ABSTRACT

O’CONNELL, SUZANNE. Short-term Nitrogen Mineralization and Soil Microbial Response to the Incorporation of Warm-season Cover Crops in Organic Farming Systems. (Under the direction of Dr. Nancy G. Creamer.)

A survey was developed and distributed to more than 200 self-identified sustainable farmers in the Southern region to determine the level of utilization, current practices, and perceptions related to cover cropping. Eighty-nine percent of participants reported having a crop rotation plan that included cover crops indicating that their use was wide-spread. Soil quality improvements, erosion control, weed management and nitrogen contributions were the most highly rated cover crop attributes. Next, a series of field and laboratory studies were conducted to assess short-term nitrogen mineralization and soil microbial response to the incorporation of warm-season cover crops. We found that all warm-season cover crops resulted in net N mineralization for ~3 months after incorporation and legume-dominated crops had greater potential for N mineralization compared to grasses. Cover-cropped soils demonstrated increased ability to retain soil inorganic N, moderated its release and reduced leaching after an intense precipitation event.

Short-term N mineralization from warm-season cover crops was affected by both plant tissue quality and environmental conditions. The soil microbial community appeared to reduce its carbon use efficiency (CUE) during a dry season, resulting in lower microbial N demand and net N mineralization from cover crops with C:N >40:1. The incorporation of cover crop residues stimulated an increase in both soil microbial biomass N and cellulase enzyme activity. In terms of distinguishing among cover crop quality, the most sensitive
enzyme was β-1,4-glucosidase (EC 3.2.1.21) which was also positively correlated with both potential C and N mineralization.

Further analysis of residue biochemical composition revealed that percent neutral detergent fiber (NDF), percent hemicellulose and lignin:N ratio in addition to shoot C:N could be used to distinguish between legume-dominated and grass cover crops suggesting their utility to predict differences among mineralization rates of relatively high quality plant residues. The C:N ratio of the free, particulate organic matter (F-POM) soil fraction was different between legume-dominated and grass cover crops and positively correlated with shoot C:N. Overall, we did not find F-POM to be a good indicator of potential N mineralization but it did appear to reflect the properties of the cover crop residues in the weeks to months after soil incorporation.
Short-term Nitrogen Mineralization and Soil Microbial Response to the Incorporation of Warm-season Cover Crops in Organic Farming Systems

by
Suzanne O’Connell

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

Horticultural Science

Raleigh, North Carolina

2013

APPROVED BY:

_______________________________
Dr. Nancy G. Creamer
Committee Chair

_______________________________
Dr. Greg D. Hoyt

_______________________________
Dr. Wei Shi

_______________________________
Dr. Julie M. Grossman
DEDICATION

This dissertation is dedicated to my loving family and friends who supported me throughout the process. It was not always easy to find the patience, energy, and positive attitude that navigating graduate school entails, you all helped make it possible! Thank you for encouraging, believing and rescuing me in your own ways from time to time. I’d also like to dedicate this work to all the farmers, mentors, and colleagues who graciously paved the way for me to pursue my interest in organic farming research, have shared their experiences and advice, and continue to inspire and challenge me. In addition, I would like to acknowledge those who introduced me to the world of farming at Appleton Farms, Ipswich MA.
BIOGRAPHY

Suzanne O’Connell graduated in 2008 with a Master of Science in Horticultural Science from North Carolina State University building on a Bachelor of Arts in Environmental and Political Science from Barnard College, Columbia University. She has received many scholarships and grants to support her research efforts during her tenure at NCSU including a Fulbright research award to Honduras in 2011. Her love of food, being outdoors, and environmental science are the motivating factors for pursuing a career in the field of sustainable agriculture.
ACKNOWLEDGMENTS

This research project would not have been possible without the help of so many people. I am very grateful to my advisor, Nancy Creamer for all of her support and encouragement, including extending the opportunity to pursue my own research interests. I would also like to sincerely thank Wei Shi and Julie Grossman for generously opening up their labs and devoting their valuable time and resources to these projects. Also, I would like to extend a big thank you to Greg Hoyt for always having a kind word, a quick editing pen, and a seasoned field research advice. All of my committee members have been wonderful role models to me in different ways, thank you.

Funding from the Southern Sustainable Agriculture Research and Education Committee (SSARE) and the Organic Crop Improvement Association (OCIA) helped make this research project possible. Additional scholarships and awards improved my graduate student quality of life and offered complementary experiences including: Barnard College Alumnae Scholarship, U.S. Student Fulbright Research Award, Doug & Ellen Sanders Graduate Research Award, the NCSU Provost Fellowship, the NCSU CALS Int’l Agric. Exchange with the Univ. of Zagreb in Croatia and NCSU CEFS Organic Agric. Exchange in Uruguay. I would also like to acknowledge my gratitude for project assistance and moral support received from Ken Fager, CEFS faculty/staff, fellow graduate students, and part-time assistants (Lee Hardy, Max Sherard and Mark Smith). And last but not least, thank you to all the farmers who responded to my inquiries/surveys, participated in research endeavors and shared their knowledge.
TABLE OF CONTENTS

LIST OF TABLES ............................................................................................................................................................................. vi
LIST OF FIGURES ............................................................................................................................................................................... vii

CHAPTER 1 – A survey of cover crop practices and perceptions of sustainable farmers in the Southern U.S.

Abstract .......................................................................................................................................................................................... 1
Introduction ..................................................................................................................................................................................... 2
Materials & Methods ........................................................................................................................................................................ 5
Statistical Analysis ............................................................................................................................................................................. 7
Results ........................................................................................................................................................................................................... 9
Discussion/Conclusion .................................................................................................................................................................. 15
Acknowledgements ............................................................................................................................................................................ 19
References ...................................................................................................................................................................................... 20

CHAPTER 2 – Short-term nitrogen mineralization from warm-season cover crops in organic farming systems

Abstract .......................................................................................................................................................................................... 33
Introduction ..................................................................................................................................................................................... 35
Materials & Methods ........................................................................................................................................................................ 37
Statistics ............................................................................................................................................................................................ 44
Results ........................................................................................................................................................................................................... 45
Discussion ......................................................................................................................................................................................... 50
Acknowledgements ............................................................................................................................................................................ 56
References ...................................................................................................................................................................................... 57

CHAPTER 3 – Soil microbial response to the incorporation of warm-season cover crops in organic farming systems

Abstract .......................................................................................................................................................................................... 70
Introduction ..................................................................................................................................................................................... 71
Materials and Methods .................................................................................................................................................................... 76
Statistical Analysis ........................................................................................................................................................................... 84
Results .............................................................................................................................................................................................. 85
Discussion and Conclusions .......................................................................................................................................................... 91
Acknowledgements ............................................................................................................................................................................ 94
References ...................................................................................................................................................................................... 96
LIST OF TABLES

Table 1.1  Demographic Profile of Survey Participants ...........................................26
Table 1.2  Farm Characteristics of Survey Participants .............................................27
Table 1.3  Factor Analysis Results for Cover Cropping Survey of Southern Sustainable Farmers ........................................................................................................31
Table 2.1  Cover Crop Planting Dates and Field Management Activities, 2010 ...............61
Table 2.2  Mean Values for End of Season, Cover Crop Quantity and Quality ..............62
Table 2.3  Mean Values of Inorganic N, Probe Adsorbed N, Soil Mineralized C and N by Field Site and Cover Crop Treatment for the Sampling Season ..............63
Table 2.4  Pearson Correlation Coefficients(r) between Mineralized Soil N and Indicators of Cover Crop Residue Quantity and Quality ........................................64
Table 3.1  Cover Crop Planting Dates and Field Management Activities, 2010 ...............100
Table 3.2  Mean Cover Crop Shoot Quantity and Quality by Field Site for the Sampling Season ...............................................................................................................101
Table 3.3  Mean Soil Enzyme Activity, Microbial Biomass N (MBN), F-POM C:N Ratio, and Soil Mineralized C & N by Field Site and Cover Crop Treatment for the Season ..................................................................................................................102
Table 3.4  Pearson Correlation Coefficients (r) between Mineralized Soil N and Indicators of Cover Crop Tissue and F-POM Quality ..............................................103
Table 3.5  Pearson Correlation Coefficients (r) between Mineralized Soil C and N and Indicators of Soil Microbial Activity .........................................................104
LIST OF FIGURES

Fig. 1.1  Survey Participants by State ................................................................. 25
Fig. 1.2  The Most Popular Cool- and Warm-season Cover Crops ......................... 27
Fig. 1.3  Survey Respondent Rankings of 22 Statements about Cover Crops ............ 30
Fig. 2.1  2010 Mean Daily Values and 30-year Normals (1971-2000) for Air Temperature and Daily Precipitation Means ................................................. 65
Fig. 2.2  Mean Inorganic N from 1 M KCl Soil Extractions .................................. 66
Fig. 2.3  Mean PRS Probe Adsorbed N ................................................................. 67
Fig. 2.4  Mean Mineralized N from a 28-day Incubation ...................................... 68
Fig. 2.5  Mean Mineralized C from a 28-day Incubation ..................................... 69
Fig. 2.6  Mean Free-Particulate Organic Matter (F-POM) C:N Ratio ....................... 71
Fig. 3.1  2010 Mean Daily Values for the 2010 Growing Season and 30-year Normals (1971-2000) for Air Temperature and Daily Precipitation .... 105
Fig. 3.2  Mean Mineralized N from a 28-day Incubation ...................................... 106
Fig. 3.3  Mean Mineralized C from a 28-day Incubation ..................................... 107
Fig. 3.4  Mean β-1,4-glucosidase Activity at 6 Sampling Dates ............................. 108
Fig. 3.5  Mean 1,4-β-cellobiosidase Activity over 6 Sampling Dates ..................... 109
Fig. 3.6  Mean Peroxidase Activity over 6 Sampling Dates .................................. 110
Fig. 3.7  Mean Microbial Biomass N over 6 Sampling Dates .............................. 111
CHAPTER 1

Title A survey of cover crop practices and perceptions of sustainable farmers in the Southern U.S.

Authors S. O’Connell\textsuperscript{1*}, N. G. Creamer\textsuperscript{1}, K. L. Fager\textsuperscript{1}, J. M. Grossman\textsuperscript{2}, G. D. Hoyt\textsuperscript{1,2}, W. Shi\textsuperscript{2}, S. Bowen\textsuperscript{3} and D. C. Marticorena\textsuperscript{1}

\textsuperscript{1} North Carolina State University, Department of Horticultural Science, Box #7609, Raleigh, NC 27695
\textsuperscript{2} North Carolina State University, Department of Soil Science, Box #7619, Raleigh, NC 27695
\textsuperscript{3} North Carolina State University, Department of Sociology and Anthropology, Box 8107, Raleigh, NC 27695

*Corresponding author: Suzanne.oconnell@gmail.com

Abstract

The environmental benefits of cover cropping are widely recognized but there is a general consensus that adoption levels are still quite low among U.S. farmers. A survey was developed and distributed to more than 200 self-identified sustainable farmers in the Southern region to determine the level of utilization, current practices, and perceptions related to cover cropping. The majority of farms were small (<8 ha and <$50,000 total gross
income) and approximately one-third had an organic component. A positive correlation between crop rotation and cover cropping was present and 89% of participants reported having a crop rotation plan that included cover crops. Soil quality improvements, erosion control, weed management and nitrogen contributions were the most highly rated cover crop attributes in our survey. Economic costs associated with cover cropping were not viewed as an obstacle to implementation. A factor analysis was conducted to identify underlying themes to a series of positive and negative statements about cover crops. Pre- and post-management challenges (i.e., seed bed preparation and incorporation of residues) were able to explain the most variability (30%) among participant responses. Farmers indicated that a lack of equipment, especially for no-till systems, influenced their decision about cover crops. Overall, farmers with less than 3 years of experience and those who reported a full-time farming status had more positive views of the benefits derived from cover crops.

**Key words:** cover crop, green manure, sustainable, organic, survey

**Introduction**

Conservation and protection of soil resources are widely recognized goals within the purview of sustainable agriculture. Cover cropping can help achieve these objectives. It is an accessible and adaptable management practice that can be integrated into many types of production systems. Cover crops were defined as “crops grown between or intercropped with cash crops, which are generally not harvested or sold for profit, but rather used as part of the overall farm management system”. They are generally comprised of grasses and/or legumes
that are grown during periods of winter or summer fallow. Multiple benefits have been attributed to cover cropping, including decreased soil erosion, improved soil and water quality, weed suppression, nutrient contributions and recycling, pest and disease management, pollinator attraction and carbon sequestration\textsuperscript{1-3}.

Although there is a lack of information about the prominence of cover crop use in the United States, the pervading opinion is that only a small percentage of farmers use them\textsuperscript{4}. There is also a lack of national-level information and existing regional surveys have found very different adoption levels. For example, in one study approximately 11% of Midwestern region farmers (Illinois, Indiana, Iowa, and Minnesota) were found to use cover crops ($n$ = 1,096)\textsuperscript{5}. A different nation-wide study with the majority of participants from the Midwest, found that 21% of farmers used cover crops regularly ($n$ = 809)\textsuperscript{6}. Approximately 56% of Utah farmers utilized cover crops ($n$ = 351)\textsuperscript{7}, while in western New York, 69% of vegetable farmers reported using cover crops ($n$ = 118)\textsuperscript{8}. In 1998, the Maryland Department of Agriculture implemented a progressive state-wide cover crop cost-share program in an effort to improve Chesapeake Bay water quality. By 2012, the program had more than 42% of eligible farmland enrolled (162,000 ha)\textsuperscript{9,10}.

The regional focus of these studies is appropriate given that a combination of environmental, political and cultural factors can heavily influence farming practices\textsuperscript{11} but many regions with important agricultural sectors are not represented in the literature, such as the Southern region of the U.S. In 2007, the Southern region as defined by the Southern Sustainable Research and Education program (S-SARE) (Fig.1.1, omitting Puerto Rico and the Virgin Islands) represented 40% of all farms in the U.S. (~890,000 farms), 30% of all
farmland (~111,000,000 ha), and 28% of the market value of agricultural products sold (~$84,000,000,000 USD)\textsuperscript{12}.

One advantage of farming in the Southern region is the predominant subtropical climate with a mean annual precipitation between 1,143-1,524 mm and average annual minimum temperatures ranging from -28.9 to 10°C (USDA plant hardiness zones 5a-11b\textsuperscript{13}). The integration of both cool and warm-season cover crops is possible due to the climate and long growing season\textsuperscript{14}. Two disadvantages of farming within this region include the highly weathered soils with a low nutrient supply capacity (e.g., Ultisols) and heavy pest and disease pressure due to mild temperatures and frequent rainfall. Including cover crops in farming rotations may help address these common regional challenges. Research efforts in the Southern region have explored the effects of cover crops on a variety of agronomic issues including weed suppression\textsuperscript{14,15}, organic matter level\textsuperscript{16}, nitrogen contribution\textsuperscript{17,18}, tillage reduction\textsuperscript{19} and run-off reduction\textsuperscript{20}.

Though cover cropping may be associated with sustainable farming, there is even less information about the level of usage within this community compared to mainstream agricultural community. The concept of sustainability has been embraced by a wide spectrum of audiences in recent years; however, the farming community in particular emphasizes the implications of agricultural practices on ecological and socioeconomic factors\textsuperscript{21}. Therefore, we created a multi-state survey to identify the current practices as well as the perceived benefits and challenges associated with cover cropping by self-identified sustainable farmers in the Southern U.S. region.
Identifying the perceived benefits and challenges of cover cropping will help understand which factors are most influential for adoption. We expected that farmers who cover crop would have higher ratings of environmental, crop management, and economic benefits derived from these practices in order to offset the associated direct and indirect costs. Previous studies have found that demographic and/or farm characteristics may influence a farmer’s perspective and management decisions\textsuperscript{22-25} and we assumed that these variables would also have an influence within the sustainable farming community. Lastly, we hypothesized that organic farmers would employ greater levels of cover cropping compared to the general survey population because it is a highly encouraged practice under the USDA National Organic Program.

**Materials and Methods**

The survey was developed by the authors in conjunction with an advisory board comprised of university personnel, extension professionals, and sustainable farmers. The advisory board provided reviews on preliminary versions of the survey that were used to improve the content and clarity of the final instrument. The survey was distributed during the winter of 2009-2010 to self-identified sustainable farmers within the Southern region of the U.S. (Fig. 1.1).

The questionnaire was distributed in two manners, both of which allowed for anonymous participation. The primary means of distribution was soliciting farmers, in-person, to fill out a paper-based survey at two popular sustainable agriculture annual meetings, 1) the Carolina Farm Stewardship Association (CFSA) conference and 2) the
Southern Sustainable Agriculture Working Group (S-SAWG) conference. The mission of CFSA is to support a regional food system by advocating for fair farm and food policy and educating communities about local and organic farming, while SSAWG’s aims to create an agricultural system that is ecologically sound, economically viable, socially just, and humane. Farmer attendance at these conferences was estimated at 200 and 650, respectively. CFSA traditionally draws audiences from North and South Carolina whereas SSAWG draws producers from the entire region. Both conference organizers, CFSA and S-SAWG, extended the invitation to participate in the survey to their membership who did not attend the conferences by advertising an online version of the same survey (Survey Builder, LeadPro247) in their respective post-conference newsletters. An online version of the survey was active from Feb. 1-March 15, 2010.

A total of 224 surveys were completed and 221 were usable. Surveys with more than 10% incomplete responses were not analyzed. The majority of survey participants were categorized as the in-person, paper format (73%) and the remainder were completed online (27%). We estimated a response rate of approximately 20% from the in-person surveys, resulting in ± 6% sampling error.

North Carolina farmer’s had by far the greater representation of any state (46%), likely due to two factors, 1) the location and membership base of CFSA is North and South Carolina and, 2) that the research team was comprised of individuals associated with North Carolina State University, a well-known public university lending credibility to in-state solicitation efforts. Although we acknowledge that this over-representation may affect our results, there is considerable farmer, crop, and climatic diversity within North Carolina.26 That
is representative of the Southern region as a whole. In addition, although the overall categorical breakdown of race and ethnicity among farmers of survey participants was similar to national averages, minority representation was very small within our dataset; therefore, we abstained from making conclusions based on these characteristics.

The survey consisted of 53 questions inquiring about farm and market operations, experiences and opinions regarding cover cropping, management practices, and demographic information. Demographic categories were aligned with 2010 U.S. Census Bureau groupings wherever possible. Questions were constructed to allow responses in one of the following formats: choose one response from a list of choices, check all responses that apply from a list of choices, fill in the blank with one or more responses, and choose a response from five levels of agreement (i.e., Likert-type scale).

**Statistical Analysis**

Data from the in-person and online survey responses appeared to be drawn from the same distribution according to a 2-sample Kolmogorov-Smirnov test and therefore were combined and analyzed as one dataset (SPSS for Windows 17.0). Descriptive statistics, including frequency counts of various responses, were conducted (SPSS for Windows 17.0). An exploration of farmer’s perceptions and beliefs about cover cropping using a factor analysis followed. Factor analysis is a data dimensionality reduction technique frequently used to analyze the underlying relationships amongst a pattern of responses. The shared variances are decomposed into their major components in order to reduce the number of variables necessary to represent the same relationships. The composite variables derived
from this analysis are called factors and represent the different considerations survey respondents make when deciding how and/or whether to integrate cover crops in their farming systems.

