# OPTIMIZING THE USE OF COVER CROPS, COMPOST, AND SUSTAINABLE PRODUCTION TECHNIQUES TO ENHANCE PRODUCTIVITY AND QUALITY OF ORGANIC CUCUMBER AND TOMATO

By

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## A DISSERTATION

Submitted to Michigan State University in partial fulfillment of the requirements for the degree of

## DOCTOR OF PHILOSOPHY

Horticulture

#### ABSTRACT

## OPTIMIZING THE USE OF COVER CROPS, COMPOST AND SUSTAINABLE PRODUCTION TECHNIQUES TO ENHANCE PRODUCTIVITY AND QUALITY OF ORGANIC CUCUMBER AND TOMATO

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#### Ajay Nair

The overall objective of this study is to focus on key aspects of organic production such as transplant production, cover cropping, biodiversity, and compost management in order to address some important and critical issues stymying the growth of this industry. In this research we hypothesize that higher level of plant biodiversity, through intercropping, along with the use of cover crops and organic amendments increases crop growth, yield, and productivity.

Healthy transplants are a key to successful organic production. Therefore, greenhouse studies were initiated to test an alfalfa-based organic amendment for tomato transplant production. A factorial experimental design with five concentrations (0.0%, 0.6%, 1.2%, 1.8%, and 2.4%) and five incubation periods (0, 1, 2, 3, and 4 weeks) was set up. We demonstrated that addition of adequate amounts of the alfalfa-based amendment could help produce healthy and vigorous tomato transplants that meet commercial standards. Large-scale field experiments were conducted from 2005 to 2009 to address a wide gamut of issues. One of the studies investigated the impact of tomato-cucumber intercropping on tomato growth and development, and soil physical and chemical properties. Effects of intercropping on tomato growth and yield characteristics were less evident; however, it significantly influenced cucumber yield and reduced cucumber beetle and bacterial wilt damage. In tomato, regardless of cropping system, compost application significantly increased plant height, stem diameter and dry weight clearly indicating a positive effect on plant growth. Repeated use of compost increased soil EC and

NO<sub>3</sub>-N concentrations, except in 2009 which received higher than normal rainfall. There were no differences in soil Ca, Mg and K levels due to intercropping or compost application. Multivariate analysis, based on variables such as soil chemical properties, crop growth and yield characteristics, separated compost and no-compost treatments, however, cropping system treatment (monocrop or intercrop) could not be clearly differentiated.

Soil respiration, microbial biomass and diversity were affected by cover crop (rye or ryevetch mixture) and compost treatments with significantly higher response in soils receiving compost applications. Highest microbial biomass (195-210  $\mu$ g g<sup>-1</sup> dry soil) was found in soils amended with rye + compost. Soil nematode populations showed a significant increase for bacterial feeding nematodes in the rye-vetch compost treatment in one of the years. Community level physiological profile based on C substrate utilization revealed higher microbial functional diversity in rye and compost amended soils. The impact of cover crops and compost on postharvest tomato fruit quality and functional food qualities was also investigated. There was minimal effect of cover crops, but, compost addition significantly increased marketable fruit quality (proportion of marketable fruit and average fruit weight). Other fruit quality aspects such as density, firmness, and total soluble solids did not differ among treatments. Percentage antioxidant activity and the functional food quality of the tomato extracts, with respect to inhibition of cyclooxygenase enzyme activity was highest in tomatoes grown on soils amended with rye-vetch and compost. A subset of the field study investigated the effect of two row covers (60% and 85% light transmission) on crop microclimate and cucumber growth. Use of row covers increased vine length, flower count, leaf area, leaf count, plant biomass, and total marketable yield. When row covers were used in conjunction with compost, no differences in plant growth and yield characteristics were observed between row covers.

#### ACKNOWLEDGEMENTS

My sincere thanks to my major professor, Dr. Mathieu Ngouajio for his encouragement, support and advice throughout the course of my dissertation projects. He has been extremely helpful in providing his critical and candid remarks on the progress of these projects. I thank him for providing me with opportunities for professional development and develop my critical thinking abilities. His efforts in involving me in various extension and outreach activities provided me with an opportunity to interact with growers, extension agents, and educators. Those interactions helped me recognize the importance of collaborative and on-farm research, and how critical they are to develop a strong research-based extension program.

I would also like to acknowledge my guidance committee members Drs. Darryl Warncke, Muraleedharan Nair, John Biernbaum, and Michael Brewer for their valuable inputs, suggestions, and advice during the conduct of my dissertation projects. The knowledge and expertise of my guidance committee members and their willingness to debate with me will be much appreciated. Thank you to Buck Counts, our lab technician, the staff at Horticulture Teaching & Research center, and the undergraduate students for their support and help in assisting me to conduct my studies. I would also like to thank Jon Dahl, Manager, Soil & Plant Nutrient Laboratory, Michigan State University and his staff for the opportunity to gain hands-on learning experience in their lab. Special thanks to my past and present colleagues and researchers: Matt Ross, Aaron Warsaw, Dr. Roberto Lopez, Dr. Matt Blanchard, Ben Henshaw, Erin Haramoto, Zack Hayden, Chad Herman, Dr. Greg Lang, Dr. Art Cameron, Dr. Sonali Padhye, and Dr. Bert Cregg, Thanks to the Horticulture Organization of Graduate Students for giving me an opportunity to serve the graduate student community and develop my leadership

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and professional skills. The amount of support and help from the staff in the main office of the Department of Horticulture is also greatly appreciated.

This dissertation would not have come true without the love, support and encourage from my family. They always supported me and gave me the courage and motivation right from the start till the end. I would especially like to thank my loving wife, Pradeepa Sukumaran, for her understanding and support throughout my PhD program. She has been enthusiastic about my dissertation research, and at times been adventurous enough to assist me in my field and greenhouse studies. Many thanks to her.

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#### INTRODUCTION

As agriculture evolved from agrarian to industrial methods, heavy reliance on synthetic fertilizers and pesticides followed. Uncontrolled and indiscriminate use of those resources lead to increase in issues related to environmental pollution, habitat destruction and risks to human health. Since last decade, there has been an increase in awareness, among growers and consumers, towards food quality, health standards and global environmental issues. Coupled with environmental concerns, rising energy costs and shrinking profit margins have motivated growers to transition and adopt environmentally sound production practices. Organic agriculture has thus emerged as a powerful tool in re-establishing production practices that are selfsufficient, biodiverse, and support practices that conserve soil and water. Organic agriculture is grounded in a holistic view of agriculture that aims to reflect a profound interrelationship between on-farm living biota, farm production and the overall environment. Organic agriculture is the fastest growing agricultural sector in the United States with certified organic land present in all 50 states. In the United States, according to Organic Trade Association, organic sales grew 15.8% in 2008. Unites States Department of Agriculture, in 2008, reported a 100% increase in certified organic vegetable cropland from 48,227 acres in 1997 to 98,525 acres in 2007.

Tomatoes (*Solanum lycopersicum* L.) constitute almost 6% of the total organic vegetable acreage in the United States. According to the Michigan Agricultural Statistics Service, 2009, Michigan harvested 2,100 and 4,400 acres of fresh market tomatoes and cucumbers (*Cucumis sativus* L.) respectively. In Michigan it is estimated that nearly 300 growers are certified organic and an unknown number are organic but not certified. Although, in the recent past, Michigan vegetable growers have indicated strong interest in organic production, they often have to go through a steep learning curve. Growers experience difficulty in acquiring technical information,

suitable equipment or inputs, and lack the knowledge to capitalize on added benefits and values in organically produced fruits and vegetables. In addition they also have to accommodate reduction in yields due to weed pressures, pest infestations, and nutrient deficiencies. Moreover, organic growers have to rely upon discussions with other farmers when dealing with production and marketing issues.

There are a number of areas in organic vegetable production that need more research and need to be optimized. The starting point for most vegetable production systems is transplant production. Benefits of using transplants are many and include: early start, uniform crop growth, and healthy root system. Production of transplants calls for early planning and optimum utilization of available resources. This is critical especially for vegetables like tomato that need sufficient growing time to attain adequate size. One of the major hurdles in organic transplant production is to develop a suitable growing medium which would provide adequate nutrients for sustained transplant growth. Commercial organic mixes are available; however they are usually expensive and may not be locally available. Growers often design their own mixes using compost and other organic amendments which may not provide the required nutrients and lead to poor quality transplants. This could detrimentally affect the growth and productivity of the crop in the field. The first chapter of this dissertation explores the opportunity of using alfalfa based organic amendment in organic tomato transplant production. The study evaluated the growing medium with respect to its chemical characteristics and its impact on seed germination and overall transplant growth.

