute of Advanced Learning and Research for access to the PsJN site and for field assistance.

5.6 Tables and Figures

5.6.1 Tables

Table 5.1 Greenhouse decomposition core soil parameter summary of ANOVA

| Factor | Level | MBC ^a | | | MBN | | | LFOM | | | NO ₃ ⁻ -N | | | NH ₄ ⁺ -N | | |
|---------------------|------------------|------------------|----------------|------|-------|------|------|-------|-------|-------|---------------------------------|-------|-------|---------------------------------|--------|-------|
| | | P > F | μ ^d | σ/√n | P > F | μ | σ/√n | P > F | μ | σ/√n | P > F | μ | σ/√n | P > F | μ | σ/√n |
| mg kg ⁻¹ | 0 ^b | 0.40 | 233 | 11.1 | 0.72 | 44.6 | 2.75 | 0.72 | 1,156 | 162.8 | <0.0001 | 0.512 | 0.080 | 0.30 | 0.107 | 0.034 |
| nitrogen(N) | 60 | | 190 | 15.3 | | 42.5 | 2.15 | | 1,140 | 150.4 | | 0.965 | 0.223 | | 0.0634 | 0.008 |
| | 90 | | 205 | 28.3 | | 42.1 | 1.83 | | 1,006 | 142.6 | | 2.62 | 0.630 | | 0.116 | 0.022 |
| Access(A) | NIC ^c | 0.76 | 197 | 14.7 | 0.53 | 43.8 | 1.42 | 0.051 | 1,392 | 146.0 | 0.0005 | 2.35 | 0.662 | 0.47 | 0.0813 | 0.010 |
| | MIC | | 212 | 10.9 | | 44.5 | 2.01 | | 1,042 | 121.6 | | 0.972 | 0.334 | | 0.0844 | 0.024 |
| | RIC | | 219 | 30.0 | | 40.8 | 3.00 | | 870.0 | 131.8 | | 0.766 | 0.151 | | 0.121 | 0.033 |
| N x A | | 0.97 | | | 0.67 | | | 0.65 | | | 0.0152 | | | 0.56 | | |

^aMBC: mg microbial biomass C kg⁻¹ soil; MBN: mg microbial biomass N kg⁻¹ soil; LFOM: mg light fraction organic matter kg⁻¹ soil; NO₃⁻-N: mg NO₃⁻-N kg⁻¹ soil; NH₄⁺-N: mg NH₄⁺-N kg⁻¹ soil.

^b mg urea-N applied kg⁻¹ soil

^c NIC: No mycorrhizal/no root ingrowth cores; MIC: Mycorrhizal ingrowth cores; RIC: Root + mycorrhizal ingrowth cores

^dμ: mean; σ/√n: standard error of the mean

Table 5.2 Greenhouse decomposition corn biomass N summary of ANOVA

| | | mg N g ⁻¹ corn biomass | | | corn mg residue ¹⁵ N pot ⁻¹ | | | mg residue N g ⁻¹ corn biomass N | | | | | |
|--------------|------------------|-----------------------------------|------|-------|---|-----|------|---|---|------|-------|--------|---|
| | | P > F | μ | σ/√n | P > F | μ | σ/√n | P > F | μ | σ/√n | | | |
| Nitrogen (N) | 0 ^a | *** | 8.86 | 0.653 | b | *** | 4.79 | 0.654 | b | *** | 0.689 | 0.100 | |
| | 60 | | 11.4 | 1.06 | c | | 5.72 | 0.622 | b | | 0.352 | 0.0470 | |
| | 120 | | 14.1 | 1.17 | a | | 9.24 | 0.983 | a | | 0.309 | 0.0327 | |
| Access (A) | NIC ^b | NS | 11.3 | 1.16 | | *** | 4.66 | 0.616 | c | *** | 0.278 | 0.0473 | c |
| | MIC | | 11.6 | 1.14 | | | 6.70 | 0.809 | b | | 0.434 | 0.0599 | b |
| | RIC | | 11.5 | 1.04 | | | 8.38 | 0.994 | a | | 0.640 | 0.0932 | a |
| Harvest (H) | 1 | *** | 15.3 | 0.632 | a | *** | 4.43 | 0.463 | b | *** | 0.335 | 0.0441 | b |
| | 2 | | 7.69 | 0.348 | b | | 8.73 | 0.700 | a | | 0.556 | 0.0711 | a |
| N x H | | ** | | | | NS | | | | ** | | | |
| N x A | | NS | | | | NS | | | | NS | | | |
| H x A | | NS | | | | NS | | | | NS | | | |
| N x H x A | | NS | | | | NS | | | | * | | | |

^a mg urea-N applied kg⁻¹ soil

^b NIC: No mycorrhizal/no root ingrowth cores; MIC: Mycorrhizal ingrowth cores; RIC: Root + mycorrhizal ingrowth cores

^c Values followed by different letters in a column for a single factor are significantly different at P ≤ 0.05 (LSD).

^d μ: mean; σ/√n: standard error of the mean

Table 5.3 Switchgrass microcosm core means for soil and residue C and N of core contents before core burial and after retrieving cores.

| | g C3-C kg ⁻¹ soil ^b | | g C kg ⁻¹ soil | | mg residue N kg ⁻¹ soil | | mg N kg ⁻¹ soil | | | | | |
|------------------|---|-------------------|---------------------------|-------------------|------------------------------------|-------------------|----------------------------|-------------------|---|------|------|----|
| | μ | σ/\sqrt{n} | μ | σ/\sqrt{n} | μ | σ/\sqrt{n} | μ | σ/\sqrt{n} | | | | |
| Before burial | 14.2 | 0.561 | 14.2 | 0.561 | 76.4 | 21.48 | 839 | 21.5 | | | | |
| After retrieval | | | | | | | | | | | | |
| NIC | 12.0 | 0.728 | ab ^c | 12.6 | 0.779 | ab | 42.7 | 4.561 | a | 918 | 49.0 | ab |
| MIC ^a | 11.8 | 0.335 | b | 12.5 | 0.358 | b | 45.2 | 2.096 | a | 910 | 23.0 | b |
| RIC | 13.2 | 0.343 | a | 14.5 | 0.368 | a | 48.4 | 2.15 | a | 1030 | 23.0 | a |

^aMIC: Mycorrhizal ingrowth cores; NIC: No mycorrhizal/ no root ingrowth cores; RIC: Root ingrowth cores.

^bg C3-C kg⁻¹ soil: g C originating in soil or residue and not root or AMF hyphae; g C kg⁻¹ soil: g total C3+C4-C or root, AMF, original soil, and residue C kg⁻¹ soil; mg residue N kg⁻¹ soil: mg N originating in ¹⁵N labelled wild oat residues kg⁻¹ soil; mg N kg⁻¹ soil: mg total N or soil and residue N kg⁻¹ soil.

^cValues followed by different letters in a column are significantly different at P≤0.05 (LSD).

^d μ : mean; σ/\sqrt{n} : standard error of the mean

Table 5.4 Switchgrass plot soil summary of ANOVA of microbial parameters

| Factor | Level | MBC ^a | | | MBN | | | K ₂ SO ₄ -C | | | NH ₄ ⁺ +NO ₃ ⁻ -N | | | PMN | | | |
|--------------|-------------------|------------------|-----|------|-------|------|------|-----------------------------------|-----|------|---|------|-------|-------|------|-------|-------|
| | | P > F | μ | σ/√n | P > F | μ | σ/√n | P > F | μ | σ/√n | P > F | μ | σ/√n | P > F | μ | σ/√n | |
| Infection(I) | Ctrl ^b | NS | 203 | 13.8 | NS | 54.8 | 2.85 | * | 127 | 3.58 | NS | 3.71 | 0.428 | NS | 2.95 | 0.566 | |
| | PsJN | | 187 | 11.4 | | 50.6 | 2.70 | | | 115 | | 2.48 | | | 3.31 | 0.524 | |
| Date(D) | June ^c | *** | 162 | 10.1 | a | NS | 51.7 | 2.20 | * | 125 | 2.97 | *** | 4.87 | 0.526 | NS | 2.83 | 0.431 |
| | Sept | | 228 | 11.2 | b | | 53.6 | 3.30 | | | 117 | | 3.00 | | | 2.14 | 0.166 |
| D x I | - | NS | - | - | NS | - | - | NS | - | - | NS | - | - | NS | - | - | |

^aMBC: mg microbial biomass C kg⁻¹ soil; MBN: mg microbial biomass N kg⁻¹ soil; K₂SO₄-C: mg extractable C kg⁻¹ soil;

NH₄⁺+NO₃⁻-N: mg NH₄⁺+NO₃⁻-N kg⁻¹ soil; PMN: mg potentially mineralizable N kg⁻¹ soil

^bCtrl: Control plants not infected with endophytic *Burkholderia phytofirmans* strain PsJN; PsJN: plants infected with *B. phytofirmans* PsJN

^cDate: day of soil sampling: June 5, 2015 and September 17, 2015

^dμ: mean; σ/√n: standard error of the mean

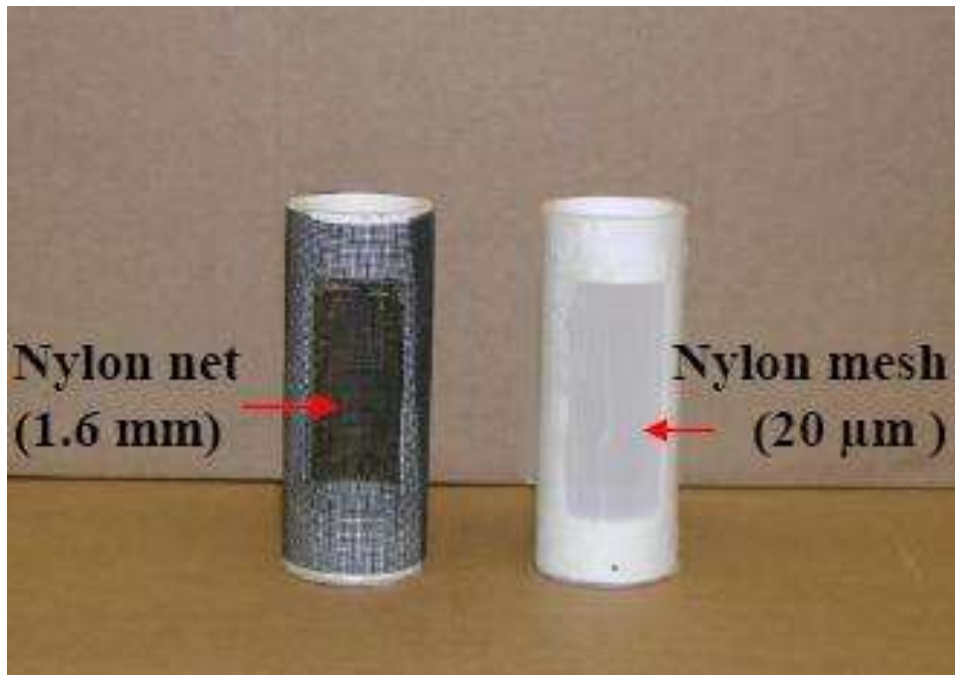
5.6.2 Figures

Figure 5.1 Microcosm root + AMF ingrowth core (RIC, left), and AMF ingrowth core (MIC, right)