The methodology of our factor analysis follows. A series of 22 statements about the benefits and challenges of cover cropping was ranked on a Likert-type scale by survey participants (strongly disagree[1], disagree[2], neutral[3], agree[4], strongly agree[5]). A Likert scale is a psychometric scale that captures the intensity of agreement for a given item; distances between each ranking are assumed to be equal. Tests were carried out to determine if our dataset was factorable and which type of factor analysis would provide the most interpretable structure. A maximum likelihood extraction with an oblique rotation (e.g. oblimin in SPSS), listwise deletion of missing data, and the Kaiser normalization method were utilized. The Kaiser-Meyer-Olkin measure of sampling adequacy was 0.828 and the Bartlett’s test of sphericity was 933.8 ($P=0.000$) indicating the appropriate application of our chosen factor analysis. The goodness-of-fit test had a chi-square value of 60.836 ($P=0.482$) and communality scores (measuring the amount of variance accounted for by the factors) ranged from 0.217 to 0.629.

The number of extracted factors was determined with a combination of three criteria: visual analysis of the scree plot, the Kaiser-Guttman rule (i.e., associated Eigenvalues >0.99), and the interpretability of the factors (i.e., those with theoretical meaningfulness). A minimum of two variables loading per factor was present and only variables with rotated factor loadings ($\geq 0.4$) were presented in our results for ease of interpretation. Each respondent included in the factor analysis was assigned a score based on a weighted linear
average combination of their responses. These scores were then used in subsequent analyses to evaluate difference among the 5 factor groupings.

One-way analysis of variance and/or planned contrasts of means were conducted to compare the practices and beliefs of different participant groups using SPSS ANOVA. Levene’s test of homogeneity of variances was utilized to evaluate equality of variance among the populations. If variances were equal, then Tukey’s honestly significant differences (HSD) was used to evaluate differences among means and minimize experiment-wise error rate using $\alpha \leq 0.05$. If variances were deemed unequal and/or sample sizes in the groups were very different, the Welch’s F test was used to evaluate significant differences among means using $\alpha \leq 0.05$. In addition, Pearson correlation coefficients were computed to assess relationships between selected variables.

**Results**

State or territory representation of participants is displayed in Figure 1. Demographic characteristics indicate that the majority of respondents were between 25-60 years old (69%), college-educated (94%), male (55%), and white (94%) (Table 1). Age and years of farming experience were positively correlated ($r=0.427$, $p<0.01$). Most farmers fell into one end of the farming experience spectrum with either “less than 3 years” (29%) or “more than 15 years” experience (29%) (Table 2). More than half of participants were full-time farmers (55%) and managed less than 8 ha of production (74%). The USDA defines a small farm as one that generates less than $250,000 USD in annual gross sales. Accordingly, 95% of our survey population could be characterized as small farms while 79% reported total gross farm
incomes less than $49,999 USD (Table 2). Total gross income was positively correlated with years of farming experience (r=0.363, n=192, p≤0.001), hectares under production (r=0.371, n=190, p≤0.001), and full-time status (r=0.465, n=182, p≤0.001).

The top five product categories in descending order included: vegetable/small fruits, livestock, cut flowers, poultry, and orchard (Table 2.1). Only farmers with more than 10 years experience produced tobacco or cotton. The majority of farmers produced 1 to 3 different types of products. Farmer’s markets, community supported agriculture programs (CSA), and wholesale outlets were the most frequently utilized marketing venues. The majority of farmers indicated that they sold products to 1 to 2 types of sales outlets. Approximately, one-third of the respondents adhered to the USDA National Organic Program (NOP) standards in some capacity while 16% adhered to alternative labels (e.g., “Certified Naturally Grown”, “Appalachian Grown”, GAP, Master farmer/cattleman and/or Grassfed) (Table 2.1).

According to the survey results, crop rotation was practiced by 89% of respondents. In addition, 89% of farmers with rotation plans used cover crops. Participants using cover crops were positively correlated with having a crop rotation plan (r=0.653, n=204, p≤0.01). Annual cover cropping was by far the most common type of cover cropping reported (87%). Full-time farmers were more likely than part-time farmers to have a crop rotation plan (p=0.005). Those that used cover crops were more likely to produce vegetables, small fruits, and cut flowers than other crop types (p=0.023) and were more likely to sell direct at farmer’s markets and through community supported agriculture programs (CSA’s) (p≤0.001) compared to those that did not use cover crops. Farmers that included cover crops in their
rotation plans also reported greater total gross incomes than those who did not use cover
crops (p=0.049). There were no significant differences among organic and non-organic
farmers in terms of implementation of crop rotation or the rate of cover cropping.

Farmers indicated that they used more than 25 different types of cover crops
indicating a wide range being used (Fig. 2.1). Ninety-one percent of farmers planted cool-
season cover crops although warm-season varieties were also widely used (55%). Eighty-six
percent of farmers who utilized cool-season cover crops also reported using warm-season
cover crops. More than 62% of farmers reported following recommended seeding rates
although only 31% of respondents applied pre-plant fertilizer or soil amendments.

Intercropping or overseeding a cover crop with a cash crop was employed by 46% of
respondents. Inoculation of legume cover crops was carried out routinely by 45% of farmers
while 16% applied inoculant only when introducing a new legume to their farm.

After the cover crop growing period, farmers waited an average of 1 to 4 weeks
between crop termination and planting of new crops. The most utilized cover crop
termination methods included mowing (70%), rototilling (51%) and diskng (48%). Fifty
percent of farmers responded that the availability of particular tools affected their
management decisions. The types of equipment they lacked included roller-crimpers (72%),
planting (31%) and incorporation (18%) tools.

Farmer age and years of farming experience were positively correlated to each other
but years of farming experience seemed to be the stronger predictor of the two variables.
First, we compared the practices or perceptions of beginning farmers, defined by the USDA
as those with more or less than 10 years experience and found very few differences between
beginner or more experienced farmers. Subsequently, we compared participants with more or less than 3 years experience. In general, farmers with less than 3 years experience, who represented 29% of our participants, expressed stronger agreement with statements about positive cover crop attributes. They were also less likely to view residue incorporation as a challenge and had stronger agreement that cover crops increase soil organic matter, suppress weeds, and break pest and disease cycles (p≤0.05). Farmers with less than 3 years experience were more likely to use no-till equipment to manage cover crops compared to farmers with more than 15 years experience (p=0.003).

Overall, farmers supported statements about both the environmental and economic benefits from cover cropping. More than half of the respondents were in strong agreement with the following statements: 1) cover crops decrease soil erosion, 2) cover crops increase soil organic matter, 3) cover crops increase soil moisture, 4) cover crops suppress weeds, and 5) selected cover crops contribute nitrogen to subsequent cash crops (Fig. 3.1). Meanwhile, more than half of the respondents disagreed that cover crops require too much water or have negative effects on subsequent cash crops (Fig. 3.1).

In the ranking of 22 statements about the benefits and challenges of cover crops, 63% of the variance among survey responses was explained by 5 key factors (Table 3.1). Factor 1 was labeled pre- and post- “management challenges” and reflected rankings of both seed bed preparation and residue incorporation statements. The 2 variables comprising this factor were able to explain almost 30% of variance within the dataset (Table 3.1). In particular, seed bed preparation was 99% positively correlated with the amount of variance within factor 1, indicating it was the main predictor for this theme. Although the mean response to the
statement that “cover crop seed bed preparation is a challenge” represented the neutral ranking, 38% of participants were in disagreement, 30% neutral, and 18% in agreement with this statement demonstrating a spectrum of opinions among participants (Fig. 3.1). Survey participants who indicated that the availability of tools influenced their decision to use cover crops were more likely to agree that seed bed prep was a challenge ($F(1,173)=12.449, \ p=0.001$). The second item in factor 1, “incorporating cover crop residues is a challenge”, was 56% positively correlated with the amount of variance. Similarly, there was a wide distribution of responses to the statement, including disagree (22%), neutral (30%), agree (36%), and strongly agree (10%). Farmers who indicated a lack of equipment and also those with less than 3 years of experience were more likely to agree with this statement ($[F(1,174)=14.054, \ p=0.000] \ [F(1,200)=5.833, \ p=0.017]$, respectively).

“Soil quality benefits” represents the 3 items comprising factor 2 and explained 12% of variance within the dataset (Table 3.1). Overall participants had strong agreement with the following statements. Participants “strongly agreed” that cover crops increase soil organic matter (81%), decrease soil erosion (79%), and increase soil moisture (62%) (Table 3.1). Full-time farmers and those with less than 3 years farming experience had greater levels of agreement that cover crops increase soil organic matter compared to part-time and more experienced farmers ($[W(1,145)=5.415, \ p=0.021], \ [W(1,182)=4.793, \ p=0.030]$). Full-time farmers also had stronger levels of agreement with the 2nd item, “cover crops decrease soil erosion” compared to part-time [$W(1,135)=7.417, \ p=0.007]$.

Factor 3 was labeled “pest management and crop yield” and had the most heterogeneous composition of item topics ranging from cover crop effects on cash crop yield,
pest and disease management, and frequency of pesticide applications. All together, these items explained approximately 9% of variance within the dataset (Table 3.1). The only inverse correlation present in the analysis was within this factor. Farmers that disagreed with the statement “the cost of establishing cover crops is prohibitive” were likely to agree with the rest of the variables in factor 3. In addition, full-time farmers had stronger agreement that cover crops increase cash crop yields compared to part-time farmers ([F(1,193)=4.387, p=0.009], respectively) and those with less than 3 years of experience had stronger agreement that cover crops break pest and disease cycles compared to those with more experience [F(1,198)=4.005, p=0.047].

“Negative effects of cover cropping” characterize the items that comprise factor 4. More than 80% of farmers disagreed with the statement that selected cover crops reduce available nitrogen for subsequent cash crops indicating that this is not a major concern. Overall, participants disagreed or were neutral about cover crops becoming weeds or resulting in a loss of cash crop opportunities. Participants that had stronger agreement with the statement that cover crops become weeds were also those who practiced rototilling [F(1,173)=5.186, p=0.014]. Farmers that reported using cover crop seeding rates lower than what was recommended, also had stronger agreement that cover crops become weeds [F(2,165)=5.275, p=0.046]. Farmers that included cover crops in their rotation plans reported less agreement with the statement that “using cover crops results in a loss of cash crop opportunities” [F(2,197)=5.010, p=0.036].

And lastly, factor 5 categorized as “additional benefits” reflects other positive attributes of cover crops (Table 3.1). Although these variables did not explain as much
variance as factor 1, more than 80% of participants were in agreement with 3 out of 4 items: cover crops reduce nutrient leaching, cover crops break hard pans with their roots, and cover crops suppress weeds. Survey participants that used intercropping or overseeding reported greater levels of agreement with all items in factor 5 compared to those who did not use these techniques [F (1,175)=3.33, p≤0.028]. Full-time farmers had stronger agreement than part-time that cover crops suppress weeds [F(1,195)=8.532, p=0.004].

Discussion/Conclusion

Our results supported the assertion that sustainable farmers assign high values to both the ecological and economic benefits\textsuperscript{21,30} from cover crops including: improved soil quality and erosion control, weed and pest management, nutrient turnover and positive effects on cash crops. Further validation of these findings was evident through farmers disagreement and/or neutrality about unfavorable impacts from cover cropping. The perceived benefits of cover cropping appeared to outweigh associated challenges and greater levels of utilization were found compared to other regional studies\textsuperscript{5-8}. In addition, the Southern region’s subtropical climate may provide both an incentive (i.e. weathered soils, high pest pressure) and added flexibility (i.e. long growing season) for the integration of cover cropping in farming systems. Reported cover crop management practices themselves were diverse which may be a reflection of the many production types represented in the study.

Survey participants who used cover crops were positively correlated with those who had crop rotation plans. Crop rotation involves growing a succession of different crops on the same land over multiple seasons in a recurring sequence. In a previous study, Liebman and
Dyck asserted that the very concept of crop rotation implies the use of cover crops and green manures, although, they are no longer included in many modern rotation schedules\textsuperscript{31}. The positive correlation suggests that the integration of cover cropping within the overall farming system was given a greater priority when long-term planning occurred or that farmers with crop rotation plans assigned more value to the long-term benefits that cover crops can provide. Farmers that included cover crops in their rotation plans were also more likely to have greater than 10 years of farming experience, indicating that it may take years for farmers to try and/or implement successful rotation schemes. The time gap may be attributed to acquiring equipment, accumulating knowledge, and/or establishing business goals.

Previous studies have suggested that demographic variables or other characteristics may help explain why certain farmers have different perceptions about topics or employ different practices\textsuperscript{22-25,32} although we did not see much evidence of this in our survey. Years of farming experience and farming status (i.e., full versus part-time) were the most influential variables on cover cropping practices. Overall, we did not find evidence that formal education level, gender, farm income, farm-scale, or organic certification resulted in significant differences in practices or perceptions related to cover cropping. This lack of separation among groups may be a result of the strong communalities that sustainable farmers in the Southern region share, the need for a larger sample size, and/or a lack of heterogeneity in our survey population.

We expected that farm-scale would be positively correlated with cover cropping based on a number of studies which assert a positive correlation between conservation or best management practices (BMP’s)\textsuperscript{24,33,34}. The reasoning behind this relationship is that there is a
relatively smaller capital investment required by larger-scale operations to adopt new technology and thus they reap higher benefits. However, we did not find differences in terms of cover crop practices or perceptions related to farm scale in our study. One possible reason was that there was not a wide enough farm size range in our study population. More than 74% of our participants farmed on less than 8 ha and 94% reported less than 81 ha. These farms are considerably smaller than the 2010 national farm size average of 162 ha and therefore may not be comparable to results from other studies that reflect larger scales of economy. Alternatively, a negative correlation between farm size and the perceived importance of environmental stewardship may exist. Other studies have found there are positive relationships between small farm size and non-economic decision-making related to environmental concerns and this trend may be reflected in our survey population.

In general, farmers with less than 3 years experience compared to more experienced farmers expressed stronger agreement with statements about positive cover crop attributes. One possible explanation is that more experienced and/or older farmers may have tempered perceptions or become more risk adverse. This result was similar to those found by Bergtold et al., evaluating perceived benefits of winter cover crops in the Southeast. Although we cannot pin point exactly when perceptions related to cover cropping may change based on our study, it does appear that this occurs during the early formative farming years. Similarly full-time farmers compared to part-time farmers expressed stronger agreement with statements about positive cover crop attributes. Parallels could be drawn from an integrated pest management study which concluded that full-time farmers may put greater effort into analyzing management decisions and seek out more educational materials.
and/or trainings compared to part-time farmers because their major income source is directly tied to farm income\textsuperscript{37}.

Overall, farmers did not appear to be consistent in efforts to maximize cover crop growth. This is surprising given that high biomass is often considered a key goal for optimizing the potential benefits from cover crops. For example, the majority followed or increased seeding rates but did not inoculate legumes consistently or provide pre-plant fertilizer to cover crops. It is plausible that farmers may choose not to optimize cover crop growth in order to avoid difficulties and/or costs in terms of management-related tasks. Alternatively, farmer objectives related to cover crops may be satisfied without crop growth being the most important factor (i.e. soil protection, weed control, nutrient scavenging, etc.). Lastly, farmers may not observe any difference in overall cover crop productivity with additional efforts to maximize cover crop growth.

Other studies have indicated that vigorous cover crop growth may lead to management challenges\textsuperscript{8,38}. Participants who indicated that the unavailability of particular tools factored into their cover crop decision-making also presented stronger agreement that pre- and post-management issues were challenging. The majority of these farmers desired access to roller-crimper tools, presumably, for no-till residue management. No-till systems that mow or crimp cover crop biomass in order to create a layer of above-ground mulch are becoming more popular\textsuperscript{19}. Our results suggest that research and education efforts associated with cover crop growth optimization must also address biomass management and equipment access/procurement issues at the same time.
Although organic and sustainable are not synonymous terms, organic farming strives to achieve an ecological balance, foster the cycling of resources, and conserve biodiversity (NOP, 2010). Organic farming objectives certainly reflect key concepts within agricultural sustainability. Cover cropping itself is a highly encouraged practice, under the USDA national organic certification program to attain soil fertility and crop nutrient management goals. We expected that organic farmers participating in the study would have greater levels of crop rotation and cover cropping but we found no significant differences compared to non-organic participants. This suggests that there were similar values and experiences related to crop rotation and cover cropping between sustainable and organic farmers or perhaps that many sustainable farmers have adopted selected organic farming practices.

Acknowledgements

The authors would like to thank Ken Dawson, Stefan Hartmann, Alex & Betsy Hitt for assistance with survey development, the Carolina Farm Stewardship Association (CSFA) and Southern Sustainable Agriculture Working Group (SSAWG) for access to their membership, and the Organic Crop Improvement Association (OCIA) for financial support.
References


Available from: http://mda.maryland.gov/Documents/12mda_ar.pdf


Fig. 1.1. Survey participants were solicited from the Sustainable Agriculture Research and Education Program, Southern Region which the darker area. The number of participants from each state or territory are reflected within the parentheses: Alabama (8), Arkansas (1), Florida (3), Georgia (18), Kentucky (17), Louisiana (10), Mississippi (3), North Carolina (96), Oklahoma (2), South Carolina (14), Tennessee (15), Texas (6), Virginia (13), Puerto Rico (0) and the U.S. Virgin Islands (2).
Table 1.1. Demographic profile of survey participants.

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>% of responses</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-25</td>
<td>15</td>
<td>7.4</td>
</tr>
<tr>
<td>25-40</td>
<td>55</td>
<td>27.1</td>
</tr>
<tr>
<td>40-60</td>
<td>85</td>
<td>41.9</td>
</tr>
<tr>
<td>&gt;60</td>
<td>48</td>
<td>23.6</td>
</tr>
<tr>
<td><strong>Highest level of education attainment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High school</td>
<td>13</td>
<td>6.4</td>
</tr>
<tr>
<td>2-year college degree</td>
<td>35</td>
<td>17.2</td>
</tr>
<tr>
<td>4-year college degree</td>
<td>85</td>
<td>41.9</td>
</tr>
<tr>
<td>Graduate degree</td>
<td>70</td>
<td>34.5</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>110</td>
<td>54.7</td>
</tr>
<tr>
<td>Female</td>
<td>91</td>
<td>45.3</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>186</td>
<td>94.4</td>
</tr>
<tr>
<td>Black</td>
<td>7</td>
<td>3.6</td>
</tr>
<tr>
<td>Amer. Indian/Alaskan</td>
<td>2</td>
<td>1.0</td>
</tr>
<tr>
<td>Native</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multi/Biracial</td>
<td>2</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hispanic or Latino</td>
<td>3</td>
<td>1.5</td>
</tr>
<tr>
<td>not Hispanic or Latino</td>
<td>197</td>
<td>98.5</td>
</tr>
</tbody>
</table>
Table 1.2. Farm characteristics of survey participants.