The focus of subsequent field studies was on suggestions and recommendations from the New Agriculture Network (http://www.new-ag.msu.edu), which is a team of farmers, researchers, and educators for sustainable and organic agriculture in the Great Lakes region. In

2004 the New Agriculture Network, which comprised of 16 growers, 10 researchers and county extension agents of Michigan, called for research that would focus on nutrient availability from organic sources, soil quality, pest management, and increase biodiversity in organic vegetable production systems. Farmers who are undergoing transition to organic production have to develop more integrated approaches to enhance productivity and maintain economic viability. Moreover, the variable climate and narrow seasonal window for growing vegetables in Michigan demands for a biodiverse cropping system that integrates cover crops, organic amendments such as compost, and efficient insect management strategies. Cover crops can reduce soil erosion, suppress weeds, improve soil structure and water holding capacity, and increase soil organic matter. Similarly, addition of compost can improve soil physical, chemical and biological properties of soil and enhance crop yields.

One of the field studies investigated the potential of use of intercropping to enhance cropping system biodiversity in tomato production. Tomato was intercropped with cucumber and data was collected on tomato growth characteristics, soil chemical and physical properties and crop yield. The effect of addition of compost was also tested. Maintaining high soil microbial biomass and microbial activity and diversity is fundamental to organic production systems. Soil microbes break down complex organic molecules and compounds and convert them to plant available forms. These organisms play an essential role in nutrient cycling, soil building, and pest suppression processes in the soil. The structure and functioning of soil microbial communities largely depends upon the quantity and quality of substrates available in the soil. One field study investigated the impact of two cover crops, Rye (*Secale cereale* L.) and hairy vetch (*Vicia villosa* Roth.), on inherent soil biological properties such as respiration, microbial

biomass, nematode population distribution, microbial community function, and functional diversity.

It has been widely proposed that organically grown fruits and vegetables have enhanced levels of photochemical/secondary plant metabolites as compared to conventionally grown ones. A large number of studies investigated differences in fruit quality and nutritional value between organically and conventionally grown food crops; however, more in depth studies within organic cropping systems are lacking. With increasing number of growers utilizing cover crops and organic amendments in their production systems, it becomes all the more important to better understand the effects of such organic inputs on food quality and health promoting properties of food produced under such systems. The cover crop study was further expanded to investigate tomato fruit quality aspects such as density, firmness, and total soluble solids. In addition, functional food properties of tomato fruits were also analyzed using antioxidant and anti-inflammatory assays.

Given the broad scope of this dissertation research, a satellite study was also conducted which involved the use of row cover materials on cucumber plants. Row covers are flexible, transparent or semitransparent materials used to enclose single or multiple rows of plants. Row covers not only act as a barrier against insects but also modify crop microclimate and influence crop growth by increasing soil and air temperature and reducing wind and insect damage. The final study of this dissertation evaluated two types of row covers (60% or 85% light transmission) with or without application of compost on microclimate modification and cucumber plant growth.

CHAPTER I

# ALFALFA-BASED ORGANIC AMENDMENT IN PEAT-COMPOST GROWING MEDIUM FOR ORGANIC TOMATO TRANSPLANT PRODUCTION

Alfalfa-based organic amendment in peat-compost growing medium for organic tomato transplant production

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This paper is part of a dissertation by the senior author in partial fulfillment of the requirements for the Ph.D. degree. This work was supported in part by Land O'Lakes Purina Feed, LLC, The Michigan Agricultural Experiment Station, and USDA grant # 2005-51300-02391.We thank Dr. Renate Snider for a critical review of an early version of this manuscript. The use of company or product in this publication does not imply any kind of endorsement.

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Manuscript published in HORTSCIENCE 46(2):253–259. 2011

#### Abstract

In the last decade, organic production has been the fastest growing segment in U.S. agriculture. With increase in organic acreages there is a strong and growing demand for organically grown transplants. Due to limited commercial availability of certified vegetable transplants, growers often produce their transplants on-farm. Commercial organic mixes for organic transplant production may not be locally available and are usually expensive. Growers often design their own mixes using compost and other organic amendments. The purpose of this study was to evaluate the incorporation of alfalfa-based amendment in a peat-compost medium for organic tomato transplant production. Growing medium of 2 peat:1 vermiculite:1 compost (by volume) was amended with 0%, 0.6%, 1.2%, 1.8%, or 2.4% weight by weight of alfalfabased organic amendment and incubated for 0, 1, 2, 3, or 4 weeks. Medium pH and EC, seed germination (untreated Solanum Lycopersicum L. 'Mountain Fresh' seed), transplant dry weight, height, stem diameter, and SPAD values were measured. Medium pH increased with addition of alfalfa-based amendment but remained within the range of 5.5 to 7.0. Germination percentages were less than 50% in amended medium that was either not incubated or incubated for 4 weeks. Germination was greater than 75% if amended media were incubated for 1, 2, or 3 weeks. Seeds grown in peat-compost without any amendments had the highest germination rates; however, severe nutrient deficiency suppressed seedling growth. Relative to growth in medium with no amendments, plants growing in the amended medium had increased stem diameters, heights, leaf chlorophyll content, and plant dry weight (90% to 160% more), provided the amended medium was incubated for at least 1 week. Application rate of 0.6% or 1.2 % of alfalfa-based amendment produced transplants with suitable growth characteristics and met commercially acceptable standards for transplanting and handling at a reasonable estimated cost.

#### Introduction

To optimize the production system, most vegetable crops are established from greenhouse-grown transplants. Transplant production is a critical phase that significantly affects growth and development of the crop in the field (Dufault, 1998). Some of the advantages of greenhouse-grown transplants are that, they can be started early, have uniformity in growth, and are robust and have healthy root systems (Cantliffe, 1993). Production of transplants in small cells in peat-based medium is the most common and widely practiced method (Raviv et al., 1986). To obtain healthy transplants, it is a common practice to fertilize medium with amendments or water-soluble fertilizer that provide N, P, K, and other nutrients to the developing seedlings (Weston and Zandstra, 1989). Nutrient management aspect for conventionally grown transplants has been extensively researched and largely optimized; however, there are challenges for organic transplant production. There is little information available on aspects such as nutrient management in organic transplant production; as a result, organically produced transplants are often of low quality (Diaz-Perez et al., 2008). With increase in demand for organically grown transplants, a number of soluble organic fertilizers and supplements have emerged in the market (Kuepper and Everett, 2004; Treadwell et al., 2007). These products are usually expensive and not always locally available (Peet et al., 2008).

Growers often design their own mixes using compost and other organic amendments. Organic growers largely depend on compost to manage nutrient requirements of growing transplants. Incorporation of large proportions of compost in the growing medium is not warranted as it can lead to increased salinity and could adversely affect seed germination, seedling growth, and yield (Sanchez-Monedero et al., 2004; Clark and Cavigelli, 2005). Compost nutrient quality also varies based on raw materials used and process and duration of composting.

Additionally, it is difficult to synchronize nitrogen mineralization from the compost-amended medium with crop N demand (Treadwell et al., 2007). Supplementing compost-amended medium with a standardized organic amendment serves as a viable alternative for nutrient management in organic transplant production. There are a number of organic nitrogen sources available such as alfalfa meal, soybean meal, and blood meal. Most of these amendments have not been tested thoroughly despite their popularity and widespread use by growers (Hochmuth et al., 2006). The addition of blood meal, rock phosphate, and greensand in the potting mix is practiced by many small-scale organic growers (Coleman, 1995). In most cases after incorporation of organic amendments, a certain period of time is required for N mineralization (Agehara and Warncke, 2005). In certain cases it is recommended that the plant-based amendment be mixed into the potting medium 2 weeks before sowing of seeds to prevent seed injury. We tested the use of a peat-compost based growing mix supplemented with an alfalfabased organic amendment derived from alfalfa, meat meal, molasses, and potash (Bradfield Organics<sup>®</sup> Tasty Tomato<sup>TM</sup> 3-3-3). The objectives of this study were to develop an efficient transplant production protocol by: (1) determining the optimal concentration of the alfalfa-based organic amendment; and (2) ascertaining the optimal incubation time of the medium with the amendment to ensure timely supply of nutrients and avoid seed or seedling injury.

#### **Materials and Methods**

The experiment was a 5 x 5 factorial with a completely randomized design. There were 25 growing medium treatments obtained through combination of five concentrations and five incubation periods of an alfalfa-based organic amendment (Bradfield Organics<sup>®</sup> Tasty Tomato<sup>TM</sup> 3-3-3, Land O'Lakes Purina Feed LLC, Gray Summit, MO). The organic amendment

is a coarse powder that has nutrients derived from alfalfa, meat meal, molasses, sulfate of potash, and contains essential nutrients (Table 1). The compost produced at the Michigan State University Student Organic Farm composed of: 1) straw and wood shaving from sheep and horse bedding, 2) 1-2 year old leaf mold collected from campus, and 3) straw and hay formulated to produce a high carbon, low nitrogen mix for transplant production. The finished compost was maintained outside for one year and later stored in a heated greenhouse for another year so it was fully mature and dry. The compost had 27.5% organic matter, 7.2 dS m<sup>-1</sup> EC (in a 1:1 v:v water extract), and 5.27 pH (in a 1:1 v:v water extract). The nutrient content was 459, 1, 45, 810, 585, 192, 169, and 235 mg kg<sup>-1</sup> of nitrate-N, ammonium-N, P, K, Ca, Mg, Na, and Cl, respectively.