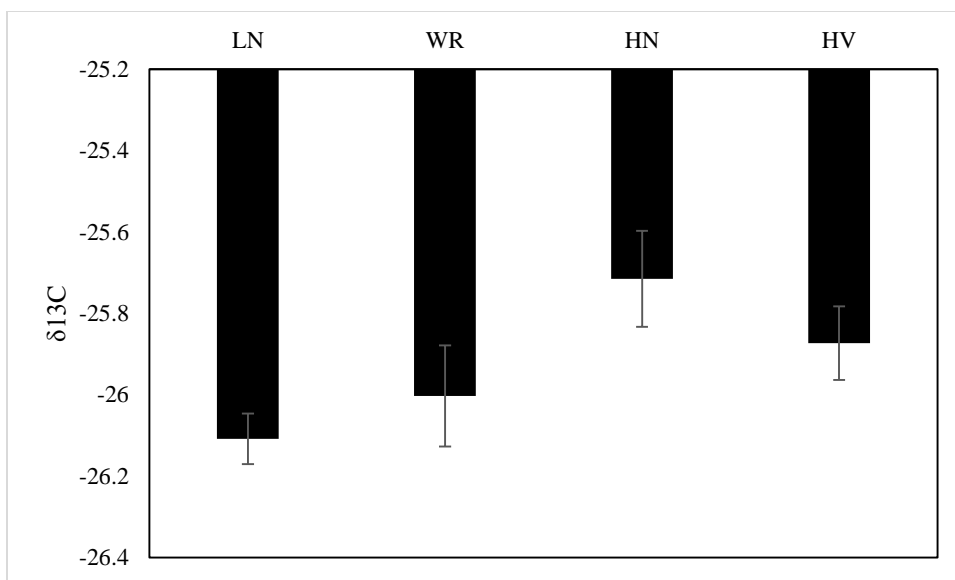


Figure 5.2 Mean isotopic signature of root + AMF and AMF cores following burial. LN: low N; WR: winter rye; HN: high N; HV: hairy vetch. Total application rates were 119.8 kg ha^{-1} in the LN, HV, and WR and 198.2 kg ha^{-1} in HN

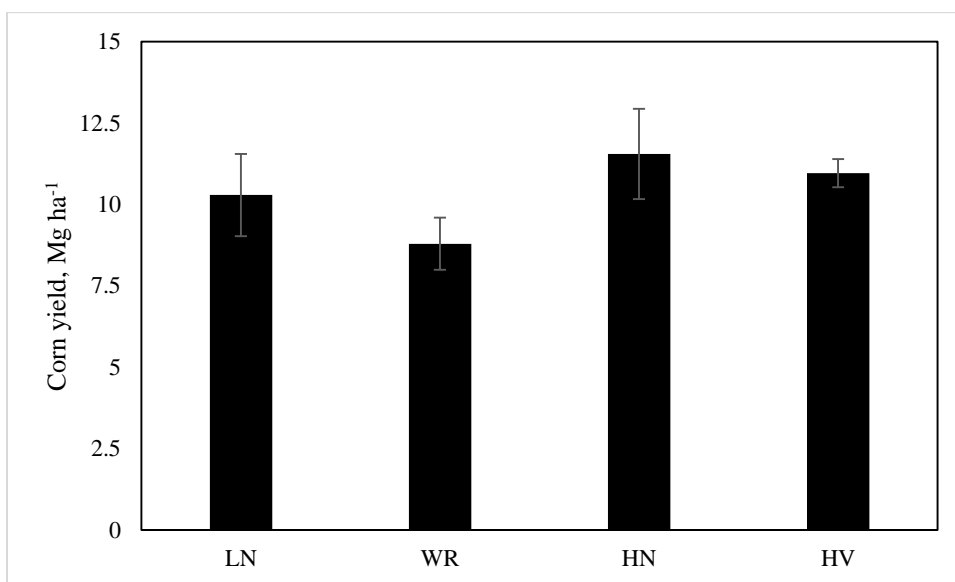


Figure 5.3 Corn yield. LN: low N; WR: winter rye; HN: high N; HV: hairy vetch. Total application rates were 119.8 kg ha^{-1} in the LN, HV, and WR and 198.2 kg ha^{-1} in HN

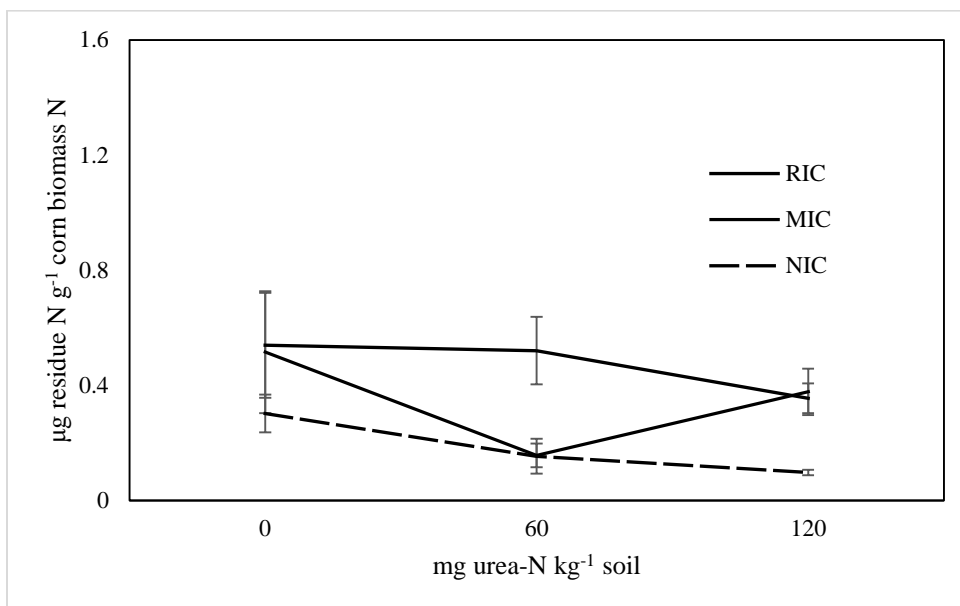


Figure 5.4 Proportion of corn biomass N originating in core residues at first destructive sampling. RIC: root + AMF ingrowth cores; MIC: mycorrhizal ingrowth cores; NIC: no root / no mycorrhizal ingrowth cores

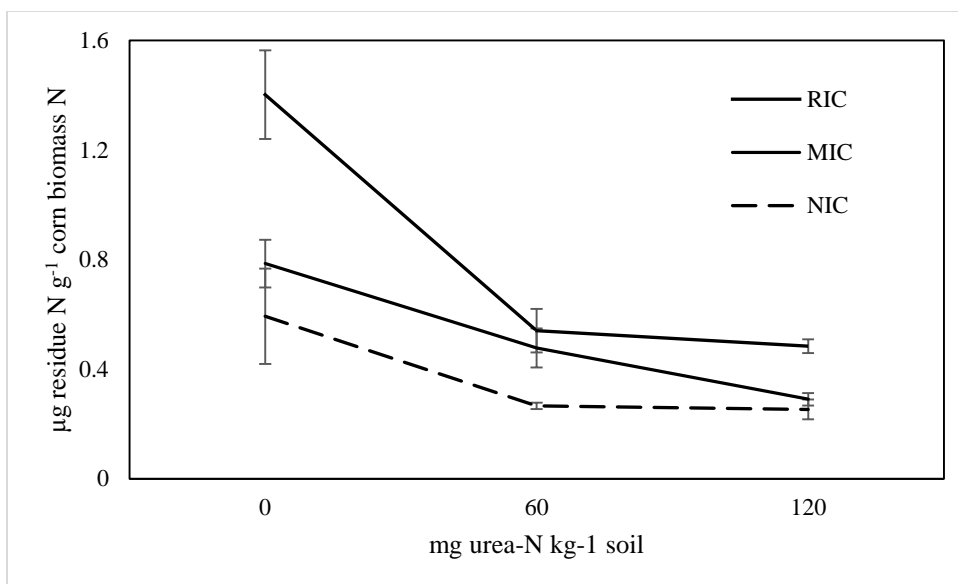


Figure 5.5 Proportion of corn biomass N originating in core residues at second destructive sampling. RIC: root + AMF ingrowth cores; MIC: mycorrhizal ingrowth cores; NIC: no root / no mycorrhizal ingrowth cores

REFERENCES

Abbott, L.K., Robson, A.D., 1985. Formation of external hyphae in soil by four species of vesicular-arbuscular mycorrhizal fungi. *New Phytologist* 99, 245–255.

doi:10.1111/j.1469-8137.1985.tb03653.x

Abdalla, M., Osborne, B., Lanigan, G., Forristal, D., Williams, M., Smith, P., Jones, M.B., 2013. Conservation tillage systems: a review of its consequences for greenhouse gas emissions. *Soil Use Manage* 29, 199–209. doi:10.1111/sum.12030

Abdollahi, L., Munkholm, L.J., 2014. Tillage System and Cover Crop Effects on Soil Quality: I. Chemical, Mechanical, and Biological Properties. *Soil Science Society of America Journal* 78, 262. doi:10.2136/sssaj2013.07.0301

- Aguilera, E., Guzmán, G., Alonso, A., 2014. Greenhouse gas emissions from conventional and organic cropping systems in Spain. II. Fruit tree orchards. *Agron. Sustain. Dev.* 35, 725–737. doi:10.1007/s13593-014-0265-y
- Akinsete, S.J., Nkongolo, N.V., 2016. Soil Carbon and Nitrogen Fractions of a Grassland in Central Missouri, USA. *Communications in Soil Science and Plant Analysis*.
- Alef, K., 1995. Soil Respiration, in: *Methods in Applied Soil Microbiology and Biochemistry*. Academic Press, San Diego, CA, USA, pp. 214–219.
- Angers, D.A., Chantigny, M.H., Rochette, P., Gagnon, B., 2006. Dynamics of soil water-extractable organic C following application of dairy cattle manures. *Can. J. Soil. Sci.* 86, 851–858. doi:10.4141/S05-092
- Asagi, N., Ueno, H., 2009. Nitrogen dynamics in paddy soil applied with various ¹⁵N-labelled green manures. *Plant and Soil* 322, 251–262. doi:10.1007/s11104-009-9913-4
- Asgedom, H., Kebreab, E., 2011. Beneficial management practices and mitigation of greenhouse gas emissions in the agriculture of the Canadian Prairie: a review. *Agronomy Sust. Developm.* 31, 433–451. doi:10.1007/s13593-011-0016-2
- Attard, E., Recous, S., Chabbi, A., De Berranger, C., Guillaumaud, N., Labreuche, J., Philippot, L., Schmid, B., Le Roux, X., 2011. Soil environmental conditions rather than denitrifier abundance and diversity drive potential denitrification after changes in land uses. *Global Change Biology* 17, 1975–1989. doi:10.1111/j.1365-2486.2010.02340.x