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>% of responses</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Full or part-time</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>full-time</td>
<td>108</td>
<td>54.5</td>
</tr>
<tr>
<td>part-time</td>
<td>90</td>
<td>45.5</td>
</tr>
<tr>
<td><strong>Years farming</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 3</td>
<td>61</td>
<td>29.3</td>
</tr>
<tr>
<td>3 to 5</td>
<td>41</td>
<td>19.7</td>
</tr>
<tr>
<td>6 to 10</td>
<td>27</td>
<td>13.0</td>
</tr>
<tr>
<td>11 to 15</td>
<td>18</td>
<td>8.7</td>
</tr>
<tr>
<td>More than 15</td>
<td>61</td>
<td>29.3</td>
</tr>
<tr>
<td><strong>Area under production (hectares)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 2</td>
<td>109</td>
<td>52.9</td>
</tr>
<tr>
<td>3 to 8</td>
<td>44</td>
<td>21.4</td>
</tr>
<tr>
<td>9 to 30</td>
<td>26</td>
<td>12.6</td>
</tr>
<tr>
<td>31 to 81</td>
<td>14</td>
<td>6.8</td>
</tr>
<tr>
<td>More than 81</td>
<td>13</td>
<td>6.3</td>
</tr>
<tr>
<td><strong>Total gross farm income</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than $10,000</td>
<td>89</td>
<td>43.4</td>
</tr>
<tr>
<td>$10,000-19,999</td>
<td>35</td>
<td>17.1</td>
</tr>
<tr>
<td>$20,000-49,999</td>
<td>37</td>
<td>18.0</td>
</tr>
<tr>
<td>$50,000-99,999</td>
<td>10</td>
<td>4.9</td>
</tr>
<tr>
<td>$100,000-249,999</td>
<td>13</td>
<td>6.3</td>
</tr>
<tr>
<td>$250,000-500,000</td>
<td>7</td>
<td>3.4</td>
</tr>
<tr>
<td>More than $500,000</td>
<td>2</td>
<td>1.0</td>
</tr>
<tr>
<td>*<em>Crop type</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vegetable/small fruit</td>
<td>179</td>
<td>85.6</td>
</tr>
<tr>
<td>livestock</td>
<td>69</td>
<td>33.0</td>
</tr>
<tr>
<td>cut flowers</td>
<td>64</td>
<td>30.6</td>
</tr>
<tr>
<td>poultry</td>
<td>59</td>
<td>28.2</td>
</tr>
<tr>
<td>other</td>
<td>41</td>
<td>19.6</td>
</tr>
<tr>
<td>orchard</td>
<td>39</td>
<td>18.7</td>
</tr>
<tr>
<td>grains</td>
<td>31</td>
<td>14.8</td>
</tr>
<tr>
<td>forage</td>
<td>29</td>
<td>13.9</td>
</tr>
<tr>
<td>nursery/ornamental</td>
<td>20</td>
<td>9.6</td>
</tr>
</tbody>
</table>
Table 1.2. (continued)

<table>
<thead>
<tr>
<th>Product</th>
<th>Count</th>
<th>Market Share</th>
</tr>
</thead>
<tbody>
<tr>
<td>tobacco</td>
<td>6</td>
<td>2.9</td>
</tr>
<tr>
<td>cotton</td>
<td>4</td>
<td>1.9</td>
</tr>
</tbody>
</table>

**Market type***

<table>
<thead>
<tr>
<th>Type</th>
<th>Count</th>
<th>Market Share</th>
</tr>
</thead>
<tbody>
<tr>
<td>farmer's markets</td>
<td>124</td>
<td>59.9</td>
</tr>
<tr>
<td>community supported agriculture (CSA)</td>
<td>71</td>
<td>34.3</td>
</tr>
<tr>
<td>wholesale</td>
<td>65</td>
<td>31.4</td>
</tr>
<tr>
<td>cooperatives</td>
<td>26</td>
<td>12.6</td>
</tr>
<tr>
<td>contracts</td>
<td>16</td>
<td>7.7</td>
</tr>
<tr>
<td>other</td>
<td>73</td>
<td>35.3</td>
</tr>
</tbody>
</table>

**Labels**

<table>
<thead>
<tr>
<th>Label</th>
<th>Count</th>
<th>Market Share</th>
</tr>
</thead>
<tbody>
<tr>
<td>certified organic</td>
<td>32</td>
<td>15.4</td>
</tr>
<tr>
<td>transitioning to organic</td>
<td>28</td>
<td>13.5</td>
</tr>
<tr>
<td>part of operation is organic</td>
<td>10</td>
<td>4.8</td>
</tr>
<tr>
<td>other (i.e., naturally grown, grassfed, Appalachian grown)</td>
<td>34</td>
<td>16.7</td>
</tr>
</tbody>
</table>
Fig. 1.2. The most popular cool- and warm-season cover crops being utilized by survey participants (N=209). Cool-season types included: crimson clover (*Secale cereale*), hairy vetch (*Vicia villosa*), annual ryegrass (*Lolium multiflorum*), arrowleaf clover (*Trifolium vesiculosum*), Austrian winter/field pea (*Pisum sativum*), cereal/winter rye (*Secale cereale*), oat (*Avena sativa*), red clover (*Trifolium pratense*), wheat (*Triticum aestivum*), oilseed radish (*Raphanus sativus*), rapeseed/canola/mustard (*Brassica sp.*), sweet clovers (*Melilotus sp.*), subterranean clover (*Trifolium sp.*), triticale (*xTriticosecale*), berseem clover (*Trifolium alexandrinum*), arrowleaf clover (*Trifolium vesiculosum*), and ‘other’. Warm-season types included: buckwheat (*Fagopyrum esculentum*), cowpea (*Vigna unguiculata*), sorghum-sudangrass (*Sorghum bicolor X S. bicolor var. sudanese*), pearl millet (*Panicum miliaceum*), soybean (*Glycine max*), sunflower (*Helianthus annuus*), Japanese millet (*Enchinochloa frumentacea*), foxtail millet (*Setaria italica*), sunn hemp (*Crotalaria juncea*), and ‘other’. Respondents were able to indicate multiple selections. *N*=168 (warm-season) *N*=182 (cool-season).
Fig. 1.3. Survey respondent rankings of 22 statements about the benefits and challenges related to cover cropping. The statements all began “cover crops…”.
Table 1.3. Factor analysis results for cover cropping survey of Southern sustainable farmers.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Description</th>
<th>Item</th>
<th>Loading</th>
<th>Communalities</th>
<th>Mean*</th>
<th>SD</th>
<th>Initial Eigenvalues</th>
<th>% of total variance explained</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Management challenges</td>
<td>Management challenges</td>
<td>Cover crop seed bed preparation is a challenge.</td>
<td>0.991**</td>
<td>0.462</td>
<td>2.73</td>
<td>0.972</td>
<td>5.04</td>
<td>29.66</td>
</tr>
<tr>
<td></td>
<td>Incorporating cover crop residues is a challenge.</td>
<td></td>
<td>0.557</td>
<td>0.352</td>
<td>3.29</td>
<td>1.101</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Soil quality benefits</td>
<td>Soil quality benefits</td>
<td>Cover crops increase soil organic matter.</td>
<td>0.951</td>
<td>0.629</td>
<td>4.79</td>
<td>0.506</td>
<td>2.05</td>
<td>12.08</td>
</tr>
<tr>
<td></td>
<td>Cover crops decrease soil erosion.</td>
<td></td>
<td>0.588</td>
<td>0.530</td>
<td>4.77</td>
<td>0.516</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cover crops increase soil moisture.</td>
<td></td>
<td>0.466</td>
<td>0.527</td>
<td>4.51</td>
<td>0.737</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Pest management &amp; crop yield</td>
<td>Pest management &amp; crop yield</td>
<td>Cover crops break pest &amp; disease cycles.</td>
<td>0.650</td>
<td>0.441</td>
<td>3.99</td>
<td>0.828</td>
<td>1.46</td>
<td>8.59</td>
</tr>
<tr>
<td></td>
<td>Cover crops reduce pesticide applications</td>
<td></td>
<td>0.530</td>
<td>0.383</td>
<td>3.84</td>
<td>1.007</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>The cost of establishing cover crops is prohibitive.</td>
<td></td>
<td>-0.538</td>
<td>0.357</td>
<td>2.39</td>
<td>1.015</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Using cover crops increases cash crop yields.</td>
<td></td>
<td>0.424</td>
<td>0.335</td>
<td>3.94</td>
<td>0.793</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 1.3. (continued)

<table>
<thead>
<tr>
<th></th>
<th>Negative effects</th>
<th>Selected cover crops reduce available nitrogen for subsequent cash crops.</th>
<th>0.637</th>
<th>0.266</th>
<th>2.85</th>
<th>1.080</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Cover crops become weeds</td>
<td></td>
<td>0.624</td>
<td>0.304</td>
<td>2.74</td>
<td>0.930</td>
</tr>
<tr>
<td></td>
<td>Using cover crops results in a loss of cash crop opportunities.</td>
<td></td>
<td>0.476</td>
<td>0.330</td>
<td>2.29</td>
<td>0.878</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Additional benefits</th>
<th>Cover crops reduce nutrient leaching</th>
<th>0.598</th>
<th>0.460</th>
<th>4.25</th>
<th>0.774</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Cover crops break hard pans with their roots.</td>
<td></td>
<td>0.576</td>
<td>0.487</td>
<td>4.28</td>
<td>0.836</td>
</tr>
<tr>
<td></td>
<td>Cover crops suppress weeds.</td>
<td></td>
<td>0.528</td>
<td>0.419</td>
<td>4.40</td>
<td>0.729</td>
</tr>
<tr>
<td></td>
<td>Cover crops increase soil moisture.</td>
<td></td>
<td>0.483</td>
<td>0.527</td>
<td>4.51</td>
<td>0.737</td>
</tr>
</tbody>
</table>

|   |                           | Cumulative %                                                              |       |       |       | 63.24 |

* Means from Likert scale ranging from 1-5 (strongly disagree to strongly agree).
** Only variables with loading factors >0.40 are presented; positive factor loadings indicated a positive correlation between the variable and factor while negative loadings indicated an inverse correlation.
CHAPTER 2

Title Short-term Nitrogen Mineralization from Warm-season Cover Crops in Organic Farming Systems

Authors S. O’Connell*, N. G. Creamer¹, W. Shi², J. M. Grossman², G. D. Hoyt¹,² and K. L. Fager¹.

(1) North Carolina State University, Department of Horticultural Science, Box #7609, Raleigh, NC 27695, USA
(2) North Carolina State University, Department of Soil Science, Box #7619, Raleigh, NC 27695, USA

* suzanne.oconnell@gmail.com

Abstract

Background and aims

There are multiple benefits derived from cover cropping yet improving our ability to predict N availability from residues remains a challenge. Further understanding and illustrating factors which influence decomposition in the field will increase the ability to tailor management practices to meet farm goals and environmental conditions.
**Methods**

We assessed short-term N mineralization from warm-season cover crops in organic farming systems with a variety of field and lab-based measures including: shoot tissue quality, extractable inorganic N, ion resin adsorbed N, potential C and N mineralization.

**Results**

All warm-season cover crops (C:N from 15-57:1) appeared to result in net N mineralization and cowpea-dominated crops had greater potential than grasses. N mineralization was strongly correlated with shoot tissue quality. Cover cropped soils demonstrated increased ability to retain soil N, moderate N release and reduce N leaching after intense precipitation.

**Conclusions**

Short-term N mineralization from warm-season cover crops was affected by both plant quality and environmental conditions. The soil microbial community appeared to reduce its carbon use efficiency (CUE) under soil moisture stress resulting in less microbial N demand and net N mineralization from cover crops of relatively low quality.

**Keywords** (4-6)

green manure, legume, carbon and nitrogen mineralization, PRS probes

**Abbreviations:** SOM soil organic matter, PRS plant resin simulator
Introduction

Cover crops are utilized in many types of farming systems and multiple benefits have been attributed to them, including: decreasing soil erosion, improving soil and water quality, suppressing weeds, contributing and recycling nutrients, managing pest and diseases, attracting pollinators, reducing fertilizer expenses, and sequestering carbon (Hartwig and Ammon 2002; Janzen and Schaalje 1992; Lal et al. 1991; Shepard et al. 2002). Organic farmers in particular, rely on cover cropping (i.e., green manures) as a nitrogen (N) source for subsequent cash crops. Cover cropping is a highly encouraged practice under the USDA National Organic Program (NOP) which includes the prohibition of synthetic inputs, inclusion of crop rotation and the continual pursuit to improve overall soil quality (7 C.F.R. § 205.203 (rev. 2013)).

One of the most challenging cover crop related issues is estimating the amount of N contribution and synchronizing N release with a subsequent cash crop from decomposing residues. Under optimum conditions, plant residue decomposition generally follows 1\textsuperscript{st} order kinetics where the rate of transformation of a substrate (i.e., the residue) is proportional to the substrate concentration (Minderman 1968). It is important to consider the effects of many biotic and abiotic variables on decomposition rates and N mineralization in field situations such as: residue quality, edaphic soil properties, environmental conditions, biological activity, and farm management (Baggie et al. 2004; Cambardella and Elliott 1993; Goh and Tutuna 2004; Johnson et al. 2007; Robertson and Groffman 2007). Improving our ability to predict N availability from cover crop residues requires further understanding of the biotic and abiotic interactions that affect C and N cycling. This knowledge will assist agricultural
professionals in becoming more efficient nutrient managers by adopting and tailoring cover crop management practices to meet farm goals and environmental conditions.

Although cool-season cover crops remain the most frequently utilized there is a growing interest in integrating warm-season cover crops into farming rotations (Creamer and Baldwin 2000; Sarrantionio and Gallandt 2003). A recent survey of more than 200 sustainable farmers in the Southern region reported that 80% used cover crops and 55% of these participants included warm-season selections (O’Connell et al. 2013, unpublished). Warm and cool-season cover crop species may have different growth and biochemical characteristics leading to varying farm applications. For example, cool-season cover crops are often selected for their cold tolerance and often utilize C3 photosynthetic pathways while warm-season cover crops typically have tropical origins, greater soil acidity tolerances and often utilize C4 photosynthetic pathways resulting in higher light, temperature, and drought tolerances (Baligar and Fageria 2007; Marschner 1995). For these reasons, the potential benefits of warm-season cover crops may be amplified in regions with long-growing seasons and/or dry conditions including the sub-tropical Southern region, drylands of the Great Plains and the Southwestern low desert in the U.S. (Creamer and Baldwin 2000; Moyer et al. 2000; Wang et al. 2008).

Many warm-season cover crops have the ability to produce large amounts of biomass over short periods of time, which can result in valuable contributions of C and N to the soil (Baligar and Fageria 2007; Creamer and Baldwin 2000). Over a dozen warm-season cover crops were examined for their potential utility in North Carolina vegetable farming systems. After a 2-month growing season, dry biomass output and N contributions ranged from 1,402
to 4,807 kg ha\(^{-1}\) for legumes (32 to 97 kg N ha\(^{-1}\)) and 3,918 to 8,792 kg ha\(^{-1}\) for grasses (39 to 88 kg N ha\(^{-1}\)) (Creamer and Baldwin 2000). Research pertaining to warm-season cover cropping has been limited and the application of cool-season cover crop results should be applied with caution. Differences between cool-season and warm-season cover crop characteristics as well as seasonal weather dynamics (e.g., temperature and precipitation patterns) could influence crop growth, management decisions, and decomposition rates. Our goal was to compare selected plant and soil-based measures for their ability to reflect short-term N mineralization from warm-season cover crops including: cover crop quantity and quality, total soil C and N content, inorganic soil N, and short-term C and N mineralization.

We hypothesized that warm-season cover crops would result in net N mineralization over the weeks-to-months following incorporation, but that the total amount and timing would be dependent on both plant quality and environmental conditions. Our objectives were: 1) to evaluate differences in short-term N mineralization among a selection of popular warm-season cover crops after soil incorporation and, 2) to assess the utility of plant root simulator (PRS) probes as an in-situ indicator of N availability.

**Materials and Methods**

*Site History and Experimental Design.* The field experiment was located at the Center for Environmental Farming Systems (CEFS) located in Goldsboro, North Carolina during 2010. The design was a randomized complete block with five warm-season cover crop treatments and a bare-ground control (i.e., no cover crop additions). Trials were conducted at two locations approximately 3 km apart from each other referred to as *site 1* and *site 2*. Both
sites were had organic management histories although only site 1 was certified. Regionally popular cover crop selections included in our study were: 1) buckwheat (*Fagopyrum esculentum*), 2) sorghum-sudangrass (*Sorghum bicolor X S. bicolor var. sudanese* (Special Effort)), 3) cowpea (*Vigna unguiculata* (Iron & Clay) (L.) Walp.), 4) German foxtail millet (*Setaria italica* (L.) P. Beauv.), 5) German foxtail millet/cowpea mix, and 6) bare-ground control. Seeding rates reflect regional practices and were the same at both sites: buckwheat (66 kg ha\(^{-1}\)), sorghum-sudangrass (39 kg ha\(^{-1}\)), cowpea (100 kg ha\(^{-1}\)), foxtail millet (22 kg ha\(^{-1}\)) and cowpea-foxtail millet mix (73 kg ha\(^{-1}\) and 6 kg ha\(^{-1}\), respectively). Treatments were replicated four times at each site to control within site variability.

Crop management and data collection were carried out by block. Each plot (i.e., experimental unit) was 12.2 x 4.0 m\(^2\). The soil at both locations was a Wickham sandy loam with a 2-6\% slope at site 1 and a 0-2\% slope at site 2 (fine-loamy, mixed, semiactive, thermic Typic Hapludults in U.S. soil taxonomy). This is a very typical soil type found in the Piedmont and Coastal Plains of the Southeastern region of the U.S. known to be well-drained and moderately permeable. *Site* 1 and *site* 2 had a starting pH of 5.9 and 6.0, cation exchange capacities (CEC) of 6.2 and 5.5, and bulk densities of 1.4 g cm\(^{-3}\) and 1.3 g cm\(^{-3}\), respectively.

**Management.** Field preparation was conducted during the month of April. Existing vegetation, fescue hay at *site* 1 and volunteer winter rye at *site* 2, was terminated by flail mowing (BEFCO Inc.; Rocky Mount, NC) and incorporated by disking (#630; John Deere, Moline, IL). One month later, each field was prepped for cover crop planting with a chisel plow (11 tine; International Harvester Co., Canton, IL) and field conditioner (4.6 m wide; Taylor-Way, Athens, TN). A drill seeder (Sukup 2055; Sukup Manufacturing Co., Sheffield,
IA) was used to plant cover crop seeds approximately 2.5-3.8 cm deep followed by a pass with a cultipacker (SS1, 3m wide, grass seeder; Brillion Farm Equipment; Brillion, WI) to ensure good contact between the soil and the seeds. All cowpea seeds were treated with *Bradyrhizobium sp. (Vigna)* inoculant prior to planting.

The length of the growing season varied at each site due to poor crop establishment at the initial *site 2* field plot that required re-planting (Table 2.1). As a result, the transitional trial was replanted, 48 days after the *site 1*, in an adjacent field with the addition of two pre-plant amendments to ensure adequate soil fertility (Table 2.1). Periodic hand-weeding and/or shallow tillage (i.e., approx. 10 cm deep) with a tractor-mounted rototiller (1.5 m wide Tilrovator; Ferguson Manufacturing Co., Suffolk, VA) was carried out in the bare-ground control plots at both sites as needed to minimize living vegetation throughout the experimental timeframe.

Irrigation was used during crop establishment and dry spells during the growing period, however, the capability to irrigate at *site 1* was more limited which resulted in a drier environment compared to *site 2*. Irrigation was not used during the post-cover crop incorporation sampling period. From mid-June to mid-July, *site 1* was subject to a grasshopper (*Melanoplus differentialis* (Thomas)) infestation. The grasshopper population was controlled with a series of organic pesticide applications but the buckwheat cover crop was lost due to heavy herbivory (Table 2.1). The establishment of the buckwheat cover crop at *site 2* was poor, likely attributable to the higher soil temperatures associated with the delayed planting date and therefore the buckwheat crop was abandoned at both sites.
The remaining crops were terminated by cutting with a flail mower (H70 Hurricane Flail Mower, BEFCO; Rocky Mount, NC) and crop residue was allowed to desiccate in place on the soil surface for 1 week. Then the cover crop residue was incorporated into the soil approximately 30 cm deep with 2 passes from a tractor-mounted rototiller (1.5 m wide Tilrovator; Ferguson Manufacturing Co.; Suffolk, VA). After rototilling, a cultipacker (SS10 Grass Seeder; Brillion Farm Equipment; Brillion, WI) was utilized to ensure good contact between the soil and the mostly buried cover crop residues.