The first batch of root medium was prepared on 2 Apr. 2007 by mixing peat (Sunshine<sup>®</sup> Professional Grade, Sun Gro Horticulture Ltd., British Columbia, Canada), compost, and No. 2 vermiculite (Michigan Growers Products, Galesburg, MI) in the ratio 2:1:1 by volume. Water was added (40% of volume) to facilitate mixing and stimulate microbial activity. Moist bulk density of the medium was estimated at 1000 kg m<sup>-3</sup>. A volume of 0.15 m<sup>3</sup> of this medium was prepared and split into five sets of 0.03 m<sup>3</sup> each. To four of these sets, one of the following amounts of organic amendment was assigned randomly: 168 g (equivalent to a rate of 10 lb·yd<sup>-3</sup> or 5.6 kg m<sup>-3</sup> of medium or 0.6% w/w), 336 g (1.2% w/w), 504 g (1.8% w/w), and 672 g (2.4% w/w). No amendment was added to the last set that served as an unamended treatment. Sets of root medium were incubated for 4 weeks. Similarly, four more sets of five treatments were prepared and incubated for 3, 2, 1, and 0 week, before seeding. Growing medium incubations were scheduled such that seeding for all media could take place the same day (30 Apr. 2007). Media were turned weekly to ensure aeration and uniformity. Air and medium temperatures were monitored during the incubation period using data logger sensors (WatchDog<sup>®</sup> Spectrum Technologies, Plainfield, IL). Sensors were placed half way inside the bucket, containing each medium, composition and outside to measure the ambient air temperature in the head house where media were stored. During the incubation phase, two medium subsamples were collected from treatments within the 4-week incubation treatment at weekly intervals to monitor pH and EC using 1:1 and 1:2 (by volume) medium-to-water method, respectively (Watson and Brown, 1998)

After the incubation period, on 30 Apr. 2007, each treatment was filled uniformly into six 49-celled flats (98-celled flats cut into half) per treatment. Each flat was labeled and seeded with untreated tomato seeds (*Solanum Lycopersicum* L. 'Mountain Fresh') (Seedway LLC, Hall, NY). Flats were then moved into a heated greenhouse. The temperature inside the greenhouse was maintained at 22 °C. Irrigation was carried out by overhead hoses and breakers, with minimal water to avoid leaching of nutrients from medium. Medium was irrigated frequently for the first week to provide constant moisture supply to facilitate seed germinating. Germination in 49 cells was assessed 2 weeks after seeding by counting emerged seedlings. Destructive sampling was carried out at two different growth stages (2 and 6 weeks after seeding) to determine total dry weight (root and shoot dry weight). Sample size for destructive sampling consisted of five transplants per treatment with six replications. Transplants were gently pulled out, and roots were washed under running water to remove the medium. Plant height, stem diameter, and chlorophyll content were also measured. Stem diameter was measured just above the point of attachment of cotyledons using vernier calipers (Avenger Products, Boulder City, NV). Leaf

chlorophyll content of the recently emerged, fully opened true leaf was recorded with a chlorophyll meter (SPAD-502 Chlorophyll Meter, Minolta Camera Co. Ltd., Osaka, Japan). The SPAD values were means of 10 leaf measurements. Prior to seeding and 2 weeks after seeding, medium samples were collected from all treatments to measure pH and EC. Entire experiment was repeated concurrently in a separate greenhouse. Statistical analyses were conducted using SAS Statistical Software (SAS Version 9.1, SAS Institute Inc., Cary, NC). Data were subjected to simple linear regression analysis using analysis of covariance (ANCOVA), with amendment rate as the covariate. Means were separated at various covariate levels by 'Ismeans' and 'pdiff' statement in SAS ( $P \le 0.05$  level). Seed germination and stem diameter data were analyzed by nonlinear regression analysis using the following logistic equation:

$$y = (a + cx)/(l + bx)$$
<sup>[1]</sup>

Where *y* is the dependent variable, *x* the amendment concentration, *a*, *b*, and *c* are the regression parameters.

## **Results and Discussion**

#### Medium characteristics

Over the course of the 4-week incubation period, medium EC increased between 3-week and 4-week incubation. For unamended medium there was initially a gradual decrease in EC during the second and third week of incubation followed by an increase by the end 4-week (Fig. 1A). Electrolytic conductivity values, by the end of 4-week incubation period, for all media treatments, except the unamended treatment, were higher than values at the start of the incubation. Increase in EC with increasing incubation is due partly to the presence of potassium sulfate in the amendment and to the release of soluble compounds from the mineralization and nitrification process due to prolonged incubation. Differences in EC values were largest at the end of 4-week incubation where medium with higher amendment rates exhibited higher values. Medium pH increased for the first two consecutive weeks of incubation, after which it started to decrease (Fig. 1B). Interestingly, irrespective of the rate, at the end of the incubation all treatments had similar pH values. Medium pH ranged from 5.2 to 6.3, a suggested optimal range for a greenhouse substrate (Herrera et al., 2008; Sanchez-Monedero et al., 2004; Warncke and Krauskopf, 1983).

For the first 2 weeks of incubation, temperatures for amended media were 2 to 4  $^{\circ}$ C higher than the unamended medium (Fig. 2). Increase in temperature after addition of a similar product to mature compost in a bioreactor, followed by incubation, has been reported by Jost (2008). Higher concentrations of alfalfa-based amendment resulted in higher medium temperatures. After 2 weeks, the difference in medium temperature among treatments gradually disappeared.

#### Seed germination

Data from both greenhouses were combined due to lack of interaction between greenhouses and treatments. Seed germination was significantly affected by amendment concentration and weeks of incubation. The effect of amendment concentration varied depending upon incubation period. For unamended medium (0% amendment), incubation time did not have a profound effect on seed germination (Fig. 3). Unincubated, 1, 2, or 3-week incubated medium had no difference in seed germination; however, 4-week incubated medium had lower germination. Differences in germination became more evident with the addition of alfalfa-based amendment. At 0.6% amendment concentration lowest germination (28%) was recorded for

unincubated medium followed by 4-week incubation treatment (46%). Similar trend was observed at 1.2, 1.8 and 2.4% amendment concentrations. Lowest seed germination (6%) was recorded for the unincubated 2.4% amendment treatment. Among all incubation treatments the highest seed germination was obtained when no amendment was added to the medium. It is evident that addition of the amendment was affecting seed germination, but this was true only when the medium was either, unincubated or incubated for 4 weeks. Within unincubated and 4week incubation treatments, drop in seed germination was sharp with increasing rates of amendment concentration. The reduction in seed germination due to the addition of amendment was counteracted by incubating the amended medium for 1, 2, or 3 weeks. To obtain satisfactory germination when incorporating the organic amendment, it is critical to incubate the medium for a minimum of 1 week and a maximum 3 weeks before seeding, based on the incubation temperature used in this study. It is important to note that the required incubation time is likely a function of temperature and could be shorter at higher temperature or longer at lower temperature than the average temperature of 18 °C in this experiment. Organic mixes containing plant and animal-based residues could lower tomato seed germination and should be carefully tested to ensure adequate germination (Peet et al., 2008). Studies on plant and animal-based amendments suggest incubation of amended medium for at least 2 weeks before sowing to prevent injury or damage to seed (Koller et al., 2004). The period of incubation also may depend on other factors such as aeration. The better the aeration, the shorter the incubation needed (Jost, 2008).

Theis (2005) observed lowest tomato seedling emergence if a peat and vermiculite medium was amended with alfalfa meal as compared to the same medium amended with composted dairy manure, dairy manure-based vermicompost, or sesame meal. Poor germination

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in media treatments that were amended and unincubated could be a result of presence of allelochemicals, or harmful microbial environment. Electrolytic conductivity also plays an important role in germination, and elevated EC is known to restrict germination and growth of tomato seedlings (Cuartero and Fernandez-Munoz, 1999; Foolad and Jones, 1991). Higher salinity could not only lower germination, but also lengthen the time needed for germination (Ayers, 1952). In our study, however, EC remained below 2 dS<sup>-m<sup>-1</sup></sup>, which is lower than values that affect tomato seed germination (Herrera et al., 2008; Ramana et al., 2002). Prior to seeding, differences in medium EC among incubation treatments were less evident in the unamended and 0.6% amendment medium, but with increasing amendment concentration, longer incubation treatments exhibited higher EC values (Fig. 4A). Those differences, however, narrowed down 2 weeks after seeding. Overall there was a strong linear relationship with medium EC increasing with increasing amendment concentration for every incubation period (Fig. 4B). Prior to seeding, with respect to medium pH, unamended medium treatments had lower pH than amended medium, except for 4-week incubation treatment (Fig. 5A). Medium pH for 4-week incubation did not increase with increasing amendment rates and did not fit the regression analysis as good as other treatments. Two weeks after seeding, within any incubation period, higher pH were recorded for unamended media (Fig. 5B). In general, most of the treatments tested in this study had pH values in the acceptable range of 5.2 to 6.5, either prior to or 2 weeks after seeding.