- Azcon, R., Gomez-Ortega, M., Barea, J.M., 1982. Comparative Effects of Foliar- or Soil-Applied Nitrate on Vesicular-Arbuscular Mycorrhizal Infection in Maize. *The New Phytologist* 92, 553–559.
- Bader, N.E., Cheng, W., 2007. Rhizosphere priming effect of *Populus fremontii* obscures the temperature sensitivity of soil organic carbon respiration. *Soil Biol. Biochem.* 39, 600–606. doi:10.1016/j.soilbio.2006.09.009
- Baisden, W.T., Amundson, R., Cook, A.C., Brenner, D.L., 2002. Turnover and storage of C and N in five density fractions from California annual grassland surface soils. *Global Biogeochem. Cycles* 16, 1117. doi:10.1029/2001GB001822
- Bajgai, Y., Hulugalle, N., Kristiansen, P., McHenry, M., Cowie, A., 2014. Residue incorporation mitigates tillage-induced loss of soil carbon in laboratory microcosms. *Soil Use and Management* 30, 328–336. doi:10.1111/sum.12130
- Ball, B.C., Crichton, I., Horgan, G.W., 2008. Dynamics of upward and downward N₂O and CO₂ fluxes in ploughed or no-tilled soils in relation to water-filled pore space, compaction and crop presence. *Soil and Tillage Research* 101, 20–30. doi:10.1016/j.still.2008.05.012
- Ban, D., Borosic, J., Znidarcic, D., Ilak Persuric, A., 2008. Potential of hairy vetch and crimson clover as cover crops. *Cereal research communications* 36, 919–922.
- Baral, K.R., Arthur, E., Olesen, J.E., Petersen, S.O., 2016. Predicting nitrous oxide emissions from manure properties and soil moisture: An incubation experiment. *Soil Biol. Biochem.* 97, 112–120. doi:10.1016/j.soilbio.2016.03.005

- Baributsa, D.N., Foster, E.F., Thelen, K.D., Kravchenko, A.N., Mutch, D.R., Ngouajio, M., 2008. Corn and Cover Crop Response to Corn Density in an Interseeding System. *Agronomy Journal* 100, 981–987. doi:10.2134/agronj2007.0110
- Barka, E.A., Nowak, J., Clement, C., 2006. Enhancement of chilling resistance of inoculated grapevine plantlets with a plant growth-promoting rhizobacterium, *Burkholderia phytofirmans* strain PsJN. *Appl. Environ. Microbiol.* 72, 7246–7252. doi:10.1128/AEM.01047-06
- Basche, A.D., Miguez, F.E., Kaspar, T.C., Castellano, M.J., 2014. Do cover crops increase or decrease nitrous oxide emissions? A meta-analysis. *Journal of Soil and Water Conservation* 69, 471–482. doi:10.2489/jswc.69.6.471
- Beare, M.H., Hendrix, P.F., Cabrera, M.L., Coleman, D.C., 1994. Aggregate-Protected and Unprotected Organic Matter Pools in Conventional- and No-Tillage Soils. *Soil Science Society of America Journal* 58, 787. doi:10.2136/sssaj1994.03615995005800030021x
- Benbi, D.K., Brar, K., Toor, A.S., Sharma, S., 2015. Sensitivity of Labile Soil Organic Carbon Pools to Long-Term Fertilizer, Straw and Manure Management in Rice-Wheat System. *Pedosphere* 25, 534–545. doi:10.1016/S1002-0160(15)30034-5
- Bergtold, J.S., Duffy, P.A., Hite, D., Raper, R.L., 2012. Demographic and Management Factors Affecting the Adoption and Perceived Yield Benefit of Winter Cover Crops in the Southeast. *Journal of Agricultural and Applied Economics* 44, 99–116.
- Bhattacharyya, R., Prakash, V., Kundu, S., Srivastva, A.K., Gupta, H.S., 2009. Soil aggregation and organic matter in a sandy clay loam soil of the Indian Himalayas

- under different tillage and crop regimes. *Agriculture, Ecosystems & Environment* 132, 126–134. doi:10.1016/j.agee.2009.03.007
- Bol, R., Poirier, N., Balesdent, J., Gleixner, G., 2009. Molecular turnover time of soil organic matter in particle-size fractions of an arable soil. *Rapid Commun. Mass Spectrom.* 23, 2551–2558. doi:10.1002/rcm.4124
- Bossuyt, H., Six, J., Hendrix, P.F., 2002. Aggregate-protected carbon in no-tillage and conventional tillage agroecosystems using carbon-14 labeled plant residue. *Soil Sci. Soc. Am. J.* 66, 1965–1973.
- Bowden, R., Melillo, J., Steudler, P., Aber, J., 1991. Effects of Nitrogen Additions on Annual Nitrous-Oxide Fluxes from Temperate Forest Soils in the Northeastern United-States. *J. Geophys. Res.-Atmos.* 96, 9321–9328. doi:10.1029/91JD00151
- Bowden, R.D., Melillo, J.M., Steudler, P.A., Aber, J.D., 1991. Effects of nitrogen additions on annual nitrous oxide fluxes from temperate forest soils in the northeastern United States. *Journal of Geophysical Research: Atmospheres* 96, 9321–9328. doi:10.1029/91JD00151
- Bowman, R.A., Reeder, J.D., Lober, R.W., 1990. Changes in soil properties in a Central Plains rangeland soil after 3, 20, and 60 years of cultivation. *Soil Science* 150, 851–857.
- Braker, G., Matthies, D., Hannig, M., Brandt, F.B., Brenzinger, K., Groengroeft, A., 2015. Impact of Land Use Management and Soil Properties on Denitrifier Communities of Namibian Savannas. *Microb. Ecol.* 70, 981–992. doi:10.1007/s00248-015-0623-6
- Bremer, E., Vankessel, C., 1992. Plant-Available Nitrogen from Lentil and Wheat Residues During a Subsequent Growing-Season. *Soil Sci. Soc. Am. J.* 56, 1155–1160.

- Brown, M., 2012. Evaluating Termination Methods of Four Winter Annual Leguminous Cover Crops for Optimizing Nitrogen Synchrony. MS Thesis. Retrieved from <http://www.lib.ncsu.edu/resolver/1840.16/8574>.
- Cabrera, M.L., Beare, M.H., 1993. Alkaline Persulfate Oxidation for Determining Total Nitrogen in Microbial Biomass Extracts. *Soil Science Society of America Journal* 57, 1007. doi:10.2136/sssaj1993.03615995005700040021x
- Calegari, A., Tourdonnet, S., Tessier, D., Rheinheimer, D.S., Ralisch, R., Hargrove, W., Guimarães, M.F., Filho, J.T., 2013. Influence of Soil Management and Crop Rotation on Physical Properties in a Long-Term Experiment in Paraná, Brazil. *Communications in Soil Science and Plant Analysis* 44, 2019–2031. doi:10.1080/00103624.2013.790406
- Campbell, C.A., Lafond, G.P., Biederbeck, V.O., Wen, G., Schoenau, J., Hahn, D., 1999. Seasonal trends in soil biochemical attributes: Effects of crop management on a Black Chernozem. *Can. J. Soil. Sci.* 79, 85–97. doi:10.4141/S98-029
- Carbone, M.S., Trumbore, S.E., 2007. Contribution of new photosynthetic assimilates to respiration by perennial grasses and shrubs: residence times and allocation patterns. *New Phytol.* 176, 124–135. doi:10.1111/j.1469-8137.2007.02153.x
- Cattanio, J.H., Kuehne, R., Vlek, P.L.G., 2008. Organic material decomposition and nutrient dynamics in a mulch system enriched with leguminous trees in the Amazon. *Revista Brasileira de Ciência do Solo* 32, 1073–1086. doi:10.1590/S0100-06832008000300016

- Cavigelli, M.A., Mirsky, S.B., Teasdale, J.R., Spargo, J.T., Doran, J., 2013. Organic grain cropping systems to enhance ecosystem services. *Renew. Agr. Food Syst.* 28, 145–159. doi:10.1017/S1742170512000439
- Chadwick, D., Sommer, S., Thorman, R., Fanguero, D., Cardenas, L., Amon, B., Misselbrook, T., 2011. Manure management: Implications for greenhouse gas emissions. *Animal Feed Science and Technology, Special Issue: Greenhouse Gases in Animal Agriculture - Finding a Balance between Food and Emissions* 166–167, 514–531. doi:10.1016/j.anifeedsci.2011.04.036
- Chamam, A., Wisniewski-Dyé, F., Comte, G., Bertrand, C., Prigent-Combaret, C., 2015. Differential responses of *Oryza sativa* secondary metabolism to biotic interactions with cooperative, commensal and phytopathogenic bacteria. *Planta* 242, 1439–1452. doi:10.1007/s00425-015-2382-5
- Chan, K.Y., Heenan, D.P., Ashley, R., 1994. Seasonal changes in surface aggregate stability under different tillage and crops. *Soil and Tillage Research* 28, 301–314. doi:10.1016/0167-1987(94)90137-6
- Chatskikh, D., Olesen, J.E., 2007. Soil tillage enhanced CO₂ and N₂O emissions from loamy sand soil under spring barley. *Soil and Tillage Research* 97, 5–18. doi:10.1016/j.still.2007.08.004
- Chen, H., Mothapo, N.V., Shi, W., 2014. The significant contribution of fungi to soil N₂O production across diverse ecosystems. *Applied Soil Ecology* 73, 70–77. doi:10.1016/j.apsoil.2013.08.011

- Chen, S., Wang, Y., Hu, Z., Gao, H., 2015. CO₂ emissions from a forest soil as influenced by amendments of different crop straws: Implications for priming effects. *CATENA* 131, 56–63. doi:10.1016/j.catena.2015.03.016
- Cheng, L., Booker, F.L., Tu, C., Burkey, K.O., Zhou, L., Shew, H.D., Ruffly, T.W., Hu, S., 2012. Arbuscular Mycorrhizal Fungi Increase Organic Carbon Decomposition Under Elevated CO₂. *Science* 337, 1084–1087. doi:10.1126/science.1224304
- Cheng, W., 2009. Rhizosphere priming effect: Its functional relationships with microbial turnover, evapotranspiration, and C-N budgets. *Soil Biol. Biochem.* 41, 1795–1801. doi:10.1016/j.soilbio.2008.04.018
- Cheng, W., Kuzyakov, Y., 2002. Root effects on decomposition of organic matter, in: Wright, S.F. (Ed.), *Roots and Soil Management : Interactions between Roots and the Soil, Agronomy ; No. 48*. American Society of Agronomy : Crop Science Society of America : Soil Science Society of America, Madison, Wis.
- Cheng, W.X., Johnson, D.W., Fu, S.L., 2003. Rhizosphere effects on decomposition: Controls of plant species, phenology, and fertilization. *Soil Sci. Soc. Am. J.* 67, 1418–1427.
- Cheshire, M., Mundie, C., 1966. Hydrolytic Extraction of Carbohydrates from Soil by Sulphuric Acid. *Journal of Soil Science* 17, 372-.
- Chung, H., Grove, J.H., Six, J., 2008. Indications for Soil Carbon Saturation in a Temperate Agroecosystem. *Soil Science Society of America Journal* 72, 1132–1139. doi:10.2136/sssaj2007.0265
- Clark, R.B., Zeto, S.K., 2000. Mineral acquisition by arbuscular mycorrhizal plants. *J. Plant Nutr.* 23, 867–902. doi:10.1080/01904160009382068