Environmental Monitoring. Non-replicated air temperature, soil temperature and soil moisture were monitored on an hourly basis at each of the two field sites with automatic data loggers (#HOBO Micro Station #H21-002 Data Logger with S-THA-M002, #S-TMB-M002 and #S-SMA-M005, Onset Computer Corp.; Bourne, MA). Precipitation data and the 30-year average for air temperature from 1971-2000 were supplied by the North Carolina State Climate Office weather station located 1.6 km from the research station (GOLD-Cherry Research Station; Lat: 35.37935° Long: -78.0448°) (Fig.1). Overall, the 2010 summer cover crop growing season was hotter and drier than average; additionally, there was a moderate drought period followed by record-breaking rainfall during the month of September (State Climate Office of NC 2010).

Above-ground biomass and tissue quality. Immediately prior to termination, the percent cover crop cover was assessed for two, 0.5 m² quadrants per plot. Above-ground biomass samples of cover crops were collected from the same quadrants. Components of cover crop mixes were separated by crop type. Fresh and then dry weights (forced-air oven at 60°C for 48 hours) of biomass from each quadrant was recorded. Dry weights were then
averaged for each plot. A sub-sample of dry plant tissue was ground to pass through a 1-mm screen (Model 4 Wiley mill, Arthur A. Thomas Co.; Philadelphia, PA), ground further with a ball mill and analyzed for elemental carbon (C) and nitrogen (N) (Perkin Elmer 2400 CHNS/O Elemental Analyzer, Perkin-Elmer Corp.; Norwalk, CT).

Soil samples were collected over a series of six sampling dates including, the day before cover crop termination and five dates post-cover crop incorporation. At each date, 40 soil cores (2.5 cm diameter x 0-15 cm depth) from each plot were homogenized in a composite sample. A 100 g sub-sample of soil was dried at 105°C for 48 hours to calculate gravimetric soil moisture content. Approximately 2,000 g of soil was packed in a labeled Ziploc bag transported in a cooler from the field, and stored at 4.4°C. Soil samples were hand-sieved to pass a 2 mm sieve within 48 hours of field collection. Half the sieved soil was dried at 50°C for 48 hours in a forced air oven, while the other half was kept field-moist and again stored at 4.4°C for use in the C and N mineralization study. Subsamples from the dried soils were ground and analyzed for elemental C and N as described previously for plant tissue.

Soil C decomposition and nitrogen mineralization were determined via a 28 day aerobic incubation experiment. Two, field-moist soil samples (sample #1=10g soil; sample #2=20 g soil) were adjusted to 45% water holding capacity and pre-incubated at room temperature (approx. 21°C) for 5 days within an incubation unit (i.e., foil-covered, 1 L glass jar) to create optimal conditions for soil microbes. Five mL of distilled water was included in each jar to maintain a humid environment and minimize loss of soil moisture throughout the experiment. After the adjustment period, one 10 g soil sample was removed, combined with
50 ml of 1 M KCl (5:1 dilution) and gently shaken. The resulting soil suspension was passed through cellulose filters (Whatman #1: 11μm) and then frozen (-20°C) until analysis representing inorganic N at day 0.

A vial containing 5 mL of 0.5 M NaOH was added to each jar at day 0 to absorb carbon dioxide (CO$_2$-C) from the remaining 20 g soil sample. The amount of CO$_2$-C evolved was estimated by titration with 0.1 M HCl at day 11 and day 28 (Zibilske 1994). The jars were flushed with fresh air for ~30 min and fresh NaOH alkaline traps installed on day 11. The sum of the CO$_2$-C evolved was estimated to be the potential C mineralization. At day 28, 10 g of the remaining soil sample was removed and analyzed for inorganic N at day 28 as described above. All extracts were analyzed for inorganic nitrogen (NH$_4^+$-N + NO$_3^-$-N) via flow injection methods with colorimetric determination (QuikChem IV; Lachat Instruments, Loveland, CO). The difference between the inorganic nitrogen before (day 0) and after incubation (day 28) was estimated to be the potential N mineralization.

*Plant root simulator probes* (PRS$^{\text{TM}}$; Western Ag Innovations Inc., Saskatoon, SK, Canada) were used to measure in situ nitrogen availability (N) from the soil solution and labile nutrient pool. The PRS probes are designed to act as nutrient sinks similar to plant roots that can measure potential soil nitrogen supply rates. Each probe was comprised of a plastic frame (3 x 15 cm) that wraps around a flat, double-sided ion exchange resin membrane (17.5 cm$^2$ of total surface area). The membranes were pre-treated so that they would continuously adsorb either the nitrate (NO$_3$-N) or ammonium (NH$_4$-N) ions. Four pairs of PRS-probes, comprised of 1 anion and 1 cation-adsorbing probe, were buried in the top 0-15 cm of soil of each plot per sample period. Care was taken to ensure good contact
between the resin membranes and the soil. At the end of each burial period, new probes were installed in the same location with the exception of the bare-ground control plots. Because shallow tillage was required to manage weeds in the control plots, probes were removed at 1 sampling date per site, tillage conducted as needed, and new probes placed in approximately the same areas. Otherwise, hand-weeding was used to control weeds in plots as needed.

Sets of PRS probes were buried and retrieved 5 times over approximately 11 weeks post-cover crop incorporation; they were not buried pre-incorporation. Protocols for burial, retrieval, washing, handling and shipping provided by the supplier were followed (Western Ag. Innovations, Inc., SK, Canada). Adsorbed ions (NH$_4$-N + NO$_3$-N) were extracted from the probe membranes by elution with of 0.5 N hydrochloric acid solution. The probe solution was analyzed for inorganic nitrogen (NH$_4^+$-N + NO$_3^-$-N) via flow injection methods with colorimetric determination via flow injection methods (FIALab 2600; FIALab Instruments Inc., Bellevue, WA). Total soluble N was calculated by dividing the average amount of nutrient adsorbed per treatment (NH$_4$-N + NO$_3$-N) per membrane surface area per burial time (e.g., μg ion per 10 cm$^2$ per burial time). Our experimental values for any given sampling point did not surpass 25% of the probe maximum ion capacity. Although the manufacturers of PRS probes suggest maintaining identical burial periods to be able to compare results on a temporally-even basis, we chose not to maintain equivalent burial periods in order to best capture anticipated N dynamics while maximizing financial resources.
Statistics

Analysis of variance was performed using proc mixed procedure (SAS Institute v. 9.2, Cary, NC). Means were separated using least-squared means post-hoc test with Tukey’s adjustment to control type 1 error at a significance level of $\alpha \leq 0.05$. First, results were analyzed for differences between experimental sites when site was considered a fixed effect. No assumptions about site differences were made if site by treatment interactions were significant. Site by treatment interactions were common and thus individual sites were also analyzed separately and results are primarily presented this way. Blocks at each site were considered random effects. Measurements that were taken from an experimental unit (i.e., plot) on more than one date were also subject to a repeated measures analysis. Variables that did not meet the assumptions of normality or homogeneity of variances were transformed by natural log prior to analysis and then back-transformed for presentation of results.

To improve the ability to distinguish among cover crop quality and minimize the confounding effects of background soil and cover crop quantity, seasonal means were normalized (i.e., $(A-B)/C$ where $A$ represents the value for a cover crop treatment, $B$ the bare-ground control value and $C$ the above-ground cover crop biomass). Analysis of variance as described above was carried out using the normalized values. Results were then compared to the original analyses in order to assess whether quantity or quality appeared to have a greater effect on treatment separations among cover crops.

Linear hypotheses were used to conduct group comparisons for fixed factors such as cowpea-dominated (i.e., cowpea and cowpea-foxtail mix) versus grass cover crops (i.e., foxtail millet and sorghum-sudangrass) (‘Estimate Statements’, SAS Institute, Cary, NC) on
normalized data at a significance level of $\alpha \leq 0.05$. Pearson’s correlation coefficient ($r$) was used to describe the degree of correlation between potential N mineralization and selected soil and cover crop tissue variables as well as plant tissue quality and FPOM quality. In addition, Pearson correlation analyses were conducted and correlation coefficients ($r$) used to describe any linear relationships between normalized, potential N mineralization and cover crop residue quality and quantity using a threshold of $\alpha \leq 0.05$.

Results

*Plant Tissue.* More cover crop biomass was generated for each treatment at *site 2* compared to *site 1* (Table 2.2). Foxtail millet, cowpea, and cowpea-millet mix produced approximately 30-35% more while sorghum-sudangrass produced almost 100% more shoot biomass at *site 2* (Table 2.2). Within each location sorghum-sudangrass produced more biomass than all other cover crops types ($p=0.0004$). The dry weight of the cover crop biomass ranged from 2,256 to 5,302 kg ha$^{-1}$ at *site 1* and from 3,195 to 10,110 kg ha$^{-1}$ at *site 2* (Table 2.2). The cowpea-millet mix was comprised of approximately 93% cowpea at the end of the growing season at both sites.

Mean shoot tissue ranged from 42 to 45% C and 1 to 3% N (Table 2.2). At both sites, the grass cover crops (i.e., foxtail millet and sorghum-sudan) had greater % C and lower % N compared to the cowpea-dominated cover crops ($p<0.0001$) (Table 2.2). A wide range of C:N ratios were present and primarily reflective of % N differences among cover crop type (i.e., grass or legume). Grass C:N ratios ranged from 30-57:1 and cowpea-dominated cover crop treatments from 15-19:1 (Table 2.2). At both sites, cowpea-dominated crops had lower C:N
ratios than grasses (p<0.0001) (Table 2.2). Foxtail millet and sorghum-sudan appeared to reach advanced stages of maturity at site 1 as evidenced by higher C:N ratios (Table 2.2).

Soil carbon and nitrogen concentration. Seasonal mean soil C and N concentrations across the two sites ranged from 0.33 to 1.27 g C 100 g⁻¹ soil and 0.02 to 0.12 g N 100 g⁻¹ soil (data not shown). As a result, the mean soil C:N ratio at site 1 was 11:1 compared to 13:1 at site 2 (data not shown). Between the 2 sites, only location effects on soil C:N were significant (p<0.0032) (p>0.05) (data not shown).

Extractable Soil N. A site comparison indicated that there were significant site and treatment effects (p<0.0001) but site by treatment interactions were not present. Within each site, treatment effects as well as treatment by time interactions were significant (p<0.0001). At both sites, bare-ground control plots had greater extractable soil N compared to sorghum-sudangrass plots, pre-incorporation and for 2-4 weeks post- incorporation (p<0.05) (Fig. 2.2). In addition, cowpea-dominated soils had greater soil N than sorghum-sudangrass soils at weeks 1 and 2 post-incorporation at both sites (p<0.05). Soil N decreased across all treatments at both sites after a heavy precipitation event occurred (9/26-9/30) and displayed very little fluctuation for the remainder of the sampling season at either site (Fig. 2.2).

At site 1, trends indicated cowpea-dominated plots had the greatest amounts of extractable inorganic soil N, 1-2 weeks post- incorporation while the same occurred in grass plots at 4 weeks post-incorporation (Fig. 2.2). At site 2, trends indicated that cowpea-dominated and foxtail millet soils had the highest amounts of extractable inorganic soil N, 1-2 weeks post- incorporation; while sorghum-sudangrass soils did not appear to peak at any one sampling date and/or before the precipitation event occurred (Fig. 2.2). At site 2 only,
cowpea-dominated plots displayed greater levels of soil N compared to the bare-ground control plots after the precipitation event (9/26-9/30) (Fig. 2.2).

A comparison of seasonal means for soil N from cowpea-dominated plots indicated no differences with bare-ground controls, approximately 25% more extractable soil N than foxtail millet plots and 100% more extractable soil N than sorghum-sudangrass plots (Table 2.3). When seasonal means were normalized to minimize the effects of background soil N and cover crop quantity donated to the soil, treatment differences were no longer significant indicating that cover crop quantity not quality was the dominant influence on treatment effects (p>0.05) (data not shown).

Resin Probe Absorbed N. A site comparison indicated that a treatment by site interaction was present (p<0.0250). Within each site, treatment effects as well as treatment by time interactions were significant (p<0.0001). The range of mean resin probe adsorbed N (i.e., NH$_4$-N + NO$_3$-N) was narrower for bare-ground control soils compared to cover crop soils (58 to 366 and 13 to 444 μg ml$^{-1}$ N 10 cm$^{-2}$ burial period$^{-1}$, respectively) across the sampling season (Fig. 2.3). The majority of the N ions extracted from the probes on all sampling dates were of the NO$_3$-N form (data not shown).

At site 1, the seasonal mean probe adsorbed N was greater in cowpea plots compared to foxtail millet and sorghum-sudangrass (p<0.05) (Table 2.4). In addition, cowpea-dominated plots had a greater N adsorption 1 and 2 weeks post-incorporation compared to both grasses at site 1 (p<0.05) (Fig. 2.3). Foxtail millet plots had greater N adsorption compared to sorghum-sudangrass for the 1$^{st}$ and 4$^{th}$ week post incorporation at this site (p<0.05) (Fig. 2.3). At site 2, there was less treatment separation over time. The mean
adsorbed N for the season from cowpea plots was greater compared to sorghum-sudangrass only at this site (p<0.05) (Table 2.4). Sorghum-sudangrass plots had lower N adsorption compared to all treatments, 1 and 5 weeks post- incorporation at site 2 (p<0.05) (Fig. 2.3).

At both sites, the seasonal mean for PRS probe adsorbed N was greater for cowpea-dominated and bare-ground control plots compared to sorghum-sudangrass plots (p<0.0001) (Table 3). When data were normalized to minimize the effects of background soil N and cover crop quantity donated to the soil, results differed by site. At site 1, treatment differences remained significant between cowpea and both grasses (p<0.0051); contrasts indicated that cowpea-dominated plots had greater values compared to the grasses (p<0.0010). At site 2, differences among treatments did not maintain a significant separation (p>0.05) indicating that either cover crop quantity had a greater influence at site 2 and/or cover crop quality which was less extreme had a smaller influence at site 2 (data not shown).

Mineralized Soil N. A lab incubation study evaluating potential N mineralization indicated that a treatment by site interaction was present (p<0.0001). Within each site, treatment effects as well as treatment by time interactions were significant (p<0.0001). The range of mineralized N values for bare-ground controls was narrower (-11.9 to 8.6 mg N kg⁻¹ soil) than cover crop plots (-16.9 to 37.0 mg N kg⁻¹ soil) (Fig. 4). At both sites, net N mineralization was predicted for all cover crops as well as bare-ground soils over the season based on our 28-day incubation experiment (Table 2.3). The seasonal means for mineralized N from cowpea plots were greater than bare-ground controls at both sites (p<0.0002) (Table 2.3). At both sites, contrasts indicated that cowpea-dominated plots had greater mineralized
soil N compared to the grasses (p<0.0005). Treatments demonstrated very little fluctuation over the last 2 sampling dates (~3 weeks) at both sites (Fig. 2.4).

At site 1, cowpea-dominated plots had greater N mineralization compared to both grasses and the bare ground controls 4 weeks post-cover crop incorporation (p<0.05) (Fig. 2.4). At site 2, cowpea-dominated plots had greater N mineralization 2 weeks after cover crop incorporation compared to the bare-ground controls (p<0.05) (Fig. 2.4). Seasonal means at site 1 were greater for cowpea-dominated plots compared to foxtail millet and bare-ground controls (p<0.0001) (Table 2.3). At site 2, seasonal means for mineralized N were greater for all cover crops compared to the bare-ground controls (p<0.0007) (Table 2.3).

When seasonal means were normalized to minimize the effects of background soil N and cover crop quantity donated to the soil, treatment differences remained (p<0.0178). At site 1, cowpea and cowpea-foxtail mix had greater mineralized N than both grasses (p=0.0004) (data not shown). At site 2, treatment differences between cowpea-foxtail mix and sorghum-sudangrass were apparent that had not previously been significant (p<0.0178) (data not shown). These results indicate that cover crop quality rather than quantity had the dominant effect on soil mineralized N. At both sites, contrasts between normalized data continued to indicate that cowpea-dominated plots had greater mineralized soil N compared to the grasses (p<0.0041). In addition, normalized, mineralized soil N was negatively correlated with shoot C:N ratio, shoot % C, and FPOM C:N ratio (established site only); it was positively correlated with shoot % N (Table 2.3).

**Mineralized Soil C.** A lab incubation study evaluating potential C mineralization indicated that site and treatment differences were significant (p<0.0433) but treatment by site
interactions were not present. Within each site, only treatment effects were significant (p<0.0001). The range of mean values for bare-ground treatments was narrower (32 to 93 mg C kg\(^{-1}\) soil) compared to cover crop plots (54 to 218 mg C kg\(^{-1}\) soil) (Fig. 2.5). At both sites, bare-ground controls had the lower mean mineralized C values compared to all cover crops for 5 out of 6 sampling dates (p<0.05) (Fig. 2.5). In addition, sorghum-sudangrass soils had greater seasonal mean mineralized C compared to cowpea and bare-ground controls (p<0.0001) (Table 2.3). Carbon mineralization appeared to decline slightly across all treatments 1-2 weeks post-incorporation at both sites.

At site 1, sorghum-sudangrass plots had greater seasonal mean mineralized C compared to the rest of the cover crops whereas at site 2, sorghum-sudangrass was only greater than cowpea (p<0.05) (Table 2.3). At site 1 only, on the pre-incorporation date, sorghum-sudangrass plots had greater mineralized C compared to foxtail millet and the bare-ground control (p<0.05) (Fig. 2.5). When seasonal means were normalized to minimize the effects of background soil N and cover crop quantity donated to the soil, treatment differences were no longer significant indicating that cover crop quantity not quality was the dominant influence on treatment effects (p>0.05) (data not shown).

**Discussion**

All cover-cropped soils subjected to a 28-day lab incubation were found to have positive mean N mineralization, including those with a C:N greater than 40:1 which was unexpected. We hypothesize that suboptimal soil conditions (i.e., low soil moisture), slowed cover crop decomposition rates by lowering the carbon utilization efficiency (CUE) of the
soil microbial community. A reduced CUE may have led to a decreased N demand. In addition, the potential N mineralization of cover crops was strongly correlated with shoot tissue quality while C mineralization and inorganic soil N pools appeared to be influenced primarily by the amount of cover crop biomass. We also found evidence that demonstrated that warm-season cover crops can help conserve and protect soil N resources thereby making agricultural systems less susceptible to nutrient losses.

Cover crop quality, represented by both the shoot C:N ratio and the shoot % N demonstrated strong correlations with potential N mineralization. Hence, soils from cowpea-dominated cover crops (i.e., lower shoot C:N and greater % N) showed greater potential N mineralization compared to soils from grass cover crops. When we inserted our values into Vigil and Kissel’s predictive equations (1991), which relate crop residue % N as a function of residue C:N ratio ($\hat{y}=408(1/N)$, $r^2=0.99$) and % N mineralized as a function of residue C:N ratio ($\hat{y}=58.89 – 1.41 \times (C/N)$, $r^2=0.75$) we found agreement with the former but not the latter. First, our cover crop shoot tissue ranged from 0.8 to 2.8% N and therefore was estimated to have C:N ratios between 51-15:1 with Vigil and Kissel’s equation, which was similar to our data (57-15:1). Next, a range of -21% to +38% N mineralization was predicted for our cover crop residues but we did not find any evidence of net N immobilization, even those with C:N ratios greater than 40:1. One plausible explanation for a difference in findings is that the carbon use efficiency (CUE) of the soil microbial community in our experiments was lowered due to sub-optimal field conditions. Reduced CUE’s, due to sub-optimal soil moisture, may have decreased the microbial demand for C and N and slowed the
decomposition process, resulting in net N mineralization even for cover crops with a C:N ratio >40:1.