Allelopathic effects of alfalfa plant residues on emergence and growth of seedlings have been reported. Alfalfa extracts inhibited seed germination in cotton (Megie et al., 1967), corn (Guenzi et al., 1964), and cucumber (Ells and McSay, 1991). Biological activity possibly could contribute to poor germination in the unincubated medium. Considerable microbial activity, in the form of a thick mycelial growth, was observed on the medium surface within 1week of

incorporation of the amendment. Concentration of fungal mycelia increased with increasing rate of the amendment and was accompanied with a distinct butyric smell. Analysis of 1-week incubated medium at Michigan State University Plant Diagnostics Laboratory indicated the presence of fungi, the dominant form being *Cephalosporium* sp. There is no available literature citing *Cephalosporium* sp. as a causal organism affecting tomato seed germination; however, proliferation of fungus, triggered by the alfalfa based amendment, could have rotten the seeds. Poor germination in 4-week incubated media can be largely attributed to higher microbial activity, and accumulation of harmful organic acids and compounds produced as a result of mineralization.

#### **Transplant Biomass**

There were no differences in total transplant dry weight (root and shoot) 2 weeks after seeding; however, differences were significant 6 weeks after seeding. Unamended treatments had lowest transplant dry weight accumulation indicating that the compost used in this study, and under the irrigation methods used, could not adequately supply nutrients up to the final stage in the greenhouse (6 to 8 weeks). Compost was 25% of the medium, by volume. To increase the long-term nutritional content of the medium there is a possibility of increasing the proportion of compost in the medium, but that action could lead to problems associated with higher salt concentration, poor physical characteristics, contamination, and low seed germination (Garcia-Gomez et al., 2002; Perez-Murcia et al., 2005; Raviv et al., 1986).

Increased amount of amendment in the medium translated to healthier transplants with 90% to 150% more transplant dry weight than plants growing in unamended medium. Due to poor germination, no data could be collected for medium to which amendment was added and

left unincubated (0-week incubation). Transplant dry weight increased as amendment rate increased, but the rate of increase was not the same between incubation periods (Fig. 6). Simple linear regression analysis explained trends for different incubation periods. Analysis of covariance with amendment concentration as the covariate revealed differences in regression slope coefficients ( $P \le 0.0001$ ). Regression slope for unincubated medium was statistically similar to 1 or 2-week incubations; however, it was different when compared to 4-week incubation ( $P \le$ 0.0001). Slopes for 2, 3, or 4-week incubations were statistically different. Unamended treatment incubated for 1, 2 or 3 weeks produced transplants with higher dry weight than 4-week incubation. At 0.6% amendment concentration 2, 3, or 4-week incubation produced higher transplant dry weight as compared to 1-week incubation. At higher amendment concentrations (1.2%, 1.8%, or 2.4%), 4-week incubation produced transplants with highest dry weight. This could be a result of enhanced nutrient availability in 4-week incubated medium due to increased mineralization during incubation.

The highest transplant dry weight was obtained with medium amended with 2.4% amendment and incubated for 4 weeks. Although this treatment produced highest transplant dry weight, germination results for 4-week incubation were lower than all other incubation treatments irrespective for amendment concentrations. The higher per plant dry weight in the 4-week incubation may be an effect of lower plant density due to void spaces created in flats by non-germinated seeds and the higher availability of nutrients per plant.

## Stem diameter, height and chlorophyll content

The effect of amendment concentration and incubation period on stem diameter was explained by non-linear regression (Fig. 7). Regression lines displayed strong coefficient of

determination values and accounted for differences in medium due to different amendment concentrations and incubation periods. At 0.6% amendment concentration there was a sharp increase in stem diameter for every incubation period. Subsequent addition of amendment did not increase stem diameter considerably. Unamended treatments in all incubation periods exhibited small stem diameters. For unamended medium, 2, 3, or 4-week incubations produced seedlings with larger stem diameters than 1 week incubation, as longer incubation resulted in enhanced mineralization of compost (part of the base medium) in those treatments. A number of researchers have reported increased plant growth after incorporation of nutrients, in the greenhouse medium (Bustamante et al., 2008; Perez-Murcia et al., 2005; Sanchez-Monedero et al., 2004). Addition of nutrients from the amendments led to increased stem diameters compared to unamended treatments, showcasing benefits to transplant growth.

Various studies demonstrated that mineral nutrition of tomato seedlings influence plant growth at the transplant and post-transplant stage (Melton and Dufault, 1991; Weston and Zandstra, 1989). Liptay et al. (1992) reported increasing tomato transplant heights with increasing nitrogen levels. No comparisons for transplant height could be made for unincubated treatments due to lack of transplants as a result of poor germination. Overall, transplant height increased with increasing amendment rates for all incubations (Fig. 8A). The response was linear with different degrees of slope. In the unamended medium, transplant heights were similar for 1 or 4-week incubations. Transplant height for 2 or 3-week incubation was higher than 1 or 4week. At 1.2% and 1.8% amendment concentration 2 and 3-week incubation treatments produced transplants with highest heights, respectively. For 2-week incubation treatment, transplant height increased with the increase in the amendment concentration up to 1.2% level,

after which it decreased. For 3-week incubation treatment, 1.8% or 2.4% amendment treatments produced taller transplants.

A direct link has been demonstrated between leaf chlorophyll content (measured indirectly through SPAD meter) and leaf nitrogen status (Wang et al., 2004; Swiader and Moore, 2002; Li et al., 1998). Leaf greenness or chlorophyll content is affected by several factors including nutrient concentration, distribution of chlorophyll in leaves, and plant genotype (Soval-Villa et al., 2002; Uddling et al., 2007). In general, SPAD values increased with increasing rates of amendment (Fig. 8B). This is due to increased nutrient concentration in the growing medium. Studies have reported increased plant height, leaf number, leaf area, and plant dry weight with increased levels of nitrogen concentration in the growing medium (Weston and Zandstra, 1989; Masson et al., 1991). Duration of incubation did influence SPAD values at 0% or 2.4% amendment level. At 0% amendment concentration, 2-week incubation had the lowest SPAD value followed by 4-week treatment. There was no difference between 1 or 3-week incubation treatment, which was higher than 2 or 4-week treatment. Differences were less evident at 0.6%, 1.2% or 1.8% amendment level; however, at 2.4% amendment level, 4-week incubation had the lowest SPAD value.

The compost and peat-based medium amended with alfalfa-based amendment produced healthy tomato transplants from direct seeding if incubation of the amended medium occurred for 1, 2, or 3 weeks. Koller et al. (2004) used several plant and animal-based fertilizers for vegetable transplant production and recommended mixing and incubating plant-based amendments with potting medium for 2 weeks to prevent seed damage. The organic amendment used in this study was derived from alfalfa, molasses, and meat meal, and it is recommended that the amendment be incubated with the growing medium prior to seeding. This process also allows

mineralization and release of nutrients from the amendment prior to seeding. Incubation also will prevent allelopathic interactions between amendment and the germinating seedlings. Allelopathic effects of alfalfa plant residue on seed germination and growth of cucumber seedlings have been reported (Ells and McSay, 1991).

Selection of a particular kind and rate of organic amendment should be made based on cost effectiveness and effects on medium pH and EC, seed germination, plant height, stem diameter, chlorophyll content, plant biomass, and sustainability of the product. To balance all those variables without compromising transplant health and quality, would be the optimum approach to organic transplant production. Overall, growing medium amended with 0.6%, 1.2%, 1.8%, or 2.4% concentration of alfalfa-based amendment produced transplants with suitable growth characteristics and met commercially acceptable standards for transplanting and handling. Higher rate of 2.4% amendment produced robust and healthy transplants but has the potential to affect seed germination and thereby needs prolonged incubation.