- Coleman, D.C., Anderson, R.V., Cole, C.V., Elliott, E.T., Woods, L., Campion, M.K., 1977. Trophic interactions in soils as they affect energy and nutrient dynamics. IV. Flows of metabolic and biomass carbon. *Microbial Ecology* 4, 373–380. doi:10.1007/BF02013280
- Compant, S., Kaplan, H., Sessitsch, A., Nowak, J., Ait Barka, E., Clément, C., 2008. Endophytic colonization of *Vitis vinifera* L. by *Burkholderia phytofirmans* strain PsJN: from the rhizosphere to inflorescence tissues. *FEMS Microbiol. Ecol.* 63, 84–93. doi:10.1111/j.1574-6941.2007.00410.x
- Conant, R.T., Easter, M., Paustian, K., Swan, A., Williams, S., 2007. Impacts of periodic tillage on soil C stocks: A synthesis. *Soil and Tillage Research* 95, 1–10. doi:10.1016/j.still.2006.12.006
- Conceição, P.C., Dieckow, J., Bayer, C., 2013. Combined role of no-tillage and cropping systems in soil carbon stocks and stabilization. *Soil and Tillage Research* 129, 40–47. doi:10.1016/j.still.2013.01.006
- Cook, J.C., Gallagher, R.S., Kaye, J.P., Lynch, J., Bradley, B., 2010. Optimizing Vetch Nitrogen Production and Corn Nitrogen Accumulation under No-Till Management. *Agronomy Journal* 102, 1491–1499. doi:10.2134/agronj2010.0165
- Cookson, W.R., Rowarth, J.S., Cameron, K.C., 2000. The fate of residual nitrogen fertiliser applied to a ryegrass (*Lolium perenne* L.) seed crop. *Aust. J. Agric. Res.* 51, 287–294.
- Coq, S., Weigel, J., Butenschoen, O., Bonal, D., Hättenschwiler, S., 2011. Litter composition rather than plant presence affects decomposition of tropical litter mixtures. *Plant Soil* 343, 273–286. doi:10.1007/s11104-011-0717-y

- Cosentino, D., Chenu, C., Le Bissonnais, Y., 2006. Aggregate stability and microbial community dynamics under drying–wetting cycles in a silt loam soil. *Soil Biology and Biochemistry* 38, 2053–2062. doi:10.1016/j.soilbio.2005.12.022
- Crews, T.E., Peoples, M.B., 2005. Can the synchrony of nitrogen supply and crop demand be improved in legume and fertilizer-based agroecosystems? A review. *Nutr. Cycl. Agroecosyst.* 72, 101–120. doi:10.1007/s10705-004-6480-1
- Curtin, D., Francis, G.S., McCallum, F.M., 2008. Decomposition rate of cereal straw as affected by soil placement. *Aust. J. Soil Res.* 46, 152–160. doi:10.1071/SR07085
- Daniel, T.C., Sharpely, A.N., Lemunyon, J.L., 1998. Agricultural phosphorus and eutrophication: A symposium overview. *J. Environ. Qual.* 27, 251–257.
- Davidson, E.A., Kanter, D., 2014. Inventories and scenarios of nitrous oxide emissions. *Environ. Res. Lett.* 9, 105012. doi:10.1088/1748-9326/9/10/105012
- Del Grosso, S.J., Parton, W.J., Mosier, A.R., Ojima, D.S., Kulmala, A.E., Phongpan, S., 2000. General model for N₂O and N₂ gas emissions from soils due to denitrification. *Glob. Biogeochem. Cycle* 14, 1045–1060.
- Dessureault-Rompere, J., Nowack, B., Schulin, R., Luster, J., 2007. Spatial and temporal variation in organic acid anion exudation and nutrient anion uptake in the rhizosphere of *Lupinus albus* L. *Plant Soil* 301, 123–134. doi:10.1007/s11104-007-9427-x
- Dhital, D., Muraoka, H., Yashiro, Y., Shizu, Y., Koizumi, H., 2010. Measurement of net ecosystem production and ecosystem respiration in a *Zoysia japonica* grassland, central Japan, by the chamber method. *Ecol. Res.* 25, 483–493. doi:10.1007/s11284-009-0678-2

- D'Hose, T., Cougnon, M., De Vlieghe, A., Van Bockstaele, E., Reheul, D., 2012. Influence of farm compost on soil quality and crop yields. *Archives of Agronomy and Soil Science* 58, S71–S75. doi:10.1080/03650340.2012.692876
- Diba, F., Shimizu, M., Hatano, R., 2011. Effects of soil aggregate size, moisture content and fertilizer management on nitrous oxide production in a volcanic ash soil. *Soil Science and Plant Nutrition* 57, 733–747. doi:10.1080/00380768.2011.604767
- Ding, G., Liu, X., Herbert, S., Novak, J., Amarasiriwardena, D., Xing, B., 2006. Effect of cover crop management on soil organic matter. *Geoderma* 130, 229–239. doi:10.1016/j.geoderma.2005.01.019
- Diochon, A., Gregorich, E.G., Kellman, L., Morrison, M., Ma, B.-L., 2015. Greater soil C inputs accelerate loss of C in cropping systems with low N input. *Plant Soil* 400, 93–105. doi:10.1007/s11104-015-2718-8
- Dong, W., Hu, C., Zhang, Y., Wu, D., 2012. Gross mineralization, nitrification and N₂O emission under different tillage in the North China Plain. *Nutr Cycl Agroecosyst* 94, 237–247. doi:10.1007/s10705-012-9536-7
- Dormaer, J., 1990. Effect of Active Roots on the Decomposition of Soil Organic Materials. *Biol. Fertil. Soils* 10, 121–126.
- Duong, T.T.T., Baumann, K., Marschner, P., 2009. Frequent addition of wheat straw residues to soil enhances carbon mineralization rate. *Soil Biology and Biochemistry* 41, 1475–1482. doi:10.1016/j.soilbio.2009.04.003
- Eagle, A.J., Olander, L.P., 2012a. Chapter Three - Greenhouse Gas Mitigation with Agricultural Land Management Activities in the United States—A Side-by-Side

- Comparison of Biophysical Potential, in: Sparks, D.L. (Ed.), *Advances in Agronomy*. Academic Press, pp. 79–179.
- Eagle, A.J., Olander, L.P., 2012b. Chapter Three - Greenhouse Gas Mitigation with Agricultural Land Management Activities in the United States—A Side-by-Side Comparison of Biophysical Potential, in: Sparks, D.L. (Ed.), *Advances in Agronomy*. Academic Press, pp. 79–179.
- Elliott, P.W., Knight, D., Anderson, J.M., 1991. Variables controlling denitrification from earthworm casts and soil in permanent pastures. *Biol Fertil Soils* 11, 24–29.
doi:10.1007/BF00335829
- Embacher, A., Zsolnay, A., Gattinger, A., Munch, J.C., 2007. The dynamics of water extractable organic matter (WEOM) in common arable topsoils: I. Quantity, quality and function over a three year period. *Geoderma* 139, 11–22.
doi:10.1016/j.geoderma.2006.12.002
- EPA, 2010. Methane and Nitrous Oxide Emissions from Natural Sources.
- Fahey, T.J., Arthur, M.A., 1994. Further Studies of Root Decomposition Following Harvest of a Northern Hardwoods Forest. *Forest Science* 40, 618–629.
- Farrar, K., Bryant, D., Cope-Selby, N., 2014. Understanding and engineering beneficial plant-microbe interactions: plant growth promotion in energy crops. *Plant Biotechnol. J.* 12, 1193–1206. doi:10.1111/pbi.12279
- Fonseca, G.C., Carneiro, M.A.C., Costa, A.R. da, Oliveira, G.C. de, Balbino, L.C., 2007. Atributos físicos, químicos e biológicos de latossolo vermelho distrófico de cerrado sob duas rotações de cultura. *Pesquisa Agropecuária Tropical (Agricultural Research in the Tropics)* 37, 22–30.

- Formowitz, B., Joergensen, R.G., Buerkert, A., 2009. Impact of legume versus cereal root residues on biological properties of West African soils. *Plant and Soil* 325, 145–156. doi:10.1007/s11104-009-9964-6
- Franzluebbers, A.J., 1999. Microbial activity in response to water-filled pore space of variably eroded southern Piedmont soils. *Appl. Soil Ecol.* 11, 91–101. doi:10.1016/S0929-1393(98)00128-0
- Franzluebbers, A.J., Hons, F.M., Zuberer, D.A., 1995. Tillage and Crop Effects on Seasonal Soil Carbon and Nitrogen Dynamics. *Soil Science Society of America Journal* 59, 1618. doi:10.2136/sssaj1995.03615995005900060016x
- Frey, B., Schüepp, H., 1993. Acquisition of nitrogen by external hyphae of arbuscular mycorrhizal fungi associated with *Zea mays* L. *New Phytologist* 124, 221–230. doi:10.1111/j.1469-8137.1993.tb03811.x
- Fu, S., Cheng, W., 2002. Rhizosphere priming effects on the decomposition of soil organic matter in C4 and C3 grassland soils. *Plant and Soil* 238, 289–294. doi:10.1023/A:1014488128054
- Gagnon, B., Lalande, R., Fahmy, S.H., 2001. Organic matter and aggregation in a degraded potato soil as affected by raw and composted pulp residue. *Biol. Fertil. Soils* 34, 441–447.
- García-Marco, S., Ravella, S.R., Chadwick, D., Vallejo, A., Gregory, A.S., Cárdenas, L.M., 2014. Ranking factors affecting emissions of GHG from incubated agricultural soils. *Eur J Soil Sci* 65, 573–583. doi:10.1111/ejss.12143
- Gavito, M.E., Olsson, P.A., Rouhier, H., Medina-Penafiel, A., Jakobsen, I., Bago, A., Azcon-Aguilar, C., 2005. Temperature constraints on the growth and functioning of root

- organ cultures with arbuscular mycorrhizal fungi. *New Phytol.* 168, 179–188.
doi:10.1111/j.1469-8137.2005.01481.x
- Gavito, M.E., Schweiger, P., Jakobsen, I., 2003. P uptake by arbuscular mycorrhizal hyphae: effect of soil temperature and atmospheric CO₂ enrichment. *Glob. Change Biol.* 9, 106–116. doi:10.1046/j.1365-2486.2003.00560.x
- Giovannetti, M., Mosse, B., 1980. An Evaluation of Techniques for Measuring Vesicular Arbuscular Mycorrhizal Infection in Roots. *The New Phytologist* 84, 489–500.
- Gomez-Rey, M.X., Couto-Vazquez, A., Gonzalez-Prieto, S.J., 2012. Nitrogen transformation rates and nutrient availability under conventional plough and conservation tillage. *Soil Tillage Res.* 124, 144–152. doi:10.1016/j.still.2012.05.010
- Grayston, S.J., Vaughan, D., Jones, D., 1997. Rhizosphere carbon flow in trees, in comparison with annual plants: The importance of root exudation and its impact on microbial activity and nutrient availability. *Appl. Soil Ecol.* 5, 29–56.
doi:10.1016/S0929-1393(96)00126-6
- Greene, C., Dimitri, C., Smith, T., 2009. Emerging Issues in the U.S. Organic Industry (No. Economic Information Bulletin No. (EIB-55)).
- Gregorich, E.G., Kachanoski, R.G., Voroney, R.P., 1989. Carbon mineralization in soil size fractions after various amounts of aggregate disruption. *Journal of Soil Science* 40, 649–659. doi:10.1111/j.1365-2389.1989.tb01306.x
- Gregorich, E.G., Rochette, P., Hopkins, D.W., McKim, U.F., St-Georges, P., 2006. Tillage-induced environmental conditions in soil and substrate limitation determine biogenic gas production. *Soil Biology and Biochemistry* 38, 2614–2628.
doi:10.1016/j.soilbio.2006.03.017