Past management histories at the two different field sites may have influenced the baseline soil qualities and potential N mineralization (i.e., site 1 had 11 years of organic management history compared to site 2 with only 4 years of organic management). But environmental conditions, primarily soil moisture, appeared to have the dominant effect on plant production and decomposition process. The 2010 growing season was hot and dry and the month-long period post-cover crop termination was considered a moderate drought. These impacts were greater at site 1 where the growing season was longer and irrigation capabilities were more limited. Low soil moisture can hinder the ability of microbes by limiting substrate solubility, decreasing the mobility of microbes and/or extracellular enzymes, and/or diverting energy towards survival pathways (e.g., production of mucilage, acquisition of osmolytes, dormancy) rather than growth activities (Broken and Matzer 2009; Manzoni et al. 2012; Schimel et al. 2007; Voroney 2007). Microbial growth yields can decrease up to 90% under osmotic stress (Killham and Firestone 1984).

The popular notion that high quality cover crops (e.g. C:N ratios <25-40:1) result in net N mineralization assumes a CUE (i.e., microbial growth yield) of 40-60%, which is typical for decomposition of readily available soluble constituents (Plante and Parton 2007). However, there is evidence to suggest that the CUE can decrease with increased environmental stress. Biogeochemical models have little consensus about the impact of soil moisture on CUE (Keiblinger et al. 2010; Manzoni et al. 2012) but a handful of studies have investigated this phenomenon and found lowered CUE in conjunction with limited soil
moisture. Tiemann and Billings, observed a decreased CUE in grassland ecosystems when there was greater variability in soil water content (i.e., more frequent dry periods) (2011). While Herron et al., documented that CUE dropped as low as 24% when soil moisture was limited in short-term gas-based stable isotope lab experiments (2009). We estimated the CUE to be an average of 15-20% in our experiment in order for cover crops with C:N ratios ranging from 42-57:1 to result in net N mineralization, when microbial C:N was assumed to be 8:1.

It has been well-documented that cool-season cover crops can retain soil N, moderate N release and reduce nitrate leaching over the fall to early spring seasons (Brinsfield and Staver 1991; Dabney et al. 2001; De Vos et al. 2000; Lewan 1994; MacDonald et al. 2005). Our data demonstrates that warm-season cover crops can provide these functions during the summer to fall months as well. At our pre-cover crop incorporation dates, cover cropped plots had lower extractable soil N compared to bare-ground controls, reflecting plant uptake of soil N during the summer growing season. For approximately 4-6 weeks after cover crop termination (i.e., 2-4 weeks post-incorporation), the grass cover crop plots most notably sorghum-sudangrass, had lower inorganic soil N values compared to bare-ground soils and the cowpea-dominated plots. This indicated the retention and subsequent moderated release of soil N from organic (i.e., cover crop plant tissue, microbial biomass, etc.) rather than inorganic sources in the bare-ground control soils.

When a record-breaking precipitation event occurred at the end of September, decreases in inorganic soil N were evident across all treatments at both sites yet the losses were of the greatest magnitude in the bare-ground control soils. Whenever high
mineralization rates are combined with high precipitation, the potential for soil N leaching is present (Lewan 1994). Pre- and post- the rain event, inorganic soil N decreased by 76-90% in bare-ground plots, 57-66% in cowpea plots and 0-33% in sorghum-sudan plots at the 2 sites. Soils that had supported warm-season cover crops were able to conserve more soil N because the soil N taken up or atmospheric N fixed by cover crops was retained in organic N forms in the soil which were less susceptible to leaching, run-off and/or denitrification than inorganic soil N. If cash crops had been present soil N losses may have been moderated by new plant uptake. The influence of cover crop biomass quantity appeared to maximize these functions.

PRS probes appeared to be an informative in-situ measure of inorganic soil N. Because the technology relies on the movement of N via mass flow or diffusion in the soil over time it was able to represent the combined effects of soil moisture on hypothetical plant-available nitrogen in the soil. PRS probes indicated that a flush of available inorganic N had occurred (i.e., increase in ion adsorption) after a record-breaking precipitation event. Separating the effects of soil moisture and N availability on probe ion absorption was not possible which limits the application of the results. Still, PRS probes may present a more dynamic representation of what is occurring in an actual field situation compared to traditional point-in-time soil extractions. Another consideration for the utility of PRS data depends on the frequency and length of probe deployment intervals. For example, we could not report with certainty when spikes in N absorption occurred beyond the parameters of between two successive deployment dates as N absorption could have been occurring primarily on one date or over the entirety of the burial period which again limits the application of the results. In agreement with other studies, which found that probe adsorbed
NO$_3$-N appeared to be a better indicator of soil N availability due to consistently low NH$_4$-N values (Bair et al. 2008; Quian and Schoenau 1995). Due to opposing results at each of our field sites after normalizing PRS probe data values, we could not conclude whether cover crop quantity or quality had stronger influence on inorganic soil N adsorption.

Our data suggested the following applications to farm management practices. Legume cover crops, as expected, appeared to have more N mineralization potential then grasses but during a dry year the differences were not as appreciable. Warm-season leguminous cover crops grown in North Carolina have been estimated to donate slightly less N to the soil system than cool-season legumes, approximately 30-100 kg N ha$^{-1}$ and 80-225 kg N ha$^{-1}$, respectively (Creamer and Baldwin 2000; Hoyt and Hargrove 1986) but the gains may be comparable when considering the short, 2-month growing season for many warm-season cover crops. In years or systems when soil moisture is not a limiting factor, net N mineralization may occur at a faster rate compared to a dry one and farmers may want to take this into consideration in regards to applying supplemental fertilizer to cash crops. When regions are susceptible to intense precipitation events (e.g., hurricane season) or susceptible to leaching, the utilization of high biomass cover crops appeared to maximize the ability to protect soil inorganic N. In these situations, cover crops can provide both environmental and economic benefits and federal and state programs which provide incentives for the use of cool-season cover crops should extend these benefits to include warm-season cover crops.
Acknowledgements

We would like to thank the Southern Sustainable Agriculture Research and Education program (SSARE) and the Organic Crop Improvement Association (OCIA) for their generous financial support. We also would like to thank NCDA/CEFS experiment station staff, the NCSU soil analytical lab, Dr. Dean Hesterberg, Dr. Michelle Wander, Dr. Carmen Ugarte as well as Joy Smith for their invaluable assistance.
References


Table 2.1. Cover Crop Planting Dates and Field Management Activities, 2010.

<table>
<thead>
<tr>
<th>Date(s)</th>
<th>Application Rate</th>
<th>Estimated Nutrient Availability$^z$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Site 1</td>
<td>Site 2</td>
</tr>
<tr>
<td><strong>Planting</strong></td>
<td>12-May</td>
<td>26-Jun</td>
</tr>
<tr>
<td><strong>Pre-plant amendments</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soybean meal</td>
<td>N/A</td>
<td>25-Jun</td>
</tr>
<tr>
<td>Potassium sulfate</td>
<td>N/A</td>
<td>25-Jun</td>
</tr>
<tr>
<td><strong>Pesticide applications</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyganic Crop Protection EC 5.0 II</td>
<td>2-Jul; 11-Jul</td>
<td>N/A</td>
</tr>
<tr>
<td>Semaspore Grasshopper Control</td>
<td>2-Jul; 19-Jul</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Termination$^y$ date</strong></td>
<td>10-Aug</td>
<td>31-Aug</td>
</tr>
<tr>
<td><strong>Incorporation date</strong></td>
<td>17-Aug</td>
<td>7-Sep</td>
</tr>
</tbody>
</table>

$^z$ Estimated nutrient availability based on total nutrient concentration for each element as reported by product manufacturers and noted in parenthesis.

$^y$ Cover crops were terminated by flail-mowing. Residues rested on the soil surface for 1 week before incorporation via rototilling.
Table 2.2. Mean Values for End of Season, Cover Crop Quantity and Quality.

<table>
<thead>
<tr>
<th>Cover Crop</th>
<th>Shoot Biomass ha(^{-1}) dm</th>
<th>(kg)</th>
<th>Shoot C (% dm)</th>
<th>Shoot N (% dm)</th>
<th>Shoot Biomass C:N Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Site 1^z)</td>
<td>(Site 2^y)</td>
<td>(Site 1)</td>
<td>(Site 2)</td>
<td>(Site 1)</td>
</tr>
<tr>
<td>Cowpea(^x)</td>
<td>2265 B(^w)</td>
<td>3276 b</td>
<td>41.96 B</td>
<td>42.85 bc</td>
<td>2.78 A</td>
</tr>
<tr>
<td>Cowpea-Millet Mix(^w)</td>
<td>2256 B</td>
<td>3195 b</td>
<td>41.97 B</td>
<td>42.62 c</td>
<td>2.53 A</td>
</tr>
<tr>
<td>Foxtail Millet</td>
<td>2632 B</td>
<td>4144 b</td>
<td>44.57 A</td>
<td>43.45 b</td>
<td>1.08 B</td>
</tr>
<tr>
<td>Sorghum-sudangrass</td>
<td>5302 A</td>
<td>10110 a</td>
<td>44.27 A</td>
<td>44.18 a</td>
<td>0.79 B</td>
</tr>
</tbody>
</table>

Variables that did not meet the assumptions of normality and homogeneity of variances were transformed by natural log prior to analysis and then back-transformed for presentation of results.

\(^z\) Total growing season at \(Site 1 = 93\) days

\(^y\) Total growing season at \(Site 2 = 67\) days

\(^x\) All cowpea seeds were treated with \(Bradyrhizobium sp. (Vigna)\) inoculant prior to planting.

\(^w\) Each site was analyzed separately. Values followed by the same letter are not significantly different within a column according to Tukey's mean separation test (\(p \leq 0.05\)).

\(^v\) At both sites cowpea comprised approximately 93% of the cowpea-foxtail millet mix.
Table 2.3. Mean Values of Inorganic N, Probe Adsorbed N, Soil Mineralized C and N by Field Site and Cover Crop Treatment for the Sampling Season.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Extractable Inorganic N (mg N kg(^{-1}) soil)</th>
<th>Probe Adsorbed N (µg N mL(^{-1}) 10 cm(^{-2}) burial period(^{-1}))</th>
<th>Mineralized N (28 day incubation) (mg N kg(^{-1}) soil)</th>
<th>Mineralized C (28 day incubation) (mg CO(_2)-C kg(^{-1}) soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Site 1</td>
<td>Site 2</td>
<td>Site 1</td>
<td>Site 2</td>
</tr>
<tr>
<td>Cowpea</td>
<td>25.0 A&lt;sup&gt;z&lt;/sup&gt;</td>
<td>20.9 a</td>
<td>276.4 A</td>
<td>208.2 a</td>
</tr>
<tr>
<td>Cowpea-Fox Mix</td>
<td>26.6 A</td>
<td>22.9 a</td>
<td>228.0 AB</td>
<td>213.3 a</td>
</tr>
<tr>
<td>Foxtail Millet</td>
<td>22.1 A</td>
<td>15.6 b</td>
<td>152.6 B</td>
<td>174.7 a</td>
</tr>
<tr>
<td>Sorghum-sudangrass</td>
<td>13.5 B</td>
<td>10.1 c</td>
<td>82.8 C</td>
<td>112.1 b</td>
</tr>
<tr>
<td>Bare-ground Control</td>
<td>27.7 A</td>
<td>20.2 a</td>
<td>227.3 AB</td>
<td>180.6 a</td>
</tr>
</tbody>
</table>

Variables that did not meet the assumptions of normality or homogeneity of variances were transformed by natural log prior to analysis and then back-transformed for presentation of results.

<sup>z</sup> Each site was analyzed separately. Values within each column followed by the same letter are not significantly different according to Tukey’s mean separation test (p≤0.05).
Table 2.4. Pearson Correlation Coefficients (r) between Mineralized Soil N and Indicators of Cover Crop Residue Quantity and Quality.

<table>
<thead>
<tr>
<th>Location</th>
<th>Shoot Biomass</th>
<th>Shoot C:N Ratio</th>
<th>Shoot N (% dm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Site 1</strong></td>
<td>n/s</td>
<td>-0.68**</td>
<td>0.77***</td>
</tr>
<tr>
<td><strong>Site 2</strong></td>
<td>n/s</td>
<td>-0.53*</td>
<td>0.58*</td>
</tr>
</tbody>
</table>

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively; the symbol - represents non-significance.

Values were normalized by subtracting the bare-ground control (B) from the cover crop treatment value (A) and dividing by cover crop biomass (C) (i.e., (A-B)/C) to maximize the effects of cover crop quality not quantity.
Fig. 2.1. 2010 Mean Daily Values and 30-year Normals (1971-2000) for Air Temperature and Daily Precipitation during the Growing and Sampling Season. Data Source: NC State Climate Office. GOLD-Cherry Research Station weather station located approx. 1.6 km from the CEFS experimental research station (Lat: 35.37935° Long: -78.0448°). The arrows indicate the growing season at Site 1 (top) and at Site 2 (bottom). Adjacent bars represent the soil sampling period at each site.
Fig. 2.2. Mean inorganic N from 1 M KCl soil extractions at 6 sampling dates. a) Represents site 1 with sampling dates: 8/10 (pre-incorporation), 8/25 (1 week post-incorporation), 8/31 (2 weeks post-), 9/14 (4 weeks post-), 10/12 (8 weeks post-) and 11/2 (11 weeks post-) and b) represents site 2 with sampling dates: 8/31 (pre-incorporation), 9/14 (1 week post-, 9/21 (2 weeks post-), 10/12 (5 weeks post-), 10/25 (7 weeks post-), 11/15 (10 weeks post-). Bars represent the standard error of 95% confidence interval (n=4 for each treatment at each sampling date).
Fig. 2.3. Mean PRS probe adsorbed N at 5 dates post-cover crop incorporation. a) Represents site 1 and b) represents site 2. No measurements were taken pre-incorporation. Bars represent the standard error of 95% confidence interval (n=4 for each treatment at each sampling date).
Fig. 2.4. Mean mineralized N from a 28-day incubation at 6 sampling dates (1st date was pre-cover crop incorporation). a) Represents site 1 and b) represents site 2. Bars represent the standard error of 95% confidence interval (n=4 for each treatment at each sampling date).
Fig. 2.5. Mean mineralized C from a 28-day incubation at 6 sampling dates (1st date was pre-cover crop incorporation). a) Represents site 1 and b) represents site 2. Bars represent the standard error of the 95% confidence interval (n=4 for each treatment at each sampling date).
CHAPTER 3

Title Soil microbial response to the incorporation of warm-season cover crops in organic farming systems

Authors S. O’Connell¹*, N. G. Creamer¹, W. Shi², J. M. Grossman², G. D. Hoyt¹, ² and K. L. Fager¹.

(1) North Carolina State University, Department of Horticultural Science, Box #7609, Raleigh, NC 27695, USA
(2) North Carolina State University, Department of Soil Science, Box #7619, Raleigh, NC 27695, USA
*Suzanne.oconnell@gmail.com

Abstract
Improving our ability to predict N availability from cover crop residues can improve methods and models to assist agricultural professionals into becoming more efficient nutrient managers. Biological measures may serve as sensitive indicators of cover crop residue decomposition. We found that the incorporation of cover crop residues stimulated an increase in both mean soil microbial biomass N and cellulase enzyme activity. Of the three enzymes examined, β-1,4-glucosidase (BG) (EC 3.2.1.21) activity appeared to be the most sensitive to differences among cover crop quality and was positively correlated with both mineralized soil C and N. Investigation into residue biochemical composition revealed that % neutral
detergent fiber (NDF), % hemicellulose and lignin:N ratio in addition to shoot C:N could distinguish between cover crop effects. Neutral detergent fiber and hemicellulose concentrations had stronger correlations with N mineralization than C:N ratio suggesting their utility to predict differences among relatively high quality cover crops.

Keywords: soil enzyme activity, residue quality, microbial biomass, green manures, free-particulate organic matter (F-POM), C and N mineralization

Introduction

Cover crops are utilized in many types of farming systems and multiple benefits have been attributed to them, including: decreased soil erosion, improved soil and water quality, weed suppression, nutrient contributions and recycling, pest and disease management, pollinator attraction, fertilizer input cost savings, and carbon sequestration (Hartwig and Ammon, 2002; Janzen and Schaalje, 1992; Lal et al., 1991). Organic farmers in particular, rely on cover cropping (i.e., green manures) as a nitrogen (N) source for subsequent cash crops. Cover cropping itself is a highly encouraged practice under the USDA National Organic Program (NOP) which helps meet other requirements such as prohibition of synthetic inputs, crop rotation and the continual pursuit to improve overall soil quality (7 C.F.R. § 205.203 (rev. 2013)).

One of the most challenging cover crop related issues is estimating the amount of N contribution and synchronizing N release with a subsequent cash crop from decomposing residues. Under optimum conditions, plant residue decomposition generally follows 1st order
kinetics where the rate of transformation of a substrate (i.e., the residue) is proportional to the substrate concentration (Minderman, 1968). But many factors such as residue quality, edaphic soil properties, environmental conditions, biological activity, and farm management can affect decomposition rates in the field (Baggie et al., 2004; Cambardella and Elliott, 1993; Goh and Tutuna, 2004; Johnson et al., 2007; Robertson and Groffman 2007; Van Veen et al., 1985). Improving our ability to predict N availability from cover crop residues in farm settings requires further understanding of the interactions between biotic and abiotic variables. The application of this knowledge could then be used to develop and improve predictive measures/models to assist agricultural professionals to become more efficient nutrient managers.

Our goal was to assess the soil microbial response to the incorporation of warm-season cover crops in organic farming systems. Because organic farming practices include additions of many natural (i.e., organic) amendments including cover crops, they generally exhibit greater levels of SOM, microbial biomass, soil C and polysaccharide content compared to conventional systems (Carter and Gregorich, 1996; Grunwald et al., 2000; Mader et al., 2002; Rosen and Allan, 2007; Werner and Dindal, 1990). Although variations are bound to exist among organic systems it makes sense to assess microbial responses with this type of farm management as the baseline biological activity may be higher and management practices are often different compared to conventional systems.

Cool-season cover crops remain the most frequently utilized in the U.S. but there is a growing interest in integrating warm-season cover crops into farming rotations (Creamer and Baldwin, 2000; Sarrantonio and Gallandt, 2003). The potential benefits of warm-season
cover crops may be amplified in regions with long-growing seasons and/or dry conditions including the sub-tropical Southern region, drylands of the Great Plains and the Southwestern low desert in the U.S. (Creamer and Baldwin 2000; Moyer et al., 2000; Wang et al., 2008). Many warm-season cover crops have the ability to produce large amounts of biomass over short amounts of time, resulting in valuable contributions of C & N to the soil (Baligar and Fageria, 2007; Creamer and Baldwin, 2000). Research pertaining to warm-season cover cropping has been limited and the application of cool-season cover crop results should be applied with caution. Differences between cool-season and warm-season cover crop characteristics as well as seasonal weather dynamics (e.g., temperature and precipitation patterns) could influence crop growth, management decisions, and decomposition rates.

The study aim was to examine the response of soil microbes to the incorporation of warm-season cover crops in organic farming systems and assess whether enzyme activity can serve as a biological indicator for cover crop decomposition and short-term N mineralization. Soil enzyme activities and plant residue decomposition rates are intricately linked given that the limiting step for microbially-mediated decomposition is depolmerizing substrates with enzyme-catalysts (Shi 2011). It is generally accepted that microbes utilize the minimum amount of resources needed to produce extracellular enzymes (EE) in order to efficiently acquire energy sources, C and nutrients (Allison et al., 2011). Microbes must generate enough EE’s to support cellular function and maintain viability but how much energy microbes allocate to EE production depends on a variety of factors including: diffusional conditions, EE survival and sorption, nature and availability of substrate, pH and temperature
Various soil enzymes activities including N-acetyl-glucosaminidase and protease, have shown correlations with potential N mineralization and soil microbial biomass after N fertilizer additions and in response to tillage (Ekenler and Tabatabai, 2002; Kandeler et al., 1999). We chose to focus on EE’s primarily associated with roles in the depolymerization of lignocellulose complexes, which are abundant in plant residues. In general, cellulases are stimulated when primary materials (i.e., lignocelluloses and polysaccharides) are available (Shi, 2011). Cellulose degradation was assessed by measuring β-1,4-glucosidase (BG) (EC 3.2.1.21) and cellulose 1,4-β-cellobiosidase (CBH) (EC 3.2.1.91) activity. Both BG and CBH are part of the hydrolytic cellulase enzyme complex that reduces the crystalline polymeric cellulose molecules into glucose and thus represent the depolymerization of labile, C-rich substrates. Lignin degradation was assessed with peroxidase (PER) (EC 1.11.1.7) activity. Peroxidase is an oxidative enzyme that catalyzes the depolymerization of polymorphic lignin molecules while using H₂O₂ as an electron acceptor and thus represents the breakdown of recalcitrant substrates.