From a grower's standpoint, feasibility for the adoption of any production system or technique is often driven by cost of production. Use of alfalfa-based amendment tested in this study was economically feasible. The unamended medium used in this study would cost  $67/m^3$ , assuming peat at  $36 (0.5 \text{ m}^3 @ \$71/m^3)$ , vermiculite at  $24 (0.25m^3 @ \$95/m^3)$  and compost at  $7 (0.25m^3 @ \$26/m^3)$ . Depending upon geographical location in United States, price for dairy compost can vary from  $16-66/m^3$  (McEntee, 2005). In case of certified organic medium it is not unusual for seedling and transplant medium to cost  $60 \text{ to } 197/m^3$  or more. Based on retail purchase of a single bag (11 kg), an estimated cost of the amendment (Bradfield Organics)

Tasty Tomato 3-3-3) used is \$20. If the product is used at 0.6% concentration (at a rate of 5.9 kg m<sup>3</sup>), it would cost  $10/m^3$  which would increase medium cost from \$67 to  $77/m^3$  (an increase of 15%). Even then, input cost for the medium is well within prevailing market value of other commercial organic blends and formulations. There are benefits to incorporation of alfalfabased amendments in compost based medium when compost alone (at 25% volume) was not able to provide sufficient nutrition for the desired transplant growth. Thus, use of plant and animal-based amendments, such as organic amendment used in this study, together with compost, have the potential to serve as nutrition supplements for sustainable greenhouse transplant production.

Nutrients	<b>Concentration (%)</b>	Nutrients	Concentration (mg/kg)
N	3.0	Fe	425.0
Р	1.3	Mn	65.0
Κ	3.0	Zn	75.0
S	0.8	Cu	20.0
Ca	2.8	Co	0.9
Mg	0.3	В	13.9
Na	0.3	Se	<5.0
Cl	0.4	-	-
Protein	19.0	-	-
Fat	3.5	-	-
Fiber	14.0	-	-
Sugar	6.5	-	-

Table 1.1. Nutrient composition of alfalfa-based organic amendment incorporated in the growing media for organic transplant production (based on data provided by Bradfield Organics, Land O'Lakes Purina Feed LLC, Gray Summit, MO).


Fig. 1.1. Changes in media EC (A) and pH (B) during 4-week incubation period with different amendment concentrations. Data collected from the 4-week incubation treatment only. Each data point is a mean of two samples. Error bars denote standard error



Fig. 1.2. Temperatures of ambient air and media (amended with 0%, 0.6%, 1.2%, 1.8%, or 2.4% alfalfa-based organic amendment) during 4 week incubation period. Media were incubated in buckets with loose lids, and temperature sensors placed in the middle.



Fig. 1.3. Effects of alfalfa-based organic amendment in peat-compost growing medium on tomato seed germination 2 weeks after seeding as analyzed by non-linear regression. Mean separation between incubation periods at any given concentration by lowercase letter(s). Values with same letter(s) are not significantly different ( $P \le 0.05$ ).



Amendment concentration (%)

Fig. 1.4. Effects of alfalfa-based organic amendment in peat-compost growing medium on medium EC prior to seeding (A) and 2 weeks after seeding (B) as analyzed by simple linear regression. Error bars denote standard error.



Fig. 1.5. Effects of alfalfa-based organic amendment in peat-compost growing medium on medium pH prior to seeding (A) and 2 weeks after seeding (B) as analyzed by simple linear regression. Error bars denote standard error.



Fig. 1.6. Effects of alfalfa-based organic amendment in peat-compost growing medium on tomato transplant biomass 6 weeks after seeding as analyzed by simple linear regression. Mean separation between incubation periods at any given concentration by lowercase letter(s). Values with same letter(s) are not significantly different ( $P \le 0.05$ ).



Fig. 1.7. Effects of alfalfa-based organic amendment in peat-compost growing medium on tomato transplant stem diameter 6 weeks after seeding as analyzed by non-linear regression. Mean separation between incubation periods at any given concentration by lowercase letter(s). Values with same letter(s) are not significantly different ( $P \le 0.05$ ). NS=Means are not significantly different.



Fig. 1.8. Effects of alfalfa-based organic amendment in peat-compost growing medium on tomato transplant height (A) and chlorophyll content (SPAD value) (B) as analyzed by simple linear regression. Error bars denote standard error.

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#### LITERATURE CITED

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# CHAPTER II

# CHANGES IN TOMATO GROWTH, YIELD CHARACTERISTICS, AND SOIL PHYSICAL AND CHEMICAL PROPERTIES IN RESPONSE TO ORGANIC AMENDMENTS AND INTERCROPPING WITH CUCUMBER UNDER A FOUR YEAR ORGANIC PRODUCTION SYSTEM

Changes in tomato growth, yield characteristics, and soil physical and chemical properties in response to organic amendments and intercropping with cucumber under a four year organic production system

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# Abstract

Development and maintenance of an efficient organic production system requires diverse strategies such as diverse plantings, crop rotation, reduced tillage, cover cropping and incorporation of composts, mulches and manures. Diversified plantings often feature intercropping-the growing of two or more crops simultaneously on the same land. Adoption of such strategies helps improve resource use, suppress pests, and may increase crop productivity. This 4-year study analyzes the effects of intercropping and application of dairy compost in an organic tomato production system. The study was arranged as a split-plot experiment within a randomized complete block design. The main plot was the cropping system (monocrop or intercrop), while the subplot was a compost or no-compost treatment. Cucumber was used as a replacement intercrop (alternate tomato rows in a monocrop were replaced by cucumber crop). Effects of intercropping on tomato growth and yield characteristics were less evident; however, intercropping significantly influenced yields in cucumber. By fourth cucumber harvest, on average 41% plants were dead in cucumber monocrop treatment. Reduced cucumber beetle and bacterial wilt damage was observed in cucumber intercrop. This resulted in land equivalent ratio values of greater than one demonstrating advantages of intercropping. In tomato, regardless of cropping system, compost application significantly increased plant height, stem diameter and dry weight clearly indicating a positive effect on plant growth. Soil organic matter (SOM) also increased over time as a result of compost applications. Soils which received no compost also increased their SOM but the increase was lower than compost amended soils. Nevertheless, this increase shows positive effects of rye cover crop on SOM buildup. Repeated use of compost increased soil EC and NO<sub>3</sub>-N concentrations, except in 2009 due to higher than normal rainfall. There were no profound differences in soil Ca, Mg and K levels due to cropping system or

compost application treatments. Multivariate analysis, based on variables such as soil chemical properties, crop growth, and yield characteristics, was able to separate compost and no-compost treatments, however, cropping system treatments could not be clearly differentiated. Overall this study demonstrates the feasibility of establishing an organic tomato production system through the use of cover crops (rye), organic amendment (dairy compost) and cucumber intercropping. Benefits of intercropping, although less obvious on tomato growth and productivity, could serve as a valuable tool in cucumber production to suppress pest incidences and enhance crop security. With regard to organic amendments, the findings of this study clearly support the use of compost as a vital component to supply essential nutrients and increase crop growth and productivity.

# Introduction

There has been a steady increase in organic production throughout the world. Growers have shown interest in transitioning to more balanced and environmentally safe production practices (Klonsky, 2004). Growing demand for organically produced fruits and vegetables have created markets and incentives for many growers to transition at least a portion of farm acreage to organic production. Transitioning to organic production often involves adjustments, technical know-how, and tools to better manage issues pertaining to soil fertility, weeds, and pest management (Dabbert and Madden, 1986). Crop rotation, cover cropping, reduced tillage, compost application, and integrated pest and nutrient management strategies are some techniques which growers adopt for transitioning to sustainable and organic production systems (Dimitri and Greene, 2002; Dorais, 2007). One of the major tenets of sustainable or organic production is the enhancement of cropping system biodiversity through cover crops, crop rotation, farmscaping (providing habitat for beneficial organisms), and intercropping (Preston, 2003). These factors

play a vital role in governing various ecological processes needed to sustain a healthy and longterm production system.

Intercropping is widely practiced in developing countries of Asia, Africa, and Central America (Vandermeer, 1989). Intercropping is defined as growing two or more crops simultaneously in the same field (spatial diversification) (Vandermeer, 1989). Meanwhile, in developed countries, including the United States, agricultural mechanization and the intensive use of chemicals led to the widespread adoption of monocultures and made intercropping less practical (Horwith, 1985; Chapman and Carter, 1976). However, in the last two decades with the increase in number of organic and sustainable farms, intercropping has started gaining relevance and importance. Organic growers practice intercropping in an attempt to achieve cropping system diversity, ecological sustainability, and economic viability in their farming enterprise. Concepts such as bio-intensive agriculture that aim to increase land use efficiency through mixed planting of crops together with the use of organic amendments such as manures and composts have started gaining momentum among a large number of organic growers (Jeavons, 2004). Establishment of ecologically balanced and sustainable production systems call for development of unique mixes of crop and practices (Russo and Webber, 2007). Production techniques that contribute towards agricultural sustainability are rapidly increasing. Adoption of intercropping practices will become more common and prevalent due to their positive effects on soil conservation and soil fertility (Jarenyama et al., 2000); weed suppression (Liebman and Dyck, 1993), and their potential to reduce pest and diseases (Smith and McSorley, 2000; Theunnissen and Shelling, 1996). An advantage of planting more than one taxa is the reduced economic loss in case of total crop failure of one of the crops (Pearce and Edmondson, 1982; Smuckler et al.,

2008). Crop failure may be either due to physical constraints of weather or biological agents like insect, bacteria, fungi, or other microorganisms (Horwith, 1985).