- Guenet, B., Neill, C., Bardoux, G., Abbadie, L., 2010. Is there a linear relationship between priming effect intensity and the amount of organic matter input? *Applied Soil Ecology* 46, 436–442. doi:10.1016/j.apsoil.2010.09.006
- Guggenberger, G., Frey, S.D., Six, J., Paustian, K., Elliott, E.T., 1999. Bacterial and Fungal Cell-Wall Residues in Conventional and No-Tillage Agroecosystems. *Soil Science Society of America Journal* 63, 1188. doi:10.2136/sssaj1999.6351188x
- Halvorson, A.D., Wienhold, B.J., Black, A.L., 2002. Tillage, Nitrogen, and Cropping System Effects on Soil Carbon Sequestration. *Soil Science Society of America Journal* 66, 906. doi:10.2136/sssaj2002.9060
- Hamer, U., Marschner, B., 2005. Priming effects in different soil types induced by fructose, alanine, oxalic acid and catechol additions. *Soil Biol. Biochem.* 37, 445–454. doi:10.1016/j.soilbio.2004.07.037
- Haque, M.M., Kim, S.Y., Ali, M.A., Kim, P.J., 2014. Contribution of greenhouse gas emissions during cropping and fallow seasons on total global warming potential in mono-rice paddy soils. *Plant Soil* 387, 251–264. doi:10.1007/s11104-014-2287-2
- Hart, S.C., Stark, J., Davidson, E., Firestone, M., 1994. Nitrogen mineralization, immobilization and nitrification, in: *Methods of Soil Analysis. Part 2: Microbiological and Biochemical Properties*. Soil Science Society of America, Madison, WI, USA, pp. 985–1018.
- Hawkins, H.-J., Johansen, A., George, E., 2009. Uptake and transport of organic and inorganic nitrogen by arbuscular mycorrhizal fungi. *Plant and Soil* 226, 275–285. doi:10.1023/A:1026500810385

- Haynes, R., Swift, R., 1990. Stability of Soil Aggregates in Relation to Organic-Constituents and Soil-Water Content. *Journal of Soil Science* 41, 73–83.
- Haynes, R.J., 1993. Effect of sample pretreatment on aggregate stability measured by wet sieving or turbidimetry on soils of different cropping history. *Journal of Soil Science* 44, 261–270. doi:10.1111/j.1365-2389.1993.tb00450.x
- Haynes, R.J., Swift, R.S., Stephen, R.C., 1991. Influence of mixed cropping rotations (pasture-arable) on organic matter content, water stable aggregation and clod porosity in a group of soils. *Soil and Tillage Research* 19, 77–87. doi:10.1016/0167-1987(91)90111-A
- Heneghan, L., Coleman, D.C., Zou, X., Crossley, D.A., Haines, B.L., 1999. Soil microarthropod contributions to decomposition dynamics: Tropical-temperate comparisons of a single substrate. *Ecology* 80, 1873–1882.
- Henriksen, T.M., Breland, T.A., 2002. Carbon mineralization, fungal and bacterial growth, and enzyme activities as affected by contact between crop residues and soil. *Biol. Fertil. Soils* 35, 41–48. doi:10.1007/s00374-001-0438-0
- Hepper, C.M., 1983. The Effect of Nitrate and Phosphate on the Vesicular-Arbuscular Mycorrhizal Infection of Lettuce. *New Phytologist* 93, 389–399. doi:10.1111/j.1469-8137.1983.tb03439.x
- Hgaza, V.K., Diby, L.N., Oberson, A., Tschannen, A., Tie, B.T., Sangakkara, U.R., Ake, S., Frossard, E., 2012. Nitrogen Use by Yam as Affected by Mineral Fertilizer Application. *Agron. J.* 104, 1558–1568. doi:10.2134/agronj2011.0387

- Higashi, T., Yunghui, M., Komatsuzaki, M., Miura, S., Hirata, T., Araki, H., Kaneko, N., Ohta, H., 2014. Tillage and cover crop species affect soil organic carbon in Andosol, Kanto, Japan. *Soil and Tillage Research* 138, 64–72. doi:10.1016/j.still.2013.12.010
- Hodge, A., 2003. Plant nitrogen capture from organic matter as affected by spatial dispersion, interspecific competition and mycorrhizal colonization. *New Phytologist* 157, 303–314. doi:10.1046/j.1469-8137.2003.00662.x
- Hodge, A., Campbell, C.D., Fitter, A.H., 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature* 413, 297–299. doi:10.1038/35095041
- Hodge, A., Helgason, T., Fitter, A.H., 2010. Nutritional ecology of arbuscular mycorrhizal fungi. *Fungal Ecol.* 3, 267–273. doi:10.1016/j.funeco.2010.02.002
- Hoffman, M.L., Regnier, E.E., Cardina, J., 1993. Weed and Corn (*Zea mays*) Responses to a Hairy Vetch (*Vicia villosa*) Cover Crop. *Weed Technology* 7, 594–599.
- Hogberg, M.N., Hogberg, P., 2002. Extramatrical ectomycorrhizal mycelium contributes one-third of microbial biomass and produces, together with associated roots, half the dissolved organic carbon in a forest soil. *New Phytol.* 154, 791–795. doi:10.1046/j.1469-8137.2002.00417.x
- Hosseini, F., Mosaddeghi, M.R., Hajabbasi, M.A., Sabzalian, M.R., 2015. Influence of tall fescue endophyte infection on structural stability as quantified by high energy moisture characteristic in a range of soils. *Geoderma* 249, 87–99. doi:10.1016/j.geoderma.2015.03.013

- Hou, Y., Velthof, G.L., Oenema, O., 2015. Mitigation of ammonia, nitrous oxide and methane emissions from manure management chains: a meta-analysis and integrated assessment. *Glob. Change Biol.* 21, 1293–1312. doi:10.1111/gcb.12767
- Hu, S., Coleman, D.C., Beare, M.H., Hendrix, P.F., 1995. Soil carbohydrates in aggrading and degrading agroecosystems: influences of fungi and aggregates. *Agriculture, Ecosystems & Environment* 54, 77–88. doi:10.1016/0167-8809(95)00588-J
- Huang, S., Lv, W., Bloszies, S., Shi, Q., Pan, X., Zeng, Y., 2016. Effects of fertilizer management practices on yield-scaled ammonia emissions from croplands in China: A meta-analysis. *Field Crop. Res.* 192, 118–125. doi:10.1016/j.fcr.2016.04.023
- Huang, Y., Zou, J., Zheng, X., Wang, Y., Xu, X., 2004. Nitrous oxide emissions as influenced by amendment of plant residues with different C:N ratios. *Soil Biology and Biochemistry* 36, 973–981. doi:10.1016/j.soilbio.2004.02.009
- Hussain, S., Peng, S., Fahad, S., Khaliq, A., Huang, J., Cui, K., Nie, L., 2014. Rice management interventions to mitigate greenhouse gas emissions: a review. *Environ Sci Pollut Res* 22, 3342–3360. doi:10.1007/s11356-014-3760-4
- Ibewiro, B., Sanginga, N., Vanlauwe, B., Merckx, R., 2000. Transformations and recovery of residue and fertilizer nitrogen-15 in a sandy Lixisol of West Africa. *Biol Fertil Soils* 31, 261–269. doi:10.1007/s003740050655
- Iqbal, J., Parkin, T.B., Helmers, M.J., Zhou, X., Castellano, M.J., 2015. Denitrification and Nitrous Oxide Emissions in Annual Croplands, Perennial Grass Buffers, and Restored Perennial Grasslands. *Soil Science Society of America Journal* 79, 239. doi:10.2136/sssaj2014.05.0221

- Jahangir, M.M.R., Minet, E.P., Johnston, P., Premrov, A., Coxon, C.E., Hackett, R., Richards, K.G., 2014. Mustard catch crop enhances denitrification in shallow groundwater beneath a spring barley field. *Chemosphere* 103, 234–239. doi:10.1016/j.chemosphere.2013.11.072
- Jakobsen, I., Rosendahl, L., 1990. Carbon flow into soil and external hyphae from roots of mycorrhizal cucumber plants. *New Phytologist* 115, 77–83. doi:10.1111/j.1469-8137.1990.tb00924.x
- Jani, A.D., Grossman, J.M., Smyth, T.J., Hu, S., 2015. Influence of soil inorganic nitrogen and root diameter size on legume cover crop root decomposition and nitrogen release. *Plant and Soil* 393, 57–68. doi:10.1007/s11104-015-2473-x
- Jastrow, J.D., 1996. Soil aggregate formation and the accrual of particulate and mineral-associated organic matter. *Soil Biology and Biochemistry* 28, 665–676. doi:10.1016/0038-0717(95)00159-X
- Jenkinson, D.S., 1988. Determination of microbial biomass carbon and nitrogen in soil, in: *Advances in Nitrogen Cycling in Agricultural Ecosystems*. CAB International, Wallingford, UK, pp. 368–386.
- Kähkölä, A.-K., Nygren, P., Leblanc, H.A., Pennanen, T., Pietikäinen, J., 2011. Leaf and root litter of a legume tree as nitrogen sources for cacaos with different root colonisation by arbuscular mycorrhizae. *Nutr Cycl Agroecosyst* 92, 51–65. doi:10.1007/s10705-011-9471-z
- Kantar, M.B., Tyl, C.E., Dorn, K.M., Zhang, X., Jungers, J.M., Kaser, J.M., Schendel, R.R., Eckberg, J.O., Runck, B.C., Bunzel, M., Jordan, N.R., Stupar, R.M., Marks, M.D., Anderson, J.A., Johnson, G.A., Sheaffer, C.C., Schoenfuss, T.C., Ismail, B., Heimpel,

- G.E., Wyse, D.L., 2016. Perennial Grain and Oilseed Crops, in: Merchant, S.S. (Ed.), Annual Review of Plant Biology, Vol 67. Annual Reviews, Palo Alto, pp. 703–729.
- Karlen, D.L., Wollenhaupt, N.C., Erbach, D.C., Berry, E.C., Swan, J.B., Eash, N.S., Jordahl, J.L., 1994. Crop residue effects on soil quality following 10-years of no-till corn. Soil and Tillage Research 31, 149–167. doi:10.1016/0167-1987(94)90077-9
- Karrou, M., Maranville, J., 1993. Seedling Vigor and Nitrogen Use Efficiency of Moroccan Wheat as Influenced by Level of Soil-Nitrogen. Commun. Soil Sci. Plant Anal. 24, 1153–1163. doi:10.1080/00103629309368867
- Ker, K., Seguin, P., Driscoll, B.T., Fyles, J.W., Smith, D.L., 2014. Evidence for enhanced N availability during switchgrass establishment and seeding year production following inoculation with rhizosphere endophytes. Arch. Agron. Soil Sci. 60, 1553–1563. doi:10.1080/03650340.2014.898840
- Kibet, L.C., Bryant, R.B., Buda, A.R., Kleinman, P.J.A., Saporito, L.S., Allen, A.L., Hashem, F.M., May, E.B., 2016. Persistence and Surface Transport of Urea-Nitrogen: A Rainfall Simulation Study. Journal of Environmental Quality 45, 1062–1070. doi:10.2134/jeq2015.09.0495
- Kim, S., Lowman, S., Hou, G., Nowak, J., Flinn, B., Mei, C., 2012. Growth promotion and colonization of switchgrass (*Panicum virgatum*) cv. Alamo by bacterial endophyte *Burkholderia phytofirmans* strain PsJN. Biotechnol. Biofuels 5, 37. doi:10.1186/1754-6834-5-37
- King, L., Ball, P., 1992. Denitrification in a White Clover Sward Killed by Plowing, Rotary Hoeing, Shallow Tillage, or with a Herbicide. N. Z. J. Agric. Res. 35, 441–450.