It has been well-documented that plant tissue quality can affect decomposition rates. The biochemical makeup of cover crops has generally found C:N ratio with or without the addition of lignin or polyphenols to be strong predictors of N mineralization (Quemada and Cabrera, 1995; Vigil and Kissel, 1991). There is also recognition that it is the availability of C and N for a given material, which may change over time, is the critical factor in decomposition kinetics (Trinsoutrot et al., 2000).
We examined the neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose, cellulose, and lignin content of our selected cover crops to gain insight into their biochemical composition as well as to assess whether lignocellulytic soil enzyme activity was related to cover crop quality. In addition, we examined free-particulate organic matter (F-POM) quality in the soil to explore whether F-POM can serve as an indicator of short-term N mineralization. We hypothesized that, 1) soil cellulase enzyme activity would be stimulated in concert with microbial biomass N after the incorporation of cover crop residues and anticipated that, 2) cellulase enzyme response would be greater when cover crops residues had a higher quality (i.e., lower C:N ratio) and that 3) F-POM quality would be positively correlated with cover crop tissue quality. Our objectives were to: 1) examine potential soil C and N mineralization, F-POM, microbial biomass N and selected soil enzyme activities following incorporation of warm-season cover crops, and 2) to evaluate whether soil enzyme activities were related to biochemical cover crop qualities and/or potential C and N mineralization.

Materials and Methods

Site History and Experimental Design. The materials and methods are detailed in Chap. 2 and therefore are only briefly covered in Chap. #3. The field experiment was located at the Center for Environmental Farming Systems (CEFS) located in Goldsboro, North Carolina during 2010. The design was a randomized complete block with five warm-season cover crop treatments and a bare-ground control (i.e., no cover crop additions). Trials were conducted at two locations approximately 3 km apart from each other referred to as site 1 and
Both sites were had organic management histories although only site 1 was certified.

Regionally popular cover crop selections included in our study were: 1) buckwheat 
(*Fagopyrum esculentum*), 2) sorghum-sudangrass (*Sorghum bicolor X S. bicolor var. sudanese* (Special Effort)), 3) cowpea (*Vigna unguiculata* (Iron & Clay) (L.) Walp.), 4) German foxtail millet (*Setaria italica* (L.) P. Beauv.), 5) German foxtail millet/cowpea mix, and 6) bare-ground control. Seeding rates reflect regional practices and were the same at both sites: buckwheat (66 kg ha$^{-1}$), sorghum-sudangrass (39 kg ha$^{-1}$), cowpea (100 kg ha$^{-1}$), foxtail millet (22 kg ha$^{-1}$) and cowpea-foxtail millet mix (73 kg ha$^{-1}$ and 6 kg ha$^{-1}$, respectively).

Treatments were replicated four times at each site to control within site variability.

Crop management and data collection were carried out by block. Each experimental unit, herein referred to as a plot, was 12.2 x 4.0 m$^2$. The soil at both locations was a Wickham sandy loam with a 2-6% slope at site 1 and a 0-2% slope at site 2 (fine-loamy, mixed, semiactive, thermic Typic Hapludults in U.S. soil taxonomy). This is a very typical soil type found in the Piedmont and Coastal Plains of the Southeastern region of the U.S., known to be well-drained and moderately permeable. Site 1 and site 2 had starting pH of 5.9 and 6.0, cation exchange capacities (CEC) of 6.2 and 5.5, and bulk densities of 1.4 g cm$^{-3}$ and 1.3 g cm$^{-3}$, respectively.

Crop Management. Field preparation was conducted during the month of April. Existing vegetation, fescue hay at site 1 and volunteer winter rye at site 2, was terminated by flail mowing (BEFCO Inc.; Rocky Mount, NC) and incorporated by disking (#630; John Deere, Moline, IL). One month later, each field was prepped for cover crop planting with a chisel plow (11 tine; International Harvester Co., Canton, IL) and field conditioner (4.6 m
wide; Taylor-Way, Athens, TN). A drill seeder (Sukup 2055; Sukup Manufacturing Co., Sheffield, IA) was used to plant cover crop seeds approximately 2.5-3.8 cm deep followed by a pass with a cultipacker (SS1, 3m wide, grass seeder; Brillion Farm Equipment; Brillion, WI) to ensure good contact between the soil and the seeds. All cowpea seeds were treated with *Bradyrhizobium sp. (Vigna)* inoculant prior to planting.

The length of the growing season varied at each site due to poor crop establishment at the initial transitional field plot that required re-planting (Table 2.1). As a result, the transitional trial was replanted, 48 days after the *site 1*, in an adjacent field with the addition of two pre-plant amendments to ensure adequate soil fertility (Table 2.1). Periodic hand-weeding and/or shallow tillage (i.e., approx. 10 cm deep) with a tractor-mounted rototiller (1.5 m wide Tilrovator; Ferguson Manufacturing Co., Suffolk, VA) was carried out in the bare-ground control plots at both sites as needed to minimize living vegetation throughout the experimental timeframe.

Irrigation was used during crop establishment and dry spells during the growing period, however, the capability to irrigate at *site 1* was more limited which resulted in a drier environment compared to *site 2*. Irrigation was not used during the post- cover crop incorporation sampling period. From mid-June to mid-July, *site 1* was subject to a grasshopper (*Melanoplus differentialis* (Thomas)) infestation. The grasshopper population was controlled with a series of organic pesticide applications but the buckwheat cover crop was lost due to heavy herbivory (Table 2.1). The establishment of the buckwheat cover crop at *site 2* was poor, likely attributable to the higher soil temperatures associated with the delayed planting date and therefore the buckwheat crop was abandoned at both sites.
The remaining crops were terminated by cutting with a flail mower (H70 Hurricane Flail Mower, BEFCO; Rocky Mount, NC) and crop residue was allowed to desiccate in place on the soil surface for 1 week. Then the cover crop residue was incorporated into the soil approximately 30 cm deep with 2 passes from a tractor-mounted rototiller (1.5 m wide Tilrovator; Ferguson Manufacturing Co.; Suffolk, VA). After rototilling, a cultipacker (SS10 Grass Seeder; Brillio Farm Equipment; Brillion, WI) was utilized to ensure good contact between the soil and the mostly buried cover crop residues.

**Environmental Monitoring.** Non-replicated air temperature, soil temperature and soil moisture were monitored on an hourly basis at each of the two field sites with automatic data loggers (#HOBO Micro Station #H21-002 Data Logger with S-THA-M002, #S-TMB-M002 and #S-SMA-M005, Onset Computer Corp.; Bourne, MA). Precipitation data and the 30-year average for air temperature from 1971-2000 were supplied by the North Carolina State Climate Office weather station located 1.6 km from the research station (GOLD-Cherry Research Station; Lat: 35.37935° Long: -78.0448°) (Fig. 1). Overall, the 2010 summer cover crop growing season was hotter and drier than average; additionally, there was a moderate drought period followed by record-breaking rainfall during the month of September (State Climate Office of NC 2010).

**Above-ground biomass and tissue quality.** Immediately prior to termination, the percent cover crop cover was assessed for two, 0.5 m² quadrants per plot. Above-ground biomass samples of cover crops were collected from the same quadrants. Components of cover crop mixes were separated by crop type. Fresh weights of all samples were taken. Biomass samples were then transported to North Carolina State University (NCSU) and
placed in a forced-air oven (60°C for 48 hours) and dried to a constant weight. Dry weights were recorded for each sample and averaged for each plot. A sub-sample of dry plant tissue was ground to pass through a 1-mm screen (Model 4 Wiley mill, Arthur A. Thomas Co.; Philadelphia, PA) then ground further using a ball mill and analyzed for elemental carbon and nitrogen (Perkin Elmer 2400 CHNS/O Elemental Analyzer, Perkin-Elmer Corp.; Norwalk, CT). Another tissue subsample was analyzed for dry matter (105°C) and ash along with the sequential determination of neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose, cellulose, and lignin using the method of Van Soest and Robertson (1980) in a batch processor (Ankom Technology 200/220 Fiber Analyzer, Fairport, NY).

Soil samples were collected over a series of six sampling dates including, the day before cover crop termination and five dates post-cover crop incorporation. At each date, 40 soil cores (2.5 cm diameter x 0-15 cm depth) from each plot were homogenized in a composite sample. A 100 g sub-sample of soil was dried at 105°C for 48 hours to calculate gravimetric soil moisture content. Approximately 2,000 g of soil was packed in a labeled Ziploc bag transported in a cooler from the field, and stored at 4.4°C. Soil samples were hand-sieved to pass a 2 mm sieve within 48 hours of field collection. Half the sieved soil was dried at 50°C for 48 hours in a forced air oven, while the other half was kept field-moist and again stored at 4.4°C for use in the C and N mineralization study. Subsamples from the dried soils were ground and analyzed for elemental C and N as described previously for plant tissue.

Soil C and N mineralization. Soil C decomposition and nitrogen mineralization were determined via a 28 day aerobic incubation experiment. Two, field-moist soil samples
(sample #1=10g soil; sample #2=20 g soil) were adjusted to 45% water holding capacity and pre-incubated at room temperature (approx. 21°C) for 5 days within an incubation unit (i.e., foil-covered, 1 L glass jar) to create optimal conditions for soil microbes. Five mL of distilled water was included in each jar to maintain a humid environment and minimize loss of soil moisture throughout the experiment. After the adjustment period, one 10 g soil sample was removed, combined with 50 ml of 1 M KCl (5:1 dilution) and gently shaken. The resulting soil suspension was passed through cellulose filters (Whatman #1: 11μm) and then frozen (-20°C) until analysis representing inorganic N at day 0.

A vial containing 5 mL of 0.5 M NaOH was added to each jar at day 0 to absorb carbon dioxide (CO₂-C) from the remaining 20 g soil sample. The amount of CO₂-C evolved was estimated by titration with 0.1 M HCl at day 11 and day 28 (Zibilske 1994). The jars were flushed with fresh air for ~30 min and fresh NaOH alkaline traps installed on day 11. The sum of the CO₂-C evolved was estimated to be the potential C mineralization. At day 28, 10 g of the remaining soil sample was removed and analyzed for inorganic N at day 28 as described above. All extracts were analyzed for inorganic nitrogen (NH₄⁺-N + NO₃⁻-N) via flow injection methods with colorimetric determination (QuikChem IV; Lachat Instruments, Loveland, CO). The difference between the inorganic nitrogen before (day 0) and after incubation (day 28) was estimated to be the potential N mineralization.

Soil enzyme activity. Colormetric techniques were used to assess cellulose degradation with both β-1,4-glucosidase (BG) (EC 3.2.1.21) and cellulose 1,4-β-cellobiosidase (CBH) (EC 3.2.1.91) activity and lignin degradation with peroxidase (PER) (EC 1.11.1.7) activity. Assays for BG, CBH, and PER used the following substrates: pNP-β-
D-glucopyranoside (pNG), pNP-β-D-cellobioside (pNC) and 3,3′,5,5′-tetramethylbenzidine (TMB), respectively. The protocols for BG and CBH were very similar; only the substrate type and incubation time differed between the two 96-well plate assays (Marx et al., 2001; Saiya-Cork et al., 2002). Enzyme activity rates were determined by incubating soil with standard concentrations of p-nitrophenol (pNP) bound substrates estimated to be above potential soil enzyme saturation levels, and then measuring the amount of pNP released (Parham and Deng, 2000; Turner et al., 2002). Assays were controlled for temperature, pH, and ionic strength of the solution (Tabatabai, 1994). Four analytical replicates as well as 4 blank controls (buffer and substrate but no soil) were included for each sample; 8 negative controls (buffer and soil but no substrate) were included on every microplate as well as a standard curve representing a range of pNP concentrations.

For BG and CBH assays, 4 grams of field moist soil (3.3–3.8 g dry weight equivalent) were combined with 10 mL of 50 mM acetate buffer (pH 5) resulting in a ~ 1:2.5 dilution rate. Then, each sample was shaken at 250 rpm for 0.5 hours. A sub-sample of the slurry (0.5 mL) was transferred to eppendorf tubes and either 0.2 mL of 50 mM pNG for the BG assay or 0.2 mL of 10 mM of pNC for the CBH assay was added. After gentle mixing, samples were incubated for 1 hour (pNG) or 2 hours (pNC) at 37°C. Post-incubation, the reaction was halted and a yellow color allowed to develop by the addition of 0.2 ml of 0.5 M CaCl$_2$ and 0.8 mL of 0.1 M Tris buffer (pH12). The reaction products were centrifuged at 11,000 rpm for 4 minutes and then 300 μl of the supernatant was immediately transferred to a microplate and absorbance (i.e., optical density) assessed at 410 nm. Soil enzyme activity was expressed as μmol of pNP produced per hour per g of dry soil.
Peroxidase activity was determined by incubating soil with the 3,3′,5,5′-tetramethylbenzidine substrate (TMB Easy Solution, Fisher Scientific Inc.) and subsequently measuring the oxidized reaction product (Johnsen and Jacobsen, 2008). Four grams of field moist soil (3.3–3.8 g dry weight equivalent) was combined with 10 mL of 50mM acetate buffer (pH 5) resulting in a ~1:2.5 dilution rate. Then, each sample was shaken at 250 rpm for 0.5 hours. A sub-sample of the slurry (0.2 ml) was transferred to eppendorf tubes and gently mixed with 0.4 mL of TMB substrate. This suspension was incubated for 20 min. at room temperature (~21°) in the dark. The enzymatic reaction was terminated by adding 1 ml of 0.3 M H₂SO₄ and then centrifuged at 11,000 rpm for 4 minutes. A subsample of the supernatant (300 μl) was immediately transferred to a microplate. The color changes associated with the breakdown of the TMB substrate was absorbance assessed at 525 nm. Four analytical replicates as well as 4 blank controls (buffer and substrate but no soil) were included for each sample; 8 negative controls (buffer and soil but no substrate) were included on every microplate (soil and buffer but no substrate). Soil enzyme activity was calculated based on an extinction coefficient (TMB = 0.059 μM cm⁻¹) and expressed as μmol of substrate converted per hour per g of dry soil.

Microbial biomass N. Soil microbial biomass N was determined by the chloroform fumigation extraction method (Brookes et al., 1985). After a 24-hour fumigation, soil samples (15 g fresh weight) were mixed with 50 ml of 0.5 M K₂SO₄ and gently shaken for 0.5 hours. The resulting soil suspension was passed through cellulose filters (Whatman #1: 11μm) and then frozen (~20°C) until digestion. A sub-sample of each extract was subjected to an oxidation reaction using an alkaline potassium persulfate reagent carried out in an
autoclave. Both the oxidized and non-oxidized extracts were analyzed for inorganic nitrogen (\(\text{NH}_4^+-\text{N} + \text{NO}_3^--\text{N}\)) via flow injection methods with colorimetric determination (QuikChem IV; Lachat Instruments, Loveland, CO) with the difference representing organic N (i.e., microbial biomass N). Microbial biomass N was then calculated as the difference between fumigated and non-fumigated soil samples using 0.54 as an extraction coefficient.

**FPOM.** The free, particulate organic matter (FPOM) from soil samples was measured following a density fractionation method (adapted from Marriot and Wander 2006). Twenty grams of sieved, dried soil and 40 ml of polytungstate solution (adjusted to a density of 1.6 g cm\(^{-3}\)) were combined in a 250 ml centrifuge bottle. The mixture was shaken for 1 hour on an orbital shaker at 200 rpm. An additional 10 ml of polytungstate solution was added in a manner that washed soil adhering to the bottle sidewalls. Samples were left to settle and separate overnight. The following morning, samples were centrifuged for 30 minutes at 5000 rpm. The resulting supernatant and floating FPOM (<1.6 g cm\(^{-3}\)) was immediately separated from the soil pellet by decanting over 1.0 \(\mu\)m polycarbonate filters under vacuum. After recovering the polytungstate solution, the FPOM material was rinsed with 50 ml of 0.5 m CaCl\(_2\) solution and then 50 ml of deionized water. FPOM samples were dried at 50°C for 24 hours in a forced air oven, weighed, ground with a ball mill. Twenty-five mg of FPOM was subjected to a combustion-based elemental analysis, as previously described, to determine N and C content.
Statistics

Analysis of variance was performed using Proc mixed procedure (SAS Institute v. 9.2, Cary, NC). Means were separated using least-squared means post-hoc test with Tukey’s adjustment to control type 1 error at a significance level of $\alpha \leq 0.05$. First, results were analyzed for differences between experimental sites when site was considered a fixed effect. No assumptions about site differences were made if site by treatment interactions were significant. Site by treatment interactions were common and thus individual sites were also always analyzed separately and results are primarily presented this way. Blocks at each site were considered random effects. Measurements that were taken from an experimental unit (i.e., plot) on more than one date were also subject to a repeated measures analysis. Variables that did not meet the assumptions of normality or homogeneity of variances were transformed by natural log prior to analysis and then back-transformed for presentation of results.

To improve the ability to distinguish among cover crop quality and minimize the confounding effects of background soil and cover crop quantity, seasonal means were normalized [i.e., $(A - B)/C$ where $A$ represents the value for a cover crop treatment, $B$ the bare-ground control value and $C$ the above-ground cover crop biomass]. Analysis of variance as described above was carried out using the normalized values. Results were then compared to the original analyses in order to assess whether quantity or quality appeared to have a greater effect on treatment separations among cover crops.

Linear hypotheses were used to conduct group comparisons for fixed factors such as cowpea-dominated (i.e., cowpea and cowpea-foxtail mix) versus grass cover crops (i.e.,
foxtail millet and sorghum-sudangrass) (‘Estimate Statements’, SAS Institute, Cary, NC) on normalized data at a significance level of $\alpha \leq 0.05$. In addition, Pearson correlation analyses between normalized, mineralized soil C and N and indicators of cover crop residue quality and soil microbial activity were conducted and correlation coefficients ($r$) used to describe any linear relationships using a threshold of $\alpha \leq 0.05$.

**Results**

*Plant Tissue.* More cover crop biomass was generated for each treatment at site 2 compared to site 1 (Table 3.2). Foxtail millet, cowpea, and cowpea-millet mix produced approximately 30-35% more while sorghum-sudangrass produced almost 100% more shoot biomass at site 2 (Table 3.2). Within each location sorghum-sudangrass produced more biomass than all other cover crops types ($p=0.0004$). The dry weight of the cover crop biomass ranged from 2,256 to 5,302 kg ha$^{-1}$ at site 1 and from 3,195 to 10,110 kg ha$^{-1}$ at site 2 (Table 3.2). The cowpea-millet mix was comprised of approximately 93% cowpea at the end of the growing season at both sites.

A wide range of C:N ratios were present and primarily reflective of % N differences among cover crop type (i.e., grass or legume). Grass C:N ratios ranged from 30-57:1 and cowpea-dominated cover crops from 15-19:1 (Table 3.2). Contrast estimates between cowpea-dominated and grass cover crops indicated that grasses had greater shoot biomass, C:N ratios, % NDF, % hemicellulose and lignin:N ratios while cowpea-dominated cover crops had greater % N and % lignin ($p<0.0016$); these trends are reflected in the means comparison summary (Table 3.2). The only significant differences for acid detergent fiber (%
ADF) was between foxtail millet and cowpea at site 2 (p=0.0253) (Table 3.2). No differences for % cellulose among cover crops were present at either site (Table 3.2).