Most intercropping studies have been conducted on field crops. Limited intercropping studies have been conducted in vegetable production systems. Our study focused on evaluating the effect of enhanced biodiversity through intercropping in organic a tomato production system. Cucumber has been suggested as one of the potential intercrop of tomato (Kuepper & Dodson, 2001). Tomatoes have been intercropped with a number of crops such as cabbage, corn, cowpea, cucumber, kale, okra, and onions (Brown et al., 1985; Pitan and Olatunde, 2006; Ramkat et al., 2008; Schultz et al., 1983). However, most of the studies involving intercropping in tomato have been conducted under non-organic versus organic production systems which are fundamentally different. A number of characteristics differ between those two systems such as soil organic matter (SOM), C and N cycling, microbial activity or biomass, microbial diversity, pest population and interaction, and crop yield (Drinkwater et al., 1995; Wander et al., 1994). In addition, organic production in temperate climate zones, where short growing season and cooler soil temperatures are prevalent, could be challenging. Nitrogen is often the limiting factor affecting yield in organic and low-input production systems (Russo and Webber, 2007). Under organic systems low availability of mineralized N could reduce crop growth and limit the crop from reaching its full yield potential (Clark et al., 1999). An efficient nutrient management program is needed which adds SOM, improves soil tilth, increases soil microbial biomass, and provides timely supply of nutrients.

Organic systems heavily rely on cover crops and organic matter based amendments like manure and compost to meet crop nutrient demand (Lammerts van Bueren et al., 2010; Russo and Webber, 2007). Composts and manures are applied to agricultural lands as a source of

essential microbes, plant nutrients, and as a source of organic matter (He et al., 2001; Schroder, 2005). Addition of cover crops and composts can reduce soil erosion, weed population, build SOM, and improve soil structure and increase soil carbon and nitrogen (Ngouajio and Mennan, 2005; Teasdale, 1996). Organic fertility amendments like compost and manures have been shown to improve soil physical, chemical and biological properties of soil and produce yields equivalent to conventional cropping systems (Bulluck et al., 2002; Drinkwater et al., 1998; Ozores-Hampton et al., 1998). Although benefits of using composts are well known, its use in conjunction with strategies such as intercropping has not been studied in detail. Possible interactions may exist between soil nutrient status and crop performance under a mixed cropping system. Our objective was to examine the impact of intercropping and an organic nutrient management program on a tomato-cucumber intercropping system. A four-year study was setup to investigate how biodiversity and nutrient management affects crop performance, growth, and yield characteristics. The soil biology aspect of this study will be covered in another article that will soon follow.

# Materials and methods

# Site description and experimental design

This study was conducted from 2005 to 2009 at the Horticulture Teaching and Research Center (HTRC), Michigan State University, Holt, MI. The soil was a Capac loam with 0% to 3% slope. Capac loam is a somewhat poorly drained, moderately to moderately slowly permeable soil formed in loamy glacial till on the low parts of moraines and till plains. The soil at the research site was previously used for a non-organic corn and soybean rotation. The fertility status of the soil before the start of treatment applications is summarized in Table 1. Mean monthly and long-term air temperature, precipitation, and relative humidity during the growing season at

HTRC are summarized in Table 2. The experimental design was a split-plot design with four replications. Crop rows (bed) and replicates ran north to south. Main plots based on cropping system (tomato monocrop, cucumber monocrop, or tomato + cucumber intercrop) were arranged east to west with the intercrop in the center. The split was the subplot with two treatments (compost or no-compost). Monocrop treatment had consecutive beds of tomato or cucumber. Intercrop treatment comprised of alternating beds of cucumber and tomato at the same spacing as in the monocrop treatment. Each bed was 7.6 m long, 0.6 m wide and 0.2 m high with one row of cucumber or tomato. Distance between transplants within the bed was 45 cm and between beds was 167 cm (center to center). Main plots contained eight beds with 14 plants each while subplot contained four beds. The middle two beds were used for data collection and one outer bed on each side served as a guard row. In addition to guard rows, each bed had two guard plants (one plant on either end of the bed). As common practice used by most organic growers in the region, a cover crop of cereal rye (Secale cereale L.) was drilled at a rate of 78 kg ha<sup>-1</sup> in the fall on 4 October, 18 September, 22 September, and 26 September in 2005, 2006, 2007, and 2008 respectively. The following spring dairy compost was hand applied to compost treatments when the rye cover crop was still growing (Table 3). In 2006 and 2009 compost application was delayed as a result of excessive rains and lack of favorable field conditions. Less compost was applied in 2009 due to higher expected availability of nutrients and to avoid phosphorus build up. Each year, after the application of compost, rye cover crop at Feekes Growth Stage 5 (Weisz, 2011) was moved and later incorporated using a chisel plow. The movement of the plow was closely monitored to minimize compost carryover to no-compost treatment plots. The amount of cover crop biomass was evaluated and varied from year to year (Table 4).

Non-treated tomato (Solanum Lycopersicum L. 'Mountain Fresh Plus', Seedway, Hall, New York) and cucumber (Cucumis sativus L. 'Dasher-II', Seedway, Hall, New York) seeds were seeded in 98-celled flats with organic growing medium. Table 5 summarizes the seeding and transplanting schedule for all growing seasons. Seedlings were transplanted to the field on raised beds covered with black plastic mulch and drip irrigated. Crops were irrigated as needed. Weeds near the plant were hand weeded while weeds growing in the aisles were managed by hoeing. Tomatoes were staked (every two plants), tied using Florida Basket Weave system up to four level high, and all cultural operations undertaken according to standard production protocol. Tobacco hornworms, the major insect pests of tomato in the region, were controlled by two applications of Dipel<sup>®</sup> (Bt formulation; Valent Biosciences Corp., California, USA). In 2007, incidence of tomato early blight (Alternaria solani) and septoria leaf spot (Septoria lycopersici) was observed late in the season and was managed using a biofungicide (Sonata<sup>®</sup>, Agraquest Inc., California, USA). Cucumber beetle infestation was observed in all growing seasons but pest pressure was most severe in 2008. A botanical insecticide (Pyganic<sup>®</sup>, Mclaughlin Gormley King Company, Minnesota, USA), was sprayed regularly throughout the growing season to manage the beetle population under control. At the end of the growing season crop residue was incorporated into the soil by disking during land preparation for seeding rye. In 2007, tomato crop residue was not incorporated and pulled out of the field to reduce septoria and early blight innoculum build up.

# Sampling and analysis

For soil chemical analysis, soil samples were collected three times (planting, mid-season, and end of the season) each year. Four soil cores from the tillage zone (0-15 cm) of each

treatment were collected, combined, dried at 38 <sup>o</sup>C for 3 d, and ground using a flail grinder. Soil cores were collected from raised beds between individual plants. Soil pH and electrical conductivity (EC) were measured using 1:1 (by volume) soil-to-water method (Watson and Brown, 1998). Water holding capacity was determined by gravimetric method (Topp, 1993). Soil organic matter and percentage carbon were determined by loss of weight-on-ignition method (Combs and Nathan, 1998). A 1N neutral ammonium acetate solution was used to extract calcium, potassium, and magnesium. Analysis of potassium and calcium was carried out by flame emission method and a colorimetric method was used for magnesium (Warncke and Brown, 1998). A 1N potassium chloride solution was used for extraction of nitrate and ammonium nitrogen. The amount of nitrate and ammonium nitrogen was determined using Latchet Nitrogen Auto Analyzer (Latchet Instrument, Milwaukee, USA) (Huffman and Barbarick, 1981; Nelson, 1983).

Each year, before incorporation of rye cover crop in the spring, four biomass subsamples (shoot and root) per replication from individual 0.25 m<sup>2</sup> area were collected and dried at 60 °C until constant weight to determine cover crop dry weight. Cucumber was harvested seven times in 2006, 2007, and 2008 and six times in 2009 with a 2-3 d interval between harvests. Fruits were graded as marketable (U.S. Fancy, U.S. Extra #1, U.S. #1, U.S. #1 Small, and U.S. #1 Large) or nonmarketable grades (deformed, overgrown, damaged by cuts, scars, sunscald, sunburn, dirt, disease, or insects) (USDA, 1958). Tomatoes were harvested five times each year from 2006-2009. Fruits were graded as marketable (U.S. #1, U.S. #1, U.S. Combination, U.S. #2, and U.S. #3) or nonmarketable (deformed, small, cracked, or damaged by cuts, scars, disease, or insects) (USDA, 1991). During the growing season data were collected on tomato plant height, stem diameter and leaf chlorophyll content (Minolta SPAD-502 Leaf Chlorophyll Meter, Japan).