- Kitur, B., Phillips, S., Olson, K., Ebelhar, S., 1994. Tillage Effects on Selected Chemical-Properties of Grantsburg Silt Loam. *Commun. Soil Sci. Plant Anal.* 25, 225–246.
doi:10.1080/00103629409369033
- Koester, J.R., Cardenas, L.M., Bol, R., Lewicka-Szczebak, D., Senbayram, M., Well, R., Gieseemann, A., Dittert, K., 2015. Anaerobic digestates lower N₂O emissions compared to cattle slurry by affecting rate and product stoichiometry of denitrification - An N₂O isotopomer case study. *Soil Biol. Biochem.* 84, 65–74.
doi:10.1016/j.soilbio.2015.01.021
- Kong, A.Y.Y., Six, J., 2012. Microbial community assimilation of cover crop rhizodeposition within soil microenvironments in alternative and conventional cropping systems. *Plant and Soil* 356, 315–330. doi:10.1007/s11104-011-1120-4
- Kong, A.Y.Y., Six, J., Bryant, D.C., Denison, R.F., van Kessel, C., 2005. The Relationship between Carbon Input, Aggregation, and Soil Organic Carbon Stabilization in Sustainable Cropping Systems. *Soil Science Society of America Journal* 69, 1078.
doi:10.2136/sssaj2004.0215
- Kuang, E., Chi, F., Jeng, A.S., Su, Q., Zhang, J., 2014. A comparison of different methods of decomposing maize straw in China. *Acta Agric. Scand. Sect. B-Soil Plant Sci.* 63, 186–194. doi:10.1080/09064710.2014.899615
- Kuo, S., Sainju, U.M., Jellum, E.J., 1997. Winter Cover Crop Effects on Soil Organic Carbon and Carbohydrate in Soil. *Soil Science Society of America Journal* 61, 145.
doi:10.2136/sssaj1997.03615995006100010022x

- Kuzyakov, Y., Cheng, W., 2001. Photosynthesis controls of rhizosphere respiration and organic matter decomposition. *Soil Biol. Biochem.* 33, 1915–1925.
doi:10.1016/S0038-0717(01)00117-1
- Kuzyakov, Y., Friedel, J.K., Stahr, K., 2000. Review of mechanisms and quantification of priming effects. *Soil Biol. Biochem.* 32, 1485–1498. doi:10.1016/S0038-0717(00)00084-5
- Laine, P., Ourry, A., Boucaud, J., Salette, J., 1998. Effects of a localized supply of nitrate on NO₃- uptake rate and growth of roots in *Lolium multiflorum* Lam. *Plant Soil* 202, 61–67. doi:10.1023/A:1004304415010
- Larkin, R.P., Honeycutt, C.W., Olanya, O.M., 2011. Management of Verticillium Wilt of Potato with Disease-Suppressive Green Manures and as Affected by Previous Cropping History. *PLANT DIS.* 95, 568–576. doi:10.1094/PDIS-09-10-0670
- Larsen, E., Grossman, J., Edgell, J., Hoyt, G., Osmond, D., Hu, S., 2014. Soil biological properties, soil losses and corn yield in long-term organic and conventional farming systems. *Soil and Tillage Research* 139, 37–45. doi:10.1016/j.still.2014.02.002
- Laudicina, V.A., Novara, A., Gristina, L., Badalucco, L., 2014. Soil carbon dynamics as affected by long-term contrasting cropping systems and tillages under semiarid Mediterranean climate. *Applied Soil Ecology* 73, 140–147.
doi:10.1016/j.apsoil.2013.09.002
- Leon, M., Laine, P., Ourry, A., Boucaud, J., 1995. Increased Uptake of Native Soil-Nitrogen by Roots of *Lolium-Multiflorum* Lam After Nitrogen-Fertilization Is Explained by a Stimulation of the Uptake Process Itself. *Plant Soil* 173, 197–203.
doi:10.1007/BF00011456

- Li, X., Sorensen, P., Olesen, J.E., Petersen, S.O., 2016. Evidence for denitrification as main source of N₂O emission from residue-amended soil. *Soil Biol. Biochem.* 92, 153–160. doi:10.1016/j.soilbio.2015.10.008
- Liang, S., Grossman, J., Shi, W., 2014. Soil microbial responses to winter legume cover crop management during organic transition. *European Journal of Soil Biology* 65, 15–22. doi:10.1016/j.ejsobi.2014.08.007
- Liu, A., Hamel, C., Hamilton, R.I., Smith, D.L., 2000. Mycorrhizae formation and nutrient uptake of new corn (*Zea mays* L.) hybrids with extreme canopy and leaf architecture as influenced by soil N and P levels. *Plant and Soil* 221, 157–166. doi:10.1023/A:1004777821422
- Liu, B., Tu, C., Hu, S., Gumpertz, M., Ristaino, J.B., 2007. Effect of organic, sustainable, and conventional management strategies in grower fields on soil physical, chemical, and biological factors and the incidence of Southern blight. *Appl. Soil Ecol.* 37, 202–214. doi:10.1016/j.apsoil.2007.06.007
- Liu, J., Qiu, W., 2016. Nitrous oxide emissions from solar greenhouses during summer fallow in the Loess Plateau of China. *Greenh. Gases* 6, 416–427. doi:10.1002/ghg.1573
- Loro, P.J., Bergstrom, D.W., Beauchamp, E.G., 1997. Intensity and duration of denitrification following application of manure and fertilizer to soil. *J. Environ. Qual.* 26, 706–713.
- Lowman, J.S., Lava-Chavez, A., Kim-Dura, S., Flinn, B., Nowak, J., Mei, C., 2014. Switchgrass Field Performance on Two Soils as Affected by Bacterization of

- Seedlings with *Burkholderia phytofirmans* Strain PsJN. *Bioenerg. Res.* 8, 440–449.
doi:10.1007/s12155-014-9536-3
- Lynch, M.J., Mulvaney, M.J., Hodges, S.C., Thompson, T.L., Thomason, W.E., 2016. Decomposition, nitrogen and carbon mineralization from food and cover crop residues in the central plateau of Haiti. *SpringerPlus* 5, 1. doi:10.1186/s40064-016-2651-1
- Mannheim, T., Braschkat, J., Marschner, H., 1995. Reduction of ammonia emissions after application of liquid cattle manure on arable land and grassland: Comparison of wide spread application, application in narrow bands and injection. *Z. Pflanzen. Bodenk.* 158, 535–542.
- Maughan, M.W., Flores, J.P.C., Anghinoni, I., Bollero, G., Fernández, F.G., Tracy, B.F., 2009. Soil Quality and Corn Yield under Crop–Livestock Integration in Illinois. *Agronomy Journal* 101, 1503. doi:10.2134/agronj2009.0068
- Mazzilli, S.R., Kemanian, A.R., Ernst, O.R., Jackson, R.B., Piñeiro, G., 2014. Priming of soil organic carbon decomposition induced by corn compared to soybean crops. *Soil Biology and Biochemistry* 75, 273–281. doi:10.1016/j.soilbio.2014.04.005
- Mbuthia, L.W., Acosta-Martínez, V., DeBruyn, J., Schaeffer, S., Tyler, D., Odoi, E., Mpheshea, M., Walker, F., Eash, N., 2015. Long term tillage, cover crop, and fertilization effects on microbial community structure, activity: Implications for soil quality. *Soil Biology and Biochemistry* 89, 24–34. doi:10.1016/j.soilbio.2015.06.016
- Mikha, M.M., Rice, C.W., 2004. Tillage and manure effects on soil and aggregate-associated carbon and nitrogen. *Soil Sci. Soc. Am. J.* 68, 809–816.

- Mirsky, S.B., Ryan, M.R., Curran, W.S., Teasdale, J.R., Maul, J., Spargo, J.T., Moyer, J., Grantham, A.M., Weber, D., Way, T.R., Camargo, G.G., 2012. Conservation tillage issues: Cover crop-based organic rotational no-till grain production in the mid-Atlantic region, USA. *Renewable Agriculture and Food Systems* 27, 31–40. doi:10.1017/S1742170511000457
- Mischler, R., Duiker, S.W., Curran, W.S., Wilson, D., 2010. Hairy Vetch Management for No-Till Organic Corn Production. *Agronomy Journal* 102, 355–362. doi:10.2134/agronj2009.0183
- Mitchell, D.C., Castellano, M.J., Sawyer, J.E., Pantoja, J., 2013. Cover Crop Effects on Nitrous Oxide Emissions: Role of Mineralizable Carbon. *Soil Science Society of America Journal* 77, 1765. doi:10.2136/sssaj2013.02.0074
- Moorhead, D.L., Sinsabaugh, R.L., 2006. A Theoretical Model of Litter Decay and Microbial Interaction. *Ecological Monographs* 76, 151–174. doi:10.1890/0012-9615(2006)076[0151:ATMOLD]2.0.CO;2
- Mousavi, H., Lack, S., Fazel, M.A., 2013. Analysis of Correlation and Stepwise Regression between Grain Protein Yield and Related Traits of Maize in Conditions of Drought Stress and Zinc Sulfate Spraying. *International Journal of Agriculture and Crop Sciences* 5, 2783.
- Mueller, C.W., Schlund, S., Prietzel, J., Koegel-Knabner, I., Gutsch, M., 2012. Soil Aggregate Destruction by Ultrasonication Increases Soil Organic Matter Mineralization and Mobility. *Soil Sci. Soc. Am. J.* 76, 1634–1643. doi:10.2136/sssaj2011.0186

- Mueller, J.P., Barbercheck, M.E., Bell, M., Brownie, C., Creamer, N.G., Hitt, A., Hu, S., King, L., Linker, H.M., Louws, F.J., Marlow, S., Marra, M., Raczkowski, C.W., Susko, D.J., Waggoner, M.G., 2002. Development and Implementation of a Long-term Agricultural Systems Study: Challenges and Opportunities. *HortTechnology* 12, 362–368.
- Negassa, W., Price, R.F., Basir, A., Snapp, S.S., Kravchenko, A., 2015. Cover crop and tillage systems effect on soil CO₂ and N₂O fluxes in contrasting topographic positions. *Soil Tillage Res.* 154, 64–74. doi:10.1016/j.still.2015.06.015
- Neogi, S., Bhattacharyya, P., Roy, K.S., Panda, B.B., Nayak, A.K., Rao, K.S., Manna, M.C., 2014. Soil respiration, labile carbon pools, and enzyme activities as affected by tillage practices in a tropical rice-maize-cowpea cropping system. *Environ. Monit. Assess.* 186, 4223–4236. doi:10.1007/s10661-014-3693-x
- Nett, L., Fuss, R., Flessa, H., Fink, M., 2015. Emissions of nitrous oxide and ammonia from a sandy soil following surface application and incorporation of cauliflower leaf residues. *J. Agric. Sci.* 153, 1341–1352. doi:10.1017/S0021859615000027
- Nguyen, B.T., Hoa, H.T., Ngo, V.T.H., Duong, T.T., Wilson, B.R., 2011. Comparative study of soil properties under various cultivation regimes of different crops. *Soil Res.* 49, 595–605.
- Ngwira, A., Sleutel, S., De Neve, S., 2012. Soil carbon dynamics as influenced by tillage and crop residue management in loamy sand and sandy loam soils under smallholder farmers' conditions in Malawi. *Nutrient Cycling in Agroecosystems* 92, 315–328. doi:10.1007/s10705-012-9492-2