Mineralized Soil N. A site comparison indicated that a treatment by site interaction was present (p<0.0001). Within each site, treatment effects as well as treatment by time interactions were significant (p<0.0001). The range of mineralized N values for bare-ground controls was narrower (-11.9 to 8.6 mg N kg$^{-1}$ soil) than cover crop plots (-16.9 to 37.0 mg N kg$^{-1}$ soil) (Fig. 3.2). At both sites, net N mineralization was predicted for all cover crops as well as bare-ground soils over the season based on our 28-day incubation experiment (Table 3.3). The seasonal means for mineralized N from cowpea plots were greater than bare-ground controls at both sites (p<0.0002) (Table 3.3). At both sites, contrasts indicated that cowpea-dominated plots had greater mineralized soil N compared to the grasses (p<0.0005). Treatments demonstrated very little fluctuation over the last 2 sampling dates (~3 weeks) at both sites (Fig. 3.2).

At site 1, cowpea-dominated plots had greater N mineralization compared to both grasses and the bare ground controls 4 weeks post-cover crop incorporation (p<0.05) (Fig. 3.2). At site 2, cowpea-dominated plots had greater N mineralization 2 weeks after cover crop incorporation compared to the bare-ground controls (p<0.05) (Fig. 3.2). Seasonal means at site 1 were greater for cowpea-dominated plots compared to foxtail millet and bare-ground controls (p<0.0001) (Table 3.3). At site 2, seasonal means for mineralized N were greater for all cover crops compared to the bare-ground controls (p<0.0007) (Table 3.3).

When seasonal means were normalized to minimize the effects of background soil N and cover crop quantity donated to the soil, treatment differences remained indicating that
cover crop quality was the dominant effect on soil mineralized N (p<0.0178). At both sites, contrasts between normalized data continued to indicate that cowpea-dominated plots had greater mineralized soil N compared to the grasses (p<0.0041). Correlation analysis showed strong negative relationships at both sites between potential soil N mineralization and the following cover crop qualities: shoot C:N, % NDF, and % hemicellulose (Table 3.4). The cover crop lignin:N ratio also had negative correlations with mineralized N at each site but it was less significant than other qualities (Table 3.4).

Mineralized carbon. A site comparison indicated that site and treatment differences were significant (p<0.0433) but treatment by site interactions were not present. Within each site, only treatment effects were significant (p<0.0001). The range of mean values for bare-ground treatments was narrower (32 to 93 mg C kg$^{-1}$ soil) compared to cover crop plots (54 to 218 mg C kg$^{-1}$ soil (Fig. 3.3). At both sites, bare-ground controls had the lower mean mineralized C values compared to all cover crops for 5 out of 6 sampling dates (p<0.05) (Fig. 3.3). In addition, sorghum-sudangrass soils had greater seasonal mean mineralized C compared to cowpea and bare-ground controls (p<0.0001) (Table 3.3). Carbon mineralization appeared to decline slightly across all treatments, 1-2 weeks post-incorporation at both sites.

At site 1, sorghum-sudangrass plots had greater seasonal mean mineralized C compared to the rest of the cover crops whereas at site 2, sorghum-sudangrass was only greater than cowpea (p<0.05) (Table 3.3). At site 1 only, on the pre-incorporation date, sorghum-sudangrass plots had greater mineralized C compared to foxtail millet and the bare-ground control (p<0.05) (Fig. 3.3). When seasonal means were normalized to minimize the effects of background soil C and cover crop quantity donated to the soil, treatment
differences were no longer significant indicating that cover crop quantity not quality was the dominant influence on treatment effects (p>0.05) (data not shown). At both sites, contrasts between normalized data did not show any differences between cowpea-dominated and grass cover crop plots.

**Enzyme Activity.** At both sites, the mean BG activity was greater for cover-cropped soils compared to bare-ground controls (p< 0.0001) (Table 3.3). At site 1, all cover crop plots had greater BG activity than the bare-ground controls and BG activity was greater in sorghum-sudangrass soils than cowpea-dominated soils (p<0.0001) (Table 3.3). At site 2, there was less separation among cover crop treatments compared to site 1 resulting in all cover crop plots having greater BG activity than the bare-ground controls (p<0.0001) (Table 3.3). Time had an effect on BG activity (p<0.0164) but time by treatment interactions were not present at either site (Fig. 3.4).

When seasonal means were normalized to minimize the effects of BG activity in control soils and cover crop quantity, separations among treatments were still significant but slightly different than pre-normalization (p<0.0143) (data not shown). This indicated that cover crop quality not quantity was the dominant influence on BG activity treatment effects. Post-normalization, cowpea-foxtail mix soils had greater BG activity than both grasses at site 1, (p<0.0001). At site 2, cowpea-foxtail mix and foxtail mix soils had greater BG activity than sorghum-sudangrass (p<0.0143). Contrasts indicated that cowpea-dominated soils had greater BG activity than grasses at both sites (p<0.0471).

Similar to BG activity, mean CBH activity was greater for cover-cropped soils compared to bare-ground controls at both sites (p< 0.0015) (Table 3.3). A site to site
comparison indicated that mean CBH activity was greater at site 1 compared to site 2 (p<0.0257). Within site 1, both grasses and cowpea soils had greater CBH activity than the bare-ground controls (p<0.0001) (Table 3.3). Within site 2, cowpea-foxtail mix and foxtail millet had greater CBH activity than the bare-ground controls (p<0.0111) (Table 3.3). Time had an effect on CBH activity (p<0.0001) but time by treatment interactions were not present at either site (Fig. 3.5). When seasonal means were normalized to minimize the effects of CBH activity in control soils and cover crop quantity, separations among treatments were no longer significant indicating that cover crop quantity not quality had the dominant influence on treatment effects (data not shown). Contrasts between legumes and grasses at site 2 reflected significant differences (p=0.05) while site 1 had a p-value of 0.06, slightly above our cut-off (α≤0.05) suggesting that cover crop quality also had an effect on CBH activity.

A site to site comparison indicated that mean PER activity was greater at site 1 compared to site 2 (p<0.0001) but mean peroxidase activity within each site did not show any differences among treatments (p<0.0001). Time had an effect on PER activity (p<0.0001) but time by treatment interactions were not present at either site (Fig. 3.5). Fluctuations in PER overtime appeared to be sensitive to soil moisture; when soil moisture was the lowest, trends indicated increased PER activity (Fig. 3.6). When seasonal means were normalized to minimize the effects of peroxidase activity in control soils and cover crop quantity donated to the soil, separations among treatments were still not significant reiterating that cover crop quality did not have an effect on peroxidase activity (data not shown).

Correlations between potential soil C and N mineralization and soil enzyme activities demonstrated positive relationships with both cellulase enzymes (i.e., BG and CBH) (Table
At both sites, activity of BG appeared to have the strongest relationships with C and N mineralization followed by CBH. Peroxidase activity did not demonstrate a significant correlation with either C or N mineralization (Table 3.5). The BG:PER ratio at site 1 did not reflect differences among treatments (p≤0.05) but at site 2 both grasses had greater BG:PER ratios compared to the bare-ground controls (p< 0.0106) (Table 3.3). Additionally, the BG:PER ratio did not demonstrate a significant correlation with either C or N mineralization (data not shown).

**FPOM C&N.** The ranges for mean FPOM-C from bare-ground control plots and cover crop plots were 0.6 to 3.6 g C fraction kg\(^{-1}\) soil and 1.0 to 2.6 g C fraction kg\(^{-1}\) soil, respectively. The ranges for mean FPOM-N from bare-ground control plots and cover crop plots were 75.2 to 165.8 mg N fraction kg\(^{-1}\) soil and 32.6 to 251.2 mg N fraction kg\(^{-1}\) soil, respectively. Within sites treatment effects were not significant for either FPOM-C or FPOM-N (Table 3.3). The FPOM C:N ratio ranged from 12-17:1 for bare-ground controls and 12-28:1 for cover crop plots across the 2 sites (Fig. 3.6). Treatment effects for FPOM C:N were only significant at site 1 (p<0.0170) but large standard errors prohibited distinguishing among which treatments. Contrasts showed that the FPOM C:N from cowpea-dominated plots was lower than from grass cover crop plots at both sites (p<0.0231). In addition, there were strong correlations present between FPOM C:N and cover crop shoot C:N at site 1 (r=0.75, p=0.0008) and site 2 (r=0.56, p=0.0241) as well as between FPOM C:N and BG:PER at site 1 (r=0.50, p=0.0236) and site 2 (r=0.44, p=0.0542)

**Soil microbial biomass nitrogen.** At both sites, the mean soil microbial biomass N (MBN) was greater for cover-cropped soils (14.8 to 23.0 mg N kg\(^{-1}\) soil) compared to bare-
ground controls (7.9 to 8.0 mg N kg\(^{-1}\) soil) (p<0.0001) (Table 3.3). Treatment separations among cover crop types were not evident at either site. Fluctuations in MBN over time appeared to be sensitive to soil moisture (Fig. 3.7). When soil moisture was the lowest, especially at site 1, trends indicated a decrease in MBN. When seasonal means were normalized to minimize the effects of soil microbial biomass N in control soils and cover crop quantity donated to the soil, separations among treatments were still not significant suggesting that cover crop quantity not quality was not the major influence on treatment effects (data not shown). Yet, contrasts on normalized data indicated that cowpea-dominated soils had greater MBN compared to grasses at both sites indicating that cover crop quality may have played a contributing role in treatment effects (p≤0.05). Positive correlations between MBN and mineralized soil C existed only at site 2 and between MBN and mineralized soil C only at site 1 (Table 3.5).

Discussion & Conclusions

The incorporation of cover crop residues stimulated an increase in both mean soil microbial biomass N and cellulase enzyme activity as hypothesized indicating that microbes were utilizing cover crop residues as a C, nutrient and energy source. Of the 3 enzymes that we examined, BG activity appeared to be the most sensitive to differences among cover crop quality (i.e., cowpea-dominated compared to grasses) and was positively correlated with both mineralized soil C and N. Our results suggested that BG was a better indicator of short-term N mineralization from crop residues than CBH or MBN.
CBH activity also increased with the addition of cover crop residues however, differences among cover crop treatments were limited after data was normalized for residue quantity indicating that CBH activity did not reflect differences among plant quality as well as BG. It is not clear why one cellulase enzyme would be more sensitive than the other as these processes are believed to occur simultaneously and synergistically to complete the hydrolysis of soil cellulose to glucose, perhaps this is due to different induction mechanisms. Savoie and Gourbiere, in a complimentary study found evidence to support that cellulase activity can be an accurate measure of cellulose degradation in litter decomposition (1989).

Peroxidase activities did not appear to be effected by cover crop residue additions nor demonstrated relationships with either C or N mineralization. This was expected as peroxidase activity is associated with low N availability and the breakdown of recalcitrant soil substrates (Carreiro et al., 2000; Waldrop et al., 2004). These conditions are not typical in annual cropping systems that receive regular fertilizer and soil amendments. Furthermore, our study indicated mean net N mineralization for all cover crop treatments indicating that N was not a limiting factor.

Past management histories at the two different field sites may have influenced the baseline biological activity; site 1 had 11 years of organic management history compared to site 2 with only 4 years of organic management. But environmental conditions, primarily soil moisture, appeared to have the dominant effect on plant production and soil microbial activity. As presented in our previous work (O’Connell et al., 2013, unpublished) all warm-season cover crops included in the study were predicted to result in net N mineralization, including those with a C:N >40:1. We hypothesized that relatively low quality residues (i.e.,
C:N ratios >40:1) could maintain net N mineralization due to a decrease in microbial carbon use efficiency (CUE). This decreased CUE coincided with a lowered resource (i.e., N) demand as a result of sub-optimal soil moisture conditions during a dry season.

Here we provide additional evidence to support the impacts that limited soil moisture had on the soil microbial community. During the month of September, we experienced a drought and irrigation capacity at site 1 was limited. Soil MBN levels decreased considerably during this time period which reemphasized that a decline in microbial N demand likely occurred as a result of stress from low soil moisture conditions. Curiously, the activity of PER and to a lesser extent CBH but not BG, appeared to increase when soil moisture was low suggesting that they were more sensitive to fluctuating soil moisture. In a review of oxidative enzyme dynamics in soil, it was postulated that drying down mineral soil could immobilize and stabilize enzyme activity (Sinsabaugh, 2009).

Cover crop quality, based on shoot C:N ratio, was previously shown to be the dominant factor related to potential soil N mineralization while cover crop quantity had the most influential effect on potential soil C mineralization in this study (O’Connell et al., 2013, unpublished). Further investigation into residue biochemical composition revealed significant differences between cowpea-dominated and grass cover crops % NDF, % hemicellulose and lignin:N ratio in addition to shoot C:N. For our cover crop residues (i.e., C:N ratios from ~15-57:1), NDF and hemicellulose concentrations had stronger correlations with N mineralization than C:N ratio. Both were negatively correlated with N mineralization which is logical as they are both indicators of tissue fractions that are relatively resistant to microbial attack (Gunnarsson et al., 2008). Although these assays may be more popular in
the animal science community for evaluating forage digestibility, NDF and hemicellulose concentrations may offer insight into the subtle differences among relatively high quality cover crops and their use should be considered in models targeting short-term C and nutrient cycling.

Free, particulate organic matter (FPOM) is believed to reflect the most active pool of soil organic matter because it minimizes the influence of recalcitrant N pools and is comprised of recently deposited substrates (Heal et al. 1997; Marriot and Wander 2004; Wander 2004). Overall, we did not find FPOM to be a good indicator of potential N mineralization as results were inconsistent across our two sites. However, FPOM C:N was positively correlated with the cover crop shoot C:N. Also, the FPOM C:N of cowpea-dominated plots was lower than grass plots indicating that FPOM did reflect the properties of the cover crop residues in the weeks to months after soil incorporation. Our results support the growing opinion that biological soil indicators can provide sensitive assessments of the impacts and benefits of agricultural management practices such as cover cropping. In particular, relationships and the predictive capacity of the response of BG soil enzyme activity to cover crop residue decomposition and net N mineralization would be a valuable pursuit.

Acknowledgements: The authors would like to thank the Southern Sustainable Agriculture Research and Education program (SSARE) and the Organic Crop Improvement Association (OCIA) for their generous financial support. We also would like to thank the NCDA/CEFS
staff, the NCSU soil analytical lab, Dr. Matt Poore, Dr. Dean Hesterberg, Dr. Tom Rufty, April Schaeffer and Joy Smith for their invaluable assistance.
References


Table 3.1. Cover Crop Planting Dates and Field Management Activities, 2010.

<table>
<thead>
<tr>
<th>Date(s)</th>
<th>Application Rate</th>
<th>Estimated Nutrient Availability</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Planting</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site 1</td>
<td>12-May</td>
<td>26-Jun</td>
</tr>
<tr>
<td>Site 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pre-plant amendments</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soybean meal</td>
<td>N/A</td>
<td>25-Jun</td>
</tr>
<tr>
<td>Potassium sulfate</td>
<td>N/A</td>
<td>25-Jun</td>
</tr>
<tr>
<td><strong>Pesticide applications</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyganic Crop Protection EC 5.0 II</td>
<td>2-Jul; 11-Jul</td>
<td>N/A</td>
</tr>
<tr>
<td>Semaspore Grasshopper Control</td>
<td>2-Jul; 19-Jul</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Termination</strong></td>
<td>10-Aug</td>
<td>31-Aug</td>
</tr>
<tr>
<td><strong>Incorporation date</strong></td>
<td>17-Aug</td>
<td>7-Sep</td>
</tr>
</tbody>
</table>

\(^x\) Estimated nutrient availability based on total nutrient concentration for each element as reported by product manufacturers and noted in parenthesis.

\(^w\) Cover crops were terminated by flail-mowing. Residues rested on the soil surface for 1 week before incorporation via rototilling.
Table 3.2. Mean Cover Crop Shoot Quantity and Quality by Field Site for the Sampling Season.

<table>
<thead>
<tr>
<th>Location</th>
<th>Treatment</th>
<th>Shoot Biomass (kg dm ha(^{-1}))</th>
<th>Shoot C (% dm)</th>
<th>Shoot N (% dm)</th>
<th>Shoot C:N Ratio</th>
<th>NDF(^y) (% dm)</th>
<th>ADF(^x) (% dm)</th>
<th>Hemi-cellulose ( % dm)</th>
<th>Cellulose (%)</th>
<th>Lignin (% dm)</th>
<th>Lignin:N Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 1(^z)</td>
<td>Cowpea(^v)</td>
<td>2208.8 B(^v)</td>
<td>41.96 B</td>
<td>2.78 A</td>
<td>15:1 B</td>
<td>51.24 B</td>
<td>38.79 A</td>
<td>12.45 B</td>
<td>31.61 A</td>
<td>7.05 A</td>
<td>2.55 C</td>
</tr>
<tr>
<td></td>
<td>Cowpea-Millet Mix(^t)</td>
<td>2163.7 B(^t)</td>
<td>41.97 B</td>
<td>2.53 A</td>
<td>17:1 B</td>
<td>52.74 B</td>
<td>39.22 A</td>
<td>13.44 B</td>
<td>32.18 A</td>
<td>6.45 A</td>
<td>3.36 BC</td>
</tr>
<tr>
<td></td>
<td>Foxtail Millet</td>
<td>2052.4 B(^u)</td>
<td>44.57 A</td>
<td>1.08 B</td>
<td>42:1 A</td>
<td>68.82 A</td>
<td>38.46 A</td>
<td>30.36 A</td>
<td>32.74 A</td>
<td>5.41 B</td>
<td>5.19 AB</td>
</tr>
<tr>
<td></td>
<td>Sorghum-sudangrass</td>
<td>5209.8 A(^v)</td>
<td>44.27 A</td>
<td>0.79 B</td>
<td>57:1 A</td>
<td>65.02 A</td>
<td>38.64 A</td>
<td>26.38 A</td>
<td>33.41 A</td>
<td>5.34 B</td>
<td>7.02 A</td>
</tr>
<tr>
<td>Site 2(^u)</td>
<td>Cowpea</td>
<td>3229.8 b(^u)</td>
<td>42.85 bc</td>
<td>2.30 a</td>
<td>19:1 c</td>
<td>52.04 b</td>
<td>38.78 a</td>
<td>13.13 b</td>
<td>31.23 a</td>
<td>7.27 a</td>
<td>3.19 b</td>
</tr>
<tr>
<td></td>
<td>Cowpea-Millet Mix</td>
<td>3178.9 b(^u)</td>
<td>42.62 c</td>
<td>2.48 a</td>
<td>17:1 c</td>
<td>50.66 b</td>
<td>37.36 ab</td>
<td>13.28 b</td>
<td>30.23 a</td>
<td>6.74 a</td>
<td>2.77 b</td>
</tr>
<tr>
<td></td>
<td>Foxtail Millet</td>
<td>4021.4 b(^u)</td>
<td>43.45 b</td>
<td>1.46 b</td>
<td>30:1 b</td>
<td>58.74 a</td>
<td>34.03 b</td>
<td>24.69 a</td>
<td>28.06 a</td>
<td>5.58 b</td>
<td>4.01 ab</td>
</tr>
<tr>
<td></td>
<td>Sorghum-sudangrass</td>
<td>9970.5 a(^v)</td>
<td>44.18 a</td>
<td>0.99 b</td>
<td>45:1 a</td>
<td>57.59 a</td>
<td>35.06 ab</td>
<td>22.51 a</td>
<td>29.86 a</td>
<td>4.96 b</td>
<td>5.10 a</td>
</tr>
</tbody>
</table>

Variables that did not meet the assumptions of normality and homogeneity of variances were transformed by natural log prior to analysis and then back-transformed for presentation of results.