Chlorophyll measurements were made on recently fully expanded leaf and 10 readings were taken per experimental unit and averaged. After the end of the season, from each treatment, two representative plants were harvested and dried at 60  $^{\circ}$ C until constant weight to record plant dry weight. In 2007, tomato disease ratings were recorded on a scale of 0 to 10 (0 for disease free and 10 if all plants in the treatment were affected). Cucumber beetle infestation and bacterial wilt incidence was high in 2008. Degree of damage due to these pathogens were estimated by recording number of dead plants every harvest. Yields were compared between intercrop and monocrop treatments using LER (Mead and Wiley, 1980; Vandermeer, 1989):

$$LER = I_T/M_T + I_C/M_C,$$

where  $I_T$  represents the yield of tomatoes in intercrop,  $M_T$  the yield of tomatoes in monocrop, and  $I_C$  and  $M_C$  are the yield of cucumber in intercrop and monocrop respectively. Yield for tomato and cucumber were calculated per hectare basis for the calculation of LER.

#### 2.3 Statistical analysis

Analysis of variance was performed using the PROC MIXED procedure of Statistical Analysis Systems Institute Inc., Version 9.2; Cary NC. Significant differences between treatment means were separated by 'Ismeans' and 'pdiff' statement in SAS ( $P \le 0.05$  level). Principal component analysis (PCA) was performed using R Statistical Software (R Development Core Team, 2008) on the entire dataset for 2007, 2008 and 2009 in order to understand variables that most influence and differentiate our production systems. Each principal component is a linear combination of all the values in the dataset and successively explains the majority of the variation in the dataset. In most cases principal component 1 (PC1) and principal component 2 (PC2) would account for most of the variance. Principal components analysis was performed using a correlation matrix because each variable consisted of different units and data ranges. As a correlation matrix was used, the magnitude of the loading variable represents its influence on the overall treatment differences and the sign indicates either a direct or inverse relationship among loading variables within each principal component. Loadings > 0.50 were considered significant (Manly, 1994).

# **Results and discussion**

#### Soil properties and nutrient dynamics

Cropping system significantly influenced concentration of soil minerals, nutrients, and chemical properties. To begin with, base line soil nutrient analysis on samples collected before the start of the study show a high range of SOM in all cropping systems. In Ingham County, where our experimental site is located, SOM in 0 to 23 cm depth can range from 2% to 6% (NRCS, 2010). Soil pH also was in the normal range (5.6-7.3). The soil had a favorable cation exchange capacity ranging from 15.8 to 17.3 meg 100  $g^{-1}$ . In 2007, two years into the experiment, there were significant differences among treatments in soil EC, but pH showed no difference among treatments (Table 6). The same trend was observed in 2008 with no difference in pH while EC showing cropping system and compost application effects. It takes time to alter soil pH especially if the soil has a large buffering capacity as was the case in our study. Castro et al. (2009) did not report significant changes in soil pH after applying 40 t ha<sup>-1</sup> of air dried sewage sludge, municipal solid waste compost, or 1 t ha<sup>-1</sup> synthetic fertilizer for three consecutive lettuce growing seasons. Flies $\beta$ bach et al. (2007) also did not observe significant differences in pH within first 7 years of their 21 years study when soils were treated with

composted manure, mineral fertilizer or no fertilizer or manure. However, a number of studies comparing organic and conventional systems have reported higher pH in organically managed soils (Drinkwater et al., 1995; Clark et al., 1998). There were minor differences in soil pH in 2009 with the intercrop no-compost treatment showing the lowest value.

In 2009 compost treatments had higher EC values as compared to no-compost treatments (Table 6). Increased EC due to application of compost and animal manure has been widely reported (Hao and Chang, 2002; Sharpley and Kamprath, 1988). Differences in soil water-holding capacity were not distinguishable among treatments until the final year, when the compost treatments, regardless of cropping system, had higher water holding capacity (Table 6). This could be partially attributed to increase in soil organic matter due to the addition of compost over time (Fig. 1) Evanylo et al. (2008) also reported higher water holding capacity in soils treated with composts.

In 2007, the monocrop no-compost treatment had significantly higher ammoniumnitrogen as compared to compost treatment. Similar trend was observed in intercrop treatment, but it was not statistically significant. After three years of application, in 2008, compost treatments had higher NO<sub>3</sub>-N in the soil as compared to no-compost treatments. This increase is likely due to continuous mineralization of compost applied in previous years which adds to the existing NO<sub>3</sub>-N levels in the soil. Soil NO<sub>3</sub>-N levels in 2008 were substantially higher than that found in 2007. Studies have shown a significant increase in nitrogen mineralization and nitrification rates through soil incorporation of animal manure and composts (Muller at al., 2003; Zaman et al., 1999). Soil nitrate levels in 2009 decreased (Table 6). The average precipitation from May to August was 400 mm as compared to an 8 year average of 306 mm for those months.

The only source of nitrogen in no-compost plots is through the decomposition of rye residue during the growing season. The incorporation of rye in the no-compost treatments does add to the soil organic N reserves but this does not always increase N availability and yields of succeeding crops (Kuo et al., 1996). In addition, incorporation of rye can also lead to net N immobilization which could affect successive crop growth and yield. High C:N ratio and low N concentration in residues of crops such as rye or oat can cause net N immobilization in the soil (Quemada and Cabrera, 1995). Therefore, tomato and cucumber monocrop and intercrop treatments without compost applications could have been affected by decreased N availability during their initial growth stages due to N-immobilization. This process would have affected cucumber more severely than tomato due to a shorter growth period. In an incubation study Kuo and Sainju (1998) observed that it took 30 weeks for the amount of N mineralized from a ryeresidue amended soil to catch up with the N mineralized from a control soil without residue amendment. They added rye residue at a rate of 10 g kg<sup>-1</sup> soil (dry weight basis) which would correspond to 23 t ha<sup>-1</sup> (assuming 1 ha of soil, 15 cm deep, will weigh 2272 t). This amount of residue is four times greater than the average amount of residue generated by rye cover crop under field conditions (Clark, 2007), as was the case in this study. Nitrogen mineralization from cover crop residue depends upon a number of factors such as soil type, moisture, temperature, and microbial activity. Based on results from Kuo and Sainju (1998), although it is not accurate to infer, we can assume that in our study the time taken for N mineralization would have been approximately 7 weeks instead of 30 weeks, which would have again affected early growth stages of both cucumber and tomato plants as they were planted generally 4 weeks after cover crop incorporation.

Soil Ca and Mg levels did not differ among treatments in 2007 and 2008. Unlike Bulluck et al. (2002), we did not observe a significant increase in soil Ca and Mg concentrations with the addition of compost. In 2009, the fourth year of the study, intercrop compost treatment had the highest concentrations of Ca and Mg, with Mg showing an increase when compared to the baseline soil concentration recorded before the start of the study in 2005. Soil K was higher in compost amended plots and effects due to cropping system were evident in 2008 and 2009. Compost amended plots in the intercrop system had higher soil K than in the monocrop system. Hao and Chang (2002) reported higher K concentration in soil after repeated annual application of cattle manure in both irrigated and non-irrigated soil. Differences in soil chemical and physical properties due to changes in soil management techniques can vary. In a 8-year study comparing organic, low-input, and conventional production system involving animal manure, winter cover crops, and synthetic fertilizers, Clark et al. (1999) did not find consistent differences in soil EC, Ca, and Mg levels; however, organic treatments led to higher soil organic C, soluble P, and exchangeable K.

Agricultural management practices can significantly influence the amount of SOM. The effect of compost application on SOM has been reviewed by Stratton and Rechcigl (1998). In general, increases in SOM is directly related to better plant nutrition, greater aggregate stability, reduced bulk density, and improved water holding capacity (Carter and Stewart, 1996). Different levels of increase in SOM have been reported depending upon the type of compost used. Schlegel (1992) reported a smaller increase (0.26%) in SOM as compared to a control plot after application of cattle manure compost for three consecutive years at an annual rate of 16 t ha<sup>-1</sup> and observed that the increase in SOM was linearly related to the rate of compost application. Evanylo et al. (2008) reported a 50% increase in soil organic carbon with annual application of

compost (45 t ha<sup>-1</sup>) as compared to control treatment (no-compost).Various studies have reported large increases in soil organic carbon with repeated compost applications (Habteselassie et al., 2006; Zaman et al., 1999). In our study, 4 years of compost application increased SOM to 5% from 3.5% in the tomato intercrop system (Fig. 1). All treatments exhibited an upward trend for SOM accumulation. Soils which received no compost also increased in SOM but the increase was lower than compost amended soils. Never the less, this increase shows the positive effects of rye cover crop on SOM buildup. Compost treatments were maintained in 2010 although no cover crop or cucumber crop was planted in 2009 or 2010, respectively. Soil samples collected in July for 2010 showed lowest SOM under tomato monocrop system.