- Nicolardot, B., Recous, S., Mary, B., 2001. Simulation of C and N mineralisation during crop residue decomposition: A simple dynamic model based on the C:N ratio of the residues. *Plant and Soil* 228, 83–103. doi:10.1023/A:1004813801728
- Novak, S.M., Fiorelli, J.L., 2011. Greenhouse Gases and Ammonia Emissions from Organic Mixed Crop-Dairy Systems: A Critical Review of Mitigation Options, in: Lichtfouse, E., Hamelin, M., Navarrete, M., Debaeke, P. (Eds.), *Sustainable Agriculture Volume 2*. Springer Netherlands, pp. 529–556. doi:10.1007/978-94-007-0394-0_24
- Novara, A., La Mantia, T., Ruehl, J., Badalucco, L., Kuzyakov, Y., Gristina, L., Laudicina, V.A., 2014. Dynamics of soil organic carbon pools after agricultural abandonment. *Geoderma* 235, 191–198. doi:10.1016/j.geoderma.2014.07.015
- Nugroho, S., Kuwatsuka, S., 1992. Concurrent Observation of Several Processes of Nitrogen-Metabolism in Soil Amended with Organic Materials .4. Regulatory Effects of Ammonium-Nitrogen and Nitrate-Nitrogen on Denitrification and N₂ Fixation. *Soil Sci. Plant Nutr.* 38, 611–617.
- Oades, J.M., 1984. Soil organic matter and structural stability: mechanisms and implications for management. *Plant and Soil* 76, 319–337. doi:10.1007/BF02205590
- O’Connell, S., Grossman, J.M., Hoyt, G.D., Shi, W., Bowen, S., Marticorena, D.C., Fager, K.L., Creamer, N.G., 2015a. A survey of cover crop practices and perceptions of sustainable farmers in North Carolina and the surrounding region. *Renew. Agr. Food Syst.* 30, 550–562. doi:10.1017/S1742170514000398
- O’Connell, S., Shi, W., Grossman, J.M., Hoyt, G.D., Fager, K.L., Creamer, N.G., 2015b. Short-term nitrogen mineralization from warm-season cover crops in organic farming systems. *Plant Soil* 396, 353–367. doi:10.1007/s11104-015-2594-2

- Palm, C.A., Sanchez, P.A., 1990. Decomposition and Nutrient Release Patterns of the Leaves of Three Tropical Legumes. *Biotropica* 22, 330. doi:10.2307/2388550
- Palma, R.M., Rímolo, M., Saubidet, M.I., Conti, M.E., 1997. Influence of tillage system on denitrification in maize-cropped soils. *Biol Fertil Soils* 25, 142–146. doi:10.1007/s003740050294
- Park, E.-J., Sul, W.J., Smucker, A.J.M., 2007. Glucose additions to aggregates subjected to drying/wetting cycles promote carbon sequestration and aggregate stability. *Soil Biol. Biochem.* 39, 2758–2768. doi:10.1016/j.soilbio.2007.06.007
- Parr, M., Grossman, J.M., Reberg-Horton, S.C., Brinton, C., Crozier, C., 2014. Roller-Crimper Termination for Legume Cover Crops in North Carolina: Impacts on Nutrient Availability to a Succeeding Corn Crop. *Commun. Soil Sci. Plant Anal.* 45, 1106–1119. doi:10.1080/00103624.2013.867061
- Parr, M., Grossman, J.M., Reberg-Horton, S.C., Brinton, C., Crozier, C., 2011. Nitrogen Delivery from Legume Cover Crops in No-Till Organic Corn Production. *Agronomy Journal* 103, 1578. doi:10.2134/agronj2011.0007
- Parrish, D.J., Fike, J.H., 2005. The biology and agronomy of switchgrass for biofuels. *Crit. Rev. Plant Sci.* 24, 423–459. doi:10.1080/07352680500316433
- Passianoto, C.C., Ahrens, T., Feigl, B.J., Steudler, P.A., Carmo, J.B. do, Melillo, J.M., 2003. Emissions of CO₂, N₂O, and NO in conventional and no-till management practices in Rondônia, Brazil. *Biol Fertil Soils* 38, 200–208. doi:10.1007/s00374-003-0653-y
- Paul, J., Beauchamp, E., 1994. Short-Term Nitrogen Dynamics in Soil Amended with Fresh and Composted Cattle Manures. *Can. J. Soil Sci.* 74, 147–155.

- Phillips, D.L., Gregg, J.W., 2001. Uncertainty in source partitioning using stable isotopes. *Oecologia* 127, 171–179. doi:10.1007/s004420000578
- Phillips, J.M., Hayman, D.S., 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* 55, 158-IN18. doi:10.1016/S0007-1536(70)80110-3
- Powlson, D.S., Bhogal, A., Chambers, B.J., Coleman, K., Macdonald, A.J., Goulding, K.W.T., Whitmore, A.P., 2012. The potential to increase soil carbon stocks through reduced tillage or organic material additions in England and Wales: A case study. *Agriculture, Ecosystems & Environment* 146, 23–33. doi:10.1016/j.agee.2011.10.004
- Pramanik, P., Haque, M.M., Kim, S.Y., Kim, P.J., 2014. C and N accumulations in soil aggregates determine nitrous oxide emissions from cover crop treated rice paddy soils during fallow season. *Science of The Total Environment* 490, 622–628. doi:10.1016/j.scitotenv.2014.05.046
- Qin, Z., Zhuang, Q., Chen, M., 2012. Impacts of land use change due to biofuel crops on carbon balance, bioenergy production, and agricultural yield, in the conterminous United States. *GCB Bioenergy* 4, 277–288. doi:10.1111/j.1757-1707.2011.01129.x
- Qiu, Q., Wu, L., Ouyang, Z., Li, B., Xu, Y., Wu, S., Gregorich, E.G., 2015. Effects of plant-derived dissolved organic matter (DOM) on soil CO₂ and N₂O emissions and soil carbon and nitrogen sequestrations. *Applied Soil Ecology* 96, 122–130. doi:10.1016/j.apsoil.2015.07.016
- Qiu, S., Ju, X., Li, L., Christie, P., Ingwersen, J., Stange, C.F., Streck, T., 2015. Nitrate Transformation and N₂O Emission in a Typical Intensively Managed Calcareous

- Fluvaquent Soil: A ¹⁵-Nitrogen Tracer Incubation Study. *Communications in Soil Science and Plant Analysis* 46, 1763–1777. doi:10.1080/00103624.2015.1044112
- Quemada, M., Cabrera, M.L., 1997. Temperature and moisture effects on C and N mineralization from surface applied clover residue. *Plant and Soil* 189, 127–137. doi:10.1023/A:1004281804058
- Ramaswamy, V., Boucher, O., Haigh, J., Hauglustaine, D., 2001. Radiative forcing of climate change, in: Houghton, J., Ding, Y., Griggs (Eds.), *Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel of Climate Change.*
- Rasse, D.P., Ritchie, J.T., Peterson, W.R., Wei, J., Smucker, A.J.M., 2000. Rye cover crop and nitrogen fertilization effects on nitrate leaching in inbred maize fields. *J. Environ. Qual.* 29, 298–304.
- Reberg-Horton, S.C., Grossman, J.M., Kornecki, T.S., Meijer, A.D., Price, A.J., Place, G.T., Webster, T.M., 2012. Utilizing cover crop mulches to reduce tillage in organic systems in the southeastern USA. *Renew. Agr. Food Syst.* 27, 41–48. doi:10.1017/S1742170511000469
- Rees, R.M., Baddeley, J.A., Bhogal, A., Ball, B.C., Chadwick, D.R., Macleod, M., Lilly, A., Pappa, V.A., Thorman, R.E., Watson, C.A., Williams, J.R., 2013. Nitrous oxide mitigation in UK agriculture. *Soil Science and Plant Nutrition* 59, 3–15. doi:10.1080/00380768.2012.733869
- Reid, J.B., Goss, M.J., 1980. Changes in the aggregate stability of a sandy loam effected by growing roots of perennial ryegrass (*Lolium perenne*). *J. Sci. Food Agric.* 31, 325–328. doi:10.1002/jsfa.2740310320

- Ribaudo, M., Kaplan, J., Christensen, L., Gollehon, N., Johansson, R., Breneman, V., Aillery, M., 2003. Manure Management for Water Quality: Costs to Animal Feeding Operations of Applying Manure Nutrients to Land. USDA Economic Research Service Agricultural Economic Report 824.
- Rice, C.W., Tiedje, J.M., 1989. Regulation of nitrate assimilation by ammonium in soils and in isolated soil microorganisms. *Soil Biology and Biochemistry* 21, 597–602. doi:10.1016/0038-0717(89)90135-1
- Rillig, M.C., Wright, S.F., Nichols, K.A., Schmidt, W.F., Torn, M.S., 2001. Large contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils. *Plant Soil* 233, 167–177. doi:10.1023/A:1010364221169
- Rivas, F.A., Tabatabai, M.A., Olk, D.C., Thompson, M.L., 2014. Kinetics of short-term carbon mineralization in roots of biofuel crops in soils. *Biology and Fertility of Soils* 50, 527–535. doi:10.1007/s00374-013-0870-y
- Robinson, D., Hodge, A., Griffiths, B.S., Fitter, A.H., 1999. Plant root proliferation in nitrogen-rich patches confers competitive advantage. *Proc. R. Soc. B-Biol. Sci.* 266, 431–435.
- Rochette, P., 2008. No-till only increases N₂O emissions in poorly-aerated soils. *Soil and Tillage Research* 101, 97–100. doi:10.1016/j.still.2008.07.011
- Roldan, A., 2003. No-tillage, crop residue additions, and legume cover cropping effects on soil quality characteristics under maize in Patzcuaro watershed (Mexico). *Soil and Tillage Research* 72, 65–73. doi:10.1016/S0167-1987(03)00051-5
- Roldán, A., Caravaca, F., Hernández, M.T., García, C., Sánchez-Brito, C., Velásquez, M., Tiscareño, M., 2003. No-tillage, crop residue additions, and legume

- cover cropping effects on soil quality characteristics under maize in Patzcuaro watershed (Mexico). *Soil and Tillage Research* 72, 65–73. doi:10.1016/S0167-1987(03)00051-5
- Ross, D.J., 1992. Influence of sieve mesh size on estimates of microbial carbon and nitrogen by fumigation-extraction procedures in soils under pasture. *Soil Biology and Biochemistry* 24, 343–350. doi:10.1016/0038-0717(92)90194-3
- Rowlings, D.W., Scheer, C., Liu, S., Grace, P.R., 2016. Annual nitrogen dynamics and urea fertilizer recoveries from a dairy pasture using 15N; effect of nitrification inhibitor DMPP and reduced application rates. *Agriculture, Ecosystems & Environment* 216, 216–225. doi:10.1016/j.agee.2015.09.025
- Ryan, J., Kapur, S., Ibrikci, H., Singh, M., 2011. Cultivation Intensity in Relation to Organic Matter and Related Properties in a Vertisol in Southern Turkey. *Journal of Sustainable Agriculture* 35, 613–623. doi:10.1080/10440046.2011.586577
- Saar, S., Semchenko, M., Barel, J.M., De Deyn, G.B., 2016. Legume presence reduces the decomposition rate of non-legume roots. *Soil Biology and Biochemistry* 94, 88–93. doi:10.1016/j.soilbio.2015.11.026
- Sainju, U.M., Lenssen, A.W., Caesar-TonThat, T., Jabro, J.D., Lartey, R.T., Evans, R.G., Allen, B.L., 2012. Dryland soil nitrogen cycling influenced by tillage, crop rotation, and cultural practice. *Nutr. Cycl. Agroecosyst.* 93, 309–322. doi:10.1007/s10705-012-9518-9
- Sainju, U.M., Schomberg, H.H., Singh, B.P., Whitehead, W.F., Tillman, P.G., Lachnicht-Weyers, S.L., 2007. Cover crop effect on soil carbon fractions under conservation tillage cotton. *Soil and Tillage Research* 96, 205–218. doi:10.1016/j.still.2007.06.006