\(^z\) Each site was analyzed separately. Values followed by the same letter are not significantly different within a column according to Tukey’s mean separation test (p≤0.05).
\(^y\) NDF = neutral detergent fiber
\(^x\) ADF = acid detergent fiber
\(^2\) Total growing season at Site 1 = 93 days
\(^3\) Total growing season at Site 2 = 67 days
\(^4\) All cowpea seeds were treated with *Bradyrhizobium sp. (Vigna)* inoculant prior to planting.
\(^5\) At both sites cowpea comprised approximately 93% of the cowpea-foxtail millet mix.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Site 1 (μmol h⁻¹ g⁻¹ soil)</th>
<th>Site 2 (μmol h⁻¹ g⁻¹ soil)</th>
<th>Site 1 (μmol h⁻¹ g⁻¹ soil)</th>
<th>Site 2 (μmol h⁻¹ g⁻¹ soil)</th>
<th>Site 1 (mg N kg⁻¹ soil)</th>
<th>Site 2 (mg N kg⁻¹ soil)</th>
<th>Site 1 (μmol h⁻¹ g⁻¹ soil)</th>
<th>Site 2 (μmol h⁻¹ g⁻¹ soil)</th>
<th>Site 1 (mg N kg⁻¹ soil)</th>
<th>Site 2 (mg N kg⁻¹ soil)</th>
<th>Site 1 (mg CO₂-C kg⁻¹)</th>
<th>Site 2 (mg CO₂-C kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cowpea</td>
<td>0.653 AB</td>
<td>0.376 a</td>
<td>0.266 A</td>
<td>0.131 ab</td>
<td>19.20 A</td>
<td>15.60 a</td>
<td>12.8 A</td>
<td>14.7 a</td>
<td>18.6 A</td>
<td>9.2 a</td>
<td>97.6 B</td>
<td>104.4 b</td>
</tr>
<tr>
<td>Cowpea-Foxtail Mix</td>
<td>0.695 AB</td>
<td>0.414 a</td>
<td>0.235 AB</td>
<td>0.143 a</td>
<td>21.77 A</td>
<td>14.78 a</td>
<td>13.3 A</td>
<td>13.8 a</td>
<td>15.5 B</td>
<td>10.6 a</td>
<td>97.3 B</td>
<td>117.0 ab</td>
</tr>
<tr>
<td>Foxtail Millet</td>
<td>0.605 B</td>
<td>0.426 a</td>
<td>0.243 A</td>
<td>0.152 a</td>
<td>18.43 A</td>
<td>15.08 a</td>
<td>15.4 A</td>
<td>17.2 a</td>
<td>3.6 C</td>
<td>8.1 a</td>
<td>93.1 B</td>
<td>119.6 ab</td>
</tr>
<tr>
<td>Sorghum-sudangrass</td>
<td>0.720 A</td>
<td>0.417 a</td>
<td>0.268 A</td>
<td>0.126 ab</td>
<td>23.02 A</td>
<td>15.20 a</td>
<td>15.4 A</td>
<td>18.6 a</td>
<td>9.8 BC</td>
<td>9.2 a</td>
<td>139.4 A</td>
<td>141.5 a</td>
</tr>
<tr>
<td>Bare-ground Control</td>
<td>0.489 C</td>
<td>0.286 b</td>
<td>0.176 B</td>
<td>0.087 b</td>
<td>7.95 B</td>
<td>7.92 b</td>
<td>13.0 A</td>
<td>15.7 a</td>
<td>3.9 C</td>
<td>4.6 b</td>
<td>56.4 C</td>
<td>64.4 c</td>
</tr>
</tbody>
</table>

Each site was analyzed separately. Values within each column followed by the same letter are not significantly different according to Tukey's mean separation test (p≤0.05).

BG = β-1,4-glucosidase; PER = Peroxidase

F-POM = free-particulate organic matter (<1.6 g cm⁻³)
Table 3.4. Pearson correlation coefficients ($r$) between mineralized soil N and indicators of cover crop tissue and F-POM quality.

<table>
<thead>
<tr>
<th>Location</th>
<th>Shoot C:N Ratio</th>
<th>NDF (% dm)</th>
<th>ADF (% dm)</th>
<th>Hemi-cellulose (% dm)</th>
<th>Cellulose (% dm)</th>
<th>Lignin:N Ratio</th>
<th>F-POM $^\gamma$ C:N</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Site 1</strong></td>
<td>-0.68**</td>
<td>-0.79***</td>
<td>n/s</td>
<td>-0.85***</td>
<td>n/s</td>
<td>-0.53*</td>
<td>-0.54*</td>
</tr>
<tr>
<td><strong>Site 2</strong></td>
<td>-0.53*</td>
<td>-0.66**</td>
<td>n/s</td>
<td>-0.62**</td>
<td>n/s</td>
<td>-0.48*</td>
<td>n/s</td>
</tr>
</tbody>
</table>

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively; n/s = non-significance.

$^\gamma$ Values were normalized by subtracting the bare-ground control (B) from the cover crop treatment value (A) and dividing by cover crop biomass (C) (i.e., (A-B)/C) to maximize the effects of cover crop quality not quantity.

$^\gamma$ F-POM = free-particulate organic matter (<1.6 g cm$^{-3}$).
Table 3.5. Pearson correlation coefficients (r) between mineralized soil C and N and indicators of soil microbial activity.

<table>
<thead>
<tr>
<th>Location</th>
<th>β-1,4-glucosidase</th>
<th>1,4-β-cellobiosidase</th>
<th>Peroxidase</th>
<th>MBN&lt;sup&gt;z&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mineralized Soil N&lt;sup&gt;y&lt;/sup&gt;</td>
<td>Site 1</td>
<td>0.54*</td>
<td>0.52*</td>
<td>n/s</td>
</tr>
<tr>
<td></td>
<td>Site 2</td>
<td>0.50*</td>
<td>n/s</td>
<td>n/s</td>
</tr>
<tr>
<td>Mineralized Soil C&lt;sup&gt;y&lt;/sup&gt;</td>
<td>Site 1</td>
<td>0.56*</td>
<td>0.51*</td>
<td>n/s</td>
</tr>
<tr>
<td></td>
<td>Site 2</td>
<td>0.68**</td>
<td>0.60*</td>
<td>n/s</td>
</tr>
</tbody>
</table>

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively; N/S = non-significance.

<sup>z</sup> MBN = Microbial biomass nitrogen

<sup>y</sup> Values were normalized by subtracting the bare-ground control (B) from the cover crop treatment value (A) and dividing by cover crop biomass (C) (i.e., (A-B)/C) to maximize the effects of cover crop quality not quantity.
Fig. 3.1. 2010 Mean Daily Values for the 2010 Growing Season and 30-year Normals (1971-2000) for Air Temperature and Daily Precipitation. Data Source: State Climate Office of NC. GOLD-Cherry Research Station weather station located approximately 1.6 km from the CEFS experimental research station (Lat: 35.37935° Long: -78.0448°).
Fig. 3.2. Mean mineralized N from a 28-day incubation at 6 sampling dates (1st date was pre-cover crop incorporation). a) Represents Site 1 and b) represents Site 2. Bars represent the standard error of 95% confidence interval (n=4 for each treatment at each sampling date).
Fig. 3.3. Mean mineralized C from a 28-day incubation at 6 sampling dates (1st date was pre-cover crop incorporation). a) Represents Site 1 and b) represents Site 2. Bars represent the standard error of 95% confidence interval (n=4 for each treatment at each sampling date).
Fig. 3.4. Mean β-1,4-glucosidase activity at 6 sampling dates. a) Represents Site 1 with sampling dates: 8/10 (pre-incorporation), 8/25 (1 week post-incorporation), 8/31 (2 weeks post-), 9/14 (4 weeks post-), 10/12 (8 weeks post-) and 11/2 (11 weeks post-) and b) represents Site 2 with sampling dates: 8/31 (pre-incorporation), 9/14 (1 week post-, 9/21 (2 weeks post-), 10/12 (5 weeks post-), 10/25 (7 weeks post-), 11/15 (10 weeks post-). Bars represent the standard error of 95% confidence interval (n=4 for each treatment at each sampling date).
Fig. 3.5. Mean 1,4-β-cellobiosidase activity over 6 sampling dates (1st date was pre-cover crop incorporation). a) Represents Site 1 and b) represents Site 2. Bars represent the standard error of the 95% confidence interval (n=4 for each treatment at each sampling date).
Fig. 3.6. Mean peroxidase activity over 6 sampling dates (1st date was pre-cover crop incorporation). a) Represents Site 1 and b) represents Site 2. Bars represent the standard error of the 95% confidence interval (n=4 for each treatment at each sampling date).
Fig. 3.7. Mean microbial biomass N over 6 sampling dates (1st date was pre-cover crop incorporation). a) Represents Site 1 and b) represents Site 2. Bars represent the standard error of the 95% confidence interval (n=4 for each treatment at each sampling date).
APPENDICES
Appendix A

SOUTHEAST FARMER COVER CROP SURVEY

Please share your views and experiences about cover cropping by volunteering to participate in the following survey. You must be greater than 18 years old and work on a farm to participate in this survey. The survey should take approximately 5-10 minutes to complete. If you do not understand a question, please ask for clarification. Results from this survey will be anonymous and used to generate information about the perceived benefits and challenges of cover crop use as well as current levels of use on Southeast farms. Thank you in advance for your participation.

SECTION A: General description of your farm and market products to help us characterize the types of operations represented in this survey.

1) Please select your current farming position? (check all that apply)
   - owner
   - manager
   - hired worker
   - OTHER: ________________________

2) Please list where your farm is located: (fill in the blank)
   - State: ________________________

3) How many years have you been farming? (check one)
   - 3 or less
   - 3-5
   - 5-10
   - 10-15
   - 15 or more

4) How many acres of your farm are currently under production? (check one)
   - 5 or less
   - 5-20
   - 20-75
   - 75-200
   - 200 or more

5) What type of products do you produce? (check all that apply)
   - vegetable/sm. fruit
   - livestock (cattle, goats, pigs, etc.)
   - poultry
   - orchard
   - grains
   - cut flowers
   - forage
   - nursery/ornamental
   - tobacco
   - cotton
   - OTHER: ________________________

6) How do you market your products? (check all that apply)
   - wholesale
   - farmer’s markets
   - CSA
   - cooperatives
   - contracts
   - OTHER: ________________________

7) Are you a USDA certified organic operation? (check one)
   - yes
   - no
   - In transition period
   - Portion of growing area is organic

8) Do you currently adhere to any other certification programs or labels? (check one)
   - yes
   - no

9) If you answered yes to the question above please list which ones below.
10) Do you have a crop rotation plan? (check one)
   □ yes □ no

11) Does your crop rotation plan include the use of cover crops? (check one)
   □ yes □ no

12) How often are cover crops included in your crop rotation plan? (check one)
    □ every year □ every other year □ every 2-3 years □ every 4 years or more

13) Please select the range which best describes your total gross farm income in 2008. Total gross farm income includes the value of products sold, farm rental income, custom work, government programs, etc. (check one)
    □ Under $10,000 □ $10,000-$20,000 □ $20,000-$50,000 □ $50,000-$100,000 □ $100,000-$250,000 □ $250,000-$499,999 □ $500,000 or more □ don’t know

SECTION B. Now, we are going to ask you a series of questions about your experiences with and opinions of cover cropping. ‘Cover crops’ are defined here as crops grown between or intercropped with cash crops, which are not harvested or sold for profit, but rather used as part of the overall farming system.

Directions: On a scale of 1 to 5, where 1 is strongly disagree and 5 is strongly agree, please tell us how much you agree or disagree with each of the following statements about cover crops.

<table>
<thead>
<tr>
<th></th>
<th>Strongly Disagree</th>
<th>Disagree</th>
<th>Neutral</th>
<th>Agree</th>
<th>Strongly Agree</th>
</tr>
</thead>
<tbody>
<tr>
<td>14. Cover crops decrease soil erosion.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>15. Cover crops increase soil organic matter.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>16. Cover crops increase soil moisture.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>17. Cover crops break hard pans with their roots.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Strongly Disagree</td>
<td>Disagree</td>
<td>Neutral</td>
<td>Agree</td>
<td>Strongly Agree</td>
</tr>
<tr>
<td>---</td>
<td>------------------</td>
<td>---------</td>
<td>--------</td>
<td>-------</td>
<td>----------------</td>
</tr>
<tr>
<td>18.</td>
<td>Cover crops suppress weeds.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>19.</td>
<td>Cover crops become weeds.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>20.</td>
<td>Cover crops reduce nutrient leaching.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>21.</td>
<td>Selected cover crops contribute nitrogen to subsequent cash crops.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>22.</td>
<td>Cover crops mine nutrients from deep in the soil.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>23.</td>
<td>Selected cover crops reduce available nitrogen for subsequent cash crops.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>24.</td>
<td>Cover crops break pest &amp; disease cycles.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>25.</td>
<td>Cover crops provide beneficial insect habitat.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>26.</td>
<td>Cover crops reduce pesticide applications.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>27.</td>
<td>Incorporating cover crop residues is a challenge.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>28.</td>
<td>Cover crop seed bed preparation is a challenge.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>29.</td>
<td>Cover crops require too much water.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>30.</td>
<td>Cover crops reduce tillage frequency.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>31.</td>
<td>Using cover crops increases cash crop yields.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>32.</td>
<td>Using cover crops results in a loss of cash crop opportunities.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>33.</td>
<td>Cover crops have negative effects on subsequent cash crops.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>34.</td>
<td>There is a lack of available information about cover crops.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>35.</td>
<td>The cost of establishing cover crops is prohibitive.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>
SECTION C. Now, we are going to ask you a series of questions about your cover crop management practices. If you do not use cover crops on your farm please skip these questions and proceed to SECTION D.

36) When you use cover crops what percentage of the time do you sow the following plant types? (check one box for each of the 4 categories listed below)

<table>
<thead>
<tr>
<th>1. grass</th>
<th>2. legume</th>
<th>3. grass/legume mixture</th>
<th>4. OTHER:</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ never use</td>
<td>□ never use</td>
<td>□ never use</td>
<td>□ never use</td>
</tr>
<tr>
<td>□ less than 25%</td>
<td>□ less than 25%</td>
<td>□ less than 25%</td>
<td>□ less than 25%</td>
</tr>
<tr>
<td>□ 25-50%</td>
<td>□ 25-50%</td>
<td>□ 25-50%</td>
<td>□ 25-50%</td>
</tr>
<tr>
<td>□ 50-75%</td>
<td>□ 50-75%</td>
<td>□ 50-75%</td>
<td>□ 50-75%</td>
</tr>
<tr>
<td>□ more than 75%</td>
<td>□ more than 75%</td>
<td>□ more than 75%</td>
<td>□ more than 75%</td>
</tr>
</tbody>
</table>

37) If you use legumes, do you apply a matching seed inoculant? (check one)

□ yes  □ no  □ only when introducing a new legume species  □ never use legumes

38) How many weeks do you typically wait between killing a cover crop and planting the next crop? (check one)

□ 0-1 week  □ 1-2 weeks  □ 3-4 weeks  □ 5-6 weeks  □ 6-8 weeks

□ more than 8 weeks

39) How do you manage your cover crops? (check all that apply)

□ mowing  □ no-till (rolling/crimping)  □ disking  □ rototilling
□ moldboard plow  □ chisel plow  □ strip tillage
□ herbicide application  □ OTHER:___________________________

40) Does the unavailability of particular tools influence your decision to use cover crops?

□ yes  □ no

41) If you answered yes to the question above, please indicate the general type of equipment that you are lacking. (check all that apply)

□ planting  □ cultivation  □ roller/crimper  □ incorporation  □ does not apply
□ OTHER:___________________________

42) When following recommended published seeding rates do you achieve satisfactory cover crop stands? (check one)

□ yes  □ no, I increase seeding rates  □ no, I decrease seeding rates

43) Do you apply fertilizer (ex: potash, compost...) prior to planting cover crops? (check one)

□ yes  □ no
44) Do you ever intercrop or overseed a cover crop with a cash crop? (check one)
   □ yes    □ no

45) When do you plant cover crops? (check all that apply)
   □ spring    □ summer    □ autumn    □ winter

46) Please select all plant species that you use as cover crops. (check all that apply)

   Warm Season:
   □ Pearl Millet (*Panicum miliaceum*)
   □ Foxtail Millet (*Setaria italica*)
   □ Buckwheat (*Fagopyrum esculentum*)
   □ Sudangrass (*Sorghum bicolor var. sudanense*)
   □ Sorghum-sudan grass (*Sorghum bicolor X S. bicolor var. sudanense*)
   □ Sunn hemp (*Crotalaria juncea*)
   □ Sunflower (*Helianthus annuus*)
   □ Japanese Millet (*Echinochloa frumentacea*)
   □ Soybean (*Glycine max*)
   □ Cowpea (*Vigna unguiculata*)
   □ OTHER: ________________________________________

   Cool Season:
   □ Austrian Winter Pea/Field Pea (*Pisum sativum*)
   □ Oat (*Avena sativa*)
   □ Rapeseed/Canola/Mustard (*Brassica sp.*)
   □ Cereal Rye (*Secale cereale*)
   □ Annual Ryegrass (*Lolium multiflorum*)
   □ Wheat (*Triticum aestivum*)
   □ Triticale (*x*Triticosecale*)
   □ Hairy vetch (*Vicia villosa*)
   □ Crimson clover (*Trifolium incarnatum*)
   □ Arrowleaf clover (*Trifolium vesiculosum*)
   □ Berseem clover (*Trifolium alexandrinum*)
   □ Subterranean clover (*Trifolium sp.*)
   □ Red clover (*Trifolium pratense*)
   □ Sweetclovers (*Melilotus sp.*)
   □ Oilseed Radish (*Raphanus sativus*)
   □ OTHER: ________________________________________
SECTION D. Finally, please provide us with some additional information about yourself. This will help us understand additional characteristics of the farmers that are integrating cover crops into their operations. The information you provide will be grouped with the responses of others and will not be associated with a single individual.

47) What is your ethnicity? (check one)
   - ☐ Hispanic or Latino ☐ Not Hispanic or Latino

48) What is your race? (check one)
   - ☐ White ☐ Black ☐ Asian ☐ American Indian/Alaskan Native
   - ☐ Native Hawaiian or Other Pacific Islander ☐ Multi/Bi Racial
   - ☐ OTHER

49) What is your sex? (check one)
   - ☐ male ☐ female

50) What is your age? (check one)
   - ☐ 18-25 years ☐ 25-40 years ☐ 40-60 years ☐ 60 years or more

51) What is your highest education level? (check one)
   - ☐ high school ☐ 2-year college ☐ 4-year college ☐ graduate degree

52) If farming a full-time (≥40 hours/week) or a part-time job for you? (check one)
   - ☐ full-time ☐ part-time

53) Which of the following sustainable agriculture conferences will you be attending this year? (check all that apply)
   - ☐ Carolina Farm Stewardship Association (CFSA)
   - ☐ Southern Sustainable Agriculture Working Group (SSAWG)
   - ☐ OTHER

Thank you very much for participating in this survey! If you have any questions or concerns, please contact: Suzanne O’Connell, NCSU, Dept. of Hort. Science, 2721 Founders Drive, Kilgore Hall, Raleigh, NC 27695-7609, email: soconnell@ncsu.edu, phone: 919-603-1838

PLEASE RETURN THE COMPLETED SURVEY TO THE PERSON WHO GAVE IT TO YOU OR PLACE IT IN THE ‘COVER-CROP SURVEY’ DROP OFF BOX LOCATED NEAR THE REGISTRATION COUNTER.