- Sainju, U.M., Whitehead, W.F., Singh, B.R., 2003. Cover crops and nitrogen fertilization effects on soil aggregation and carbon and nitrogen pools. *Can. J. Soil Sci.* 83, 155–165.
- Sarkodie-Addo, J., Lee, H. c., Baggs, E. m., 2003. Nitrous oxide emissions after application of inorganic fertilizer and incorporation of green manure residues. *Soil Use and Management* 19, 331–339. doi:10.1111/j.1475-2743.2003.tb00323.x
- Schweiger, P.F., Thingstrup, I., Jakobsen, I., 1999. Comparison of two test systems for measuring plant phosphorus uptake via arbuscular mycorrhizal fungi. *Mycorrhiza* 8, 207–213. doi:10.1007/s005720050236
- Shcherbak, I., Millar, N., Robertson, G.P., 2014. Global metaanalysis of the nonlinear response of soil nitrous oxide (N₂O) emissions to fertilizer nitrogen. *Proc. Natl. Acad. Sci. U. S. A.* 111, 9199–9204. doi:10.1073/pnas.1322434111
- Singh, R.K., Malik, N., Singh, S., 2013. Improved Nutrient Use Efficiency Increases Plant Growth of Rice with the Use of IAA-Overproducing Strains of Endophytic *Burkholderia cepacia* Strain RRE25. *Microb. Ecol.* 66, 375–384. doi:10.1007/s00248-013-0231-2
- Six, J., Elliott, E., Paustian, K., 2000. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. *Soil Biology and Biochemistry* 32, 2099–2103. doi:10.1016/S0038-0717(00)00179-6
- Skiba, U., van Dijk, S., Ball, B.C., 2002. The influence of tillage on NO and N₂O fluxes under spring and winter barley. *Soil Use Manage.* 18, 340–345. doi:10.1079/SUM2002141

- Smith, A.N., Reberg-Horton, C., Place, G.T., Meijer, A.D., Arellano, C., Mueller, J.P., 2011. Rolled Rye Mulch for Weed Suppression in Organic No-Tillage Soybeans. *Weed Sci.* 59, 224–231. doi:10.1614/WS-D-10-00112.1
- Smith, S.E., Read, D.J. (David J.), c2008. *Mycorrhizal symbiosis*, 3rd ed. ed. Academic Press, Amsterdam.
- Sommer, R., Mukalama, J., Kihara, J., Koala, S., Winowiecki, L., Bossio, D., 2015. Nitrogen dynamics and nitrous oxide emissions in a long-term trial on integrated soil fertility management in Western Kenya. *Nutr Cycl Agroecosyst* 105, 229–248. doi:10.1007/s10705-015-9693-6
- Sparling, G.P., West, A.W., 1988. A direct extraction method to estimate soil microbial C: calibration in situ using microbial respiration and ¹⁴C labelled cells. *Soil Biology and Biochemistry* 20, 337–343. doi:10.1016/0038-0717(88)90014-4
- Srivastava, R., Khalid, A., Singh, U.S., Sharma, A.K., 2010. Evaluation of arbuscular mycorrhizal fungus, fluorescent *Pseudomonas* and *Trichoderma harzianum* formulation against *Fusarium oxysporum* f. sp *lycopersici* for the management of tomato wilt. *Biol. Control* 53, 24–31. doi:10.1016/j.biocontrol.2009.11.012
- Steenwerth, K.L., Belina, K.M., 2010. Vineyard weed management practices influence nitrate leaching and nitrous oxide emissions. *Agriculture, Ecosystems & Environment* 138, 127–131. doi:10.1016/j.agee.2010.03.016
- Stipesevic, B., Kladvko, E.J., 2005. Effects of winter wheat cover crop desiccation times on soil moisture, temperature and early maize growth. *Plant Soil Environ.* 51, 255–261.
- TerAvest, D., Smith, J.L., Carpenter-Boggs, L., Granatstein, D., Hoagland, L., Reganold, J.P., 2011. Soil Carbon Pools, Nitrogen Supply, and Tree Performance under Several

- Groundcovers and Compost Rates in a Newly Planted Apple Orchard. *HortScience* 46, 1687–1694.
- Thomsen, I., 1993. Nitrogen Uptake in Barley After Spring Incorporation of N-15-Labeled Italian Ryegrass into Sandy Soils. *Plant Soil* 150, 193–201. doi:10.1007/BF00013016
- Tisdall, J.M., Oades, J.M., 1982. Organic matter and water-stable aggregates in soils. *Journal of Soil Science* 33, 141–163. doi:10.1111/j.1365-2389.1982.tb01755.x
- Tivet, F., de Moraes Sá, J.C., Lal, R., Briedis, C., Borszowskei, P.R., dos Santos, J.B., Farias, A., Eurich, G., Hartman, D. da C., Nadolny Junior, M., Bouzinac, S., Séguy, L., 2013. Aggregate C depletion by plowing and its restoration by diverse biomass-C inputs under no-till in sub-tropical and tropical regions of Brazil. *Soil and Tillage Research* 126, 203–218. doi:10.1016/j.still.2012.09.004
- Torbert, H.A., Prior, S.A., Reeves, D.W., 1999. Land management effects on nitrogen and carbon cycling in an Ultisol. *Communications in Soil Science and Plant Analysis* 30, 1345–1349. doi:10.1080/00103629909370290
- Treseder, K.K., Allen, M.F., 2002. Direct Nitrogen and Phosphorus Limitation of Arbuscular Mycorrhizal Fungi: A Model and Field Test. *The New Phytologist* 155, 507–515.
- Tu, C., Booker, F.L., Watson, D.M., Chen, X., Rufty, T.W., Shi, W., Hu, S., 2006. Mycorrhizal mediation of plant N acquisition and residue decomposition: Impact of mineral N inputs. *Global Change Biology* 12, 793–803. doi:10.1111/j.1365-2486.2006.01149.x
- Tucker, C.L., Tamang, S., Pendall, E., Ogle, K., 2016. Shallow snowpack inhibits soil respiration in sagebrush steppe through multiple biotic and abiotic mechanisms. *Ecosphere* 7, e01297. doi:10.1002/ecs2.1297

- Van Der Krift, T.A.J., Kuikman, P.J., Berendse, F., 2002. The effect of living plants on root decomposition of four grass species. *Oikos* 96, 36–45. doi:10.1034/j.1600-0706.2002.10978.x
- Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry* 19, 703–707. doi:10.1016/0038-0717(87)90052-6
- Venkateswarlu, B., Srinivasarao, C., Ramesh, G., Venkateswarlu, S., Katyal, J.C., 2007. Effects of long-term legume cover crop incorporation on soil organic carbon, microbial biomass, nutrient build-up and grain yields of sorghum/sunflower under rain-fed conditions. *Soil Use and Management* 23, 100–107. doi:10.1111/j.1475-2743.2006.00068.x
- Vitti, A.C., Trivelin, P.C.O., Cantarella, H., Franco, H.C.J., Faroni, C.E., Otto, R., Trivelin, M.O., Tovajar, J.G., 2008. Straw mineralization and root growth of sugar cane as related to nitrogen fertilization at planting. *Revista Brasileira de Ciência do Solo* 32, 2757–2762. doi:10.1590/S0100-06832008000700020
- Voisin, A.S., Salon, C., Munier-Jolain, N.G., Ney, B., 2002. Effect of mineral nitrogen on nitrogen nutrition and biomass partitioning between the shoot and roots of pea (*Pisum sativum* L.). *Plant Soil* 242, 251–262. doi:10.1023/A:1016214223900
- von Lützow, M., Kögel-Knabner, I., Ludwig, B., Matzner, E., Flessa, H., Ekschmitt, K., Guggenberger, G., Marschner, B., Kalbitz, K., 2008. Stabilization mechanisms of organic matter in four temperate soils: Development and application of a conceptual model. *Z. Pflanzenernähr. Bodenk.* 171, 111–124. doi:10.1002/jpln.200700047

- Wander, M.M., Bidart, M.G., 2000. Tillage practice influences on the physical protection, bioavailability and composition of particulate organic matter. *Biology and Fertility of Soils* 32, 360–367. doi:10.1007/s003740000260
- Wang, X., Tang, C., Severi, J., Butterly, C.R., Baldock, J.A., 2016. Rhizosphere priming effect on soil organic carbon decomposition under plant species differing in soil acidification and root exudation. *New Phytol.* 211, 864–873. doi:10.1111/nph.13966
- Weilharter, A., Mitter, B., Shin, M.V., Chain, P.S.G., Nowak, J., Sessitsch, A., 2011. Complete Genome Sequence of the Plant Growth-Promoting Endophyte *Burkholderia phytofirmans* Strain PsJN. *J. Bacteriol.* 193, 3383–3384. doi:10.1128/JB.05055-11
- Wilson, G.W.T., Rice, C.W., Rillig, M.C., Springer, A., Hartnett, D.C., 2009. Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: results from long-term field experiments. *Ecol. Lett.* 12, 452–461. doi:10.1111/j.1461-0248.2009.01303.x
- Wright, S.R., Coble, H.D., Raper, C.D., Rufty, T.W., 1999. Comparative responses of soybean (*Glycine max*), sicklepod (*Senna obtusifolia*), and Palmer amaranth (*Amaranthus palmeri*) to root zone and aerial temperatures. *Weed Sci.* 47, 167–174.
- Wu, Q.-S., Xia, R.-X., 2006. Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. *Journal of Plant Physiology* 163, 417–425. doi:10.1016/j.jplph.2005.04.024
- Xu, Z., Myers, R., Saffigna, P., Chapman, A., 1993. Nitrogen Cycling in *Leucaena* (*leucaena-Leucocephala*) Alley Cropping in Semiarid Tropics .2. Response of Maize

- Growth to Addition of Nitrogen-Fertilizer and Plant Residues. *Plant Soil* 148, 73–82.
doi:10.1007/BF02185386
- Yang, L.F., Cai, Z.C., 2006. Soil respiration during a soybean-growing season. *Pedosphere* 16, 192–200. doi:10.1016/S1002-0160(06)60043-X
- Yoder, R.E., 1936. A Direct Method of Aggregate Analysis of Soils and a Study of the Physical Nature of Erosion Losses¹. *Agronomy Journal* 28, 337.
doi:10.2134/agronj1936.00021962002800050001x
- Zandersen, M., Jorgensen, S.L., Nainggolan, D., Gyldenkaerne, S., Winding, A., Greve, M.H., Termansen, M., 2016. Potential and economic efficiency of using reduced tillage to mitigate climate effects in Danish agriculture. *Ecol. Econ.* 123, 14–22.
doi:10.1016/j.ecolecon.2015.12.002
- Zhang, B., He, H., Ding, X., Zhang, X., Zhang, X., Yang, X., Filley, T.R., 2012. Soil microbial community dynamics over a maize (*Zea mays* L.) growing season under conventional- and no-tillage practices in a rainfed agroecosystem. *Soil and Tillage Research* 124, 153–160. doi:10.1016/j.still.2012.05.011
- Zhou, X., Wu, H., Koetz, E., Xu, Z., Chen, C., 2012. Soil labile carbon and nitrogen pools and microbial metabolic diversity under winter crops in an arid environment. *Applied Soil Ecology* 53, 49–55. doi:10.1016/j.apsoil.2011.11.002
- Zhu, B., Cheng, W., 2012. Nodulated soybean enhances rhizosphere priming effects on soil organic matter decomposition more than non-nodulated soybean. *Soil Biol. Biochem.* 51, 56–65. doi:10.1016/j.soilbio.2012.04.016