# Crop growth

Monocropping and intercropping systems differ due to light, nutrient dynamics, pest and disease pressure, and microclimate modifications including air flow, relative humidity, and canopy temperature. Some changes in soil environment due to cropping system (monocropping or intercropping) may be of a short duration (change in soil temperature, moisture, etc.) while some, such as soil fertility, may persist for a long time (Fukai and Trenbath, 1993). Intercropping is most productive when intercrops differ in growth duration and stages so that maximum requirements for resources occur at different times (Fukai and Trenbath, 1993). This was the case with the cucumber season lasting for 60 d and tomato from 90 to 95 d. We believe that in our intercropping system competition for soil nutrients between cucumber and tomato plants would have been minimal. This is based on the fact that cucumber and tomato beds were approximately 1.7 m apart and their root systems were confined within raised beds on plastic mulch. However, there is a possibility that cucumber growth could have been affected due to lower photosynthetic

active radiation (PAR) interception and reduced air flow in intercropping system. Tomato on the other hand could have benefited due to increased air flow and lower degree of shade in the intercrop system.

Response to cropping system and compost treatment on tomato growth characteristics varied. There were no differences in tomato SPAD readings in 2008 or 2009. SPAD readings have been correlated with yield of crops such as potato (Gianquinto et al., 2003) and cabbage (Westerveld et al., 2003) but did not correlate with tomato yields in our study. Studies have indicated varied results on predictions based on SPAD readings. Martini et al. (2004) reported higher SPAD readings in conventionally grown tomato plants than organic, but the final yield was higher in organic production. An intercrop study conducted on cauliflower reported no significant differences in growth characteristics (leaf number, leaf weight, stem height, curd height, curd diameter, or curd weight) when cauliflower was intercropped with beans, lettuce or onions. However, growth characteristics were affected when cauliflower was intercropped with radish (Yildrim and Guvenc, 2005).

Plant height and stem diameter were significantly different among treatments in 2008. There was no impact of cropping system, but compost application produced taller and larger stem diameter plants (Table 7). Dry weight was higher for plants receiving compost in monoculture system but not in the intercrop system. Similar to 2008, plants receiving compost had larger stem diameter and dry weight when compared to no-compost plants in 2009. Compost use as a soil amendment for vegetable crop production significantly influences plant growth response (Stoffella and Kahn, 2001). Gopinath et al., (2009) reported significant increases in plant height in bell pepper with the addition of composted farmyard manure, poultry manure, or

vermicompost. Studies have also reported no significant increase in shoot and root dry weight and total fruit weight in tomato after addition of compost and manure (Hasna et al., 2007).

In 2007 tomato plants were affected by early blight or septoria leaf spot disease. There was no difference in the severity of tomato early blight or septoria leaf spot between compost and no-compost treatments, however, cropping system significantly influenced disease incidence (Table 8). There are examples of foliar disease suppression with the use of composted organic by-products (Khan et al., 2004; Stone et al., 2003), but their efficacy has varied (Abbasi et al., 2002; Hasna et al., 2007). Intercrop treatment showed higher disease severity as compared to monoculture treatment. Crop diversity could have influenced this occurrence as increase in pest numbers due to crop diversity has been reported (Kass, 1978).

# Yield characteristics

The yield of tomato in 2006 was not significantly affected when intercropped with cucumber, with or without compost amendments (Table 9). The presence of considerable soil reserves of essential plant nutrients from fertilization of previous crops likely limited yield responses to the treatments in 2006. It is also important to indicate the entire plot was in soybean in 2005 prior to the initiation of this study. There was a lot of variability in tomato marketable yield in 2006. Even though there were no statistically significant differences among treatments, compost treatments seemed to perform poorly as compared to no-compost treatment. This could be attributed to the condition of compost used in 2006 which was water saturated and not fully mature at the time of application. Six weeks after transplanting plants growing in compost amended plots showed symptoms of stress. Immature and poorly stabilized composts may cause negative impact on plant growth due to reduced oxygen, N-immobilization and/or presence of phyto-toxic compounds (Cooperband et al., 2002;

http://www.anr.state.vt.us/dec/wastediv/compost/documents/CompMaturity.pdf). In 2006, intercrop treatments produced higher number and weight of non-marketable tomato fruits. There were no treatment effects on marketable fruit number and yield in 2007; however, nonmarketable fruit count and weight were higher in no-compost intercrop treatment. Teasdale & Deahl (1987) reported reduced yield and canopy width of tomato when intercropped with snap bean. Other studies also did not report significant yield differences between monocrop and intercrop systems. Examples of those studies include Brown et al. (1985) in tomato:cabbage, Gliessman (1998) in broccoli:lettuce, and Natarjan (1992) in chilli:onion. Brown et al. (1985) reported higher total plant N, plant height, and tomato yield in tomatoes grown alone than those of tomato intercropped with cabbage.

In 2008 monocrop system with compost application produced the highest total number of fruits (Table 9). There was no difference in marketable fruit numbers among other treatments. In our study monocrop no-compost treatment produced lower marketable fruit weight as compared to compost treatment but this difference was not found in intercrop system where both compost and no-compost treatments produced similar marketable fruit weights suggesting improve performance of tomato in intercrop system. Application of compost has been shown to increase crop growth and yield (Roe and Cornforth, 1997; Smukler et al. 2008). Non-marketable fruit numbers and weights were higher for intercrop treatments in 2008. A reason for higher non-marketable fruits in intercrop treatments in 2008 is attributed to the damage caused by birds. As tomatoes were maturing, more red colored fruits appeared earlier in intercrop treatment which attracted more birds. There was no consistent trend in non-marketable fruit number or weight in 2009 growing season.

Treatments did not have a significant effect on cucumber yield in 2006 (Table 9). Yields were generally low as compared to subsequent years. In 2007 marketable fruit number and weight were the lowest in intercrop no-compost treatment. All other treatments were statistically similar to each other but were different than the intercrop no-compost treatment. These trends did not translate to non-marketable fruits as there were no differences among treatments. Cucumber marketable yield in 2008 was significantly affected by cropping system and compost applications. Intercrop system had higher marketable yields than monocrop system. This is primarily due to reduced cucumber beetle and bacterial wilt incidence in the intercrop system. Both in 2008 and 2009 highest marketable fruit weight was obtained for intercrop compost treatment.

# Land equivalent ratio

LER was below 1 for the first two years (Table 10). There was a substantial increase in LER for marketable yield in 2008 demonstrating advantages of intercropping (Table 10). High LER values were a direct consequence of reduced cucumber beetle damage in cucumber intercrop, especially in 2008. Monoculture cucumber plots were heavily infested with cucumber beetle and bacterial wilt which significantly reduced relative yield from plants in the monoculture system. By fourth cucumber harvest, in cucumber monocrop, 35% and 47% of plants were dead in compost and no-compost treatments respectively (Table 8). In contrast, when cucumber was intercropped with tomato, only 12% and 14% plants were dead in compost and no-compost treatments, respectively. These differences between monocrop and intercrop treatments were highly significant ( $P \le 0.0002$ ). In 2009 LER was more than 1 for compost treatment but was lower for no-compost treatment. An earlier study conducted in Michigan by Schultz et al., (1983) demonstrated higher yields and benefits of crop security when tomato was

intercropped with cucumber. Tomato intercrop consistently yielded higher than monocrop in 15 out of 16 replicated plots used in the study. They reported an LER value of 1.14 which means a single unit of intercrop yielded 14% more than a unit of cucumber or tomato monoculture. When the values of LER appear to be greater than 1, this usually indicates the efficiency of intercropping over monocropping (Vandermeer, 1989). In contrast to our 2007 data, intercropping has shown great potential for pest and disease reduction (Theunnissen and Shelling, 1996). One of the most obvious advantages of growing two or more crops is the reduced risk of total crop failure (Anderson et al., 1977). Additionally, intercropping has been shown to reduce the population of numerous herbivore species. A 53% reduction in herbivore species was reported under intercropping system (Risch et al., 1983). Reduction in pest population in most cases is the result of confused visual and olfactory stimuli perceived or presence of mechanical barriers which lead to low oviposition and poor colonization (Theunnissen, 1994). In our study, as the season progressed, intercrop treatments were also affected by beetle and bacterial wilt damage. By the end of the seventh harvest highest damage was observed in cucumber monocrop compost treatment and the least in cucumber intercrop nocompost treatment.

Practical significance of LER can only be fully accessed when related to the actual economic yield (Willey, 1979). The sustainability of any production system is influenced by the economic return, which determines the commercial feasibility of different intercropping systems. Highest LER values do not always reflect highest monetary return to the grower (Muoneke and Asiegbu, 1997). Thus a thorough economic analysis is needed to better evaluate the significance and relevance of cropping systems in production.

# Principal component analysis

Principal component analysis provided unique information regarding the interrelationships among soil chemical properties, crop growth and yield characteristics. The proportion of variation in the data explained by principal component 1 (PC1) and principal component 2 (PC2) ranged from 31 to 45% and 20 to 31%, respectively (Table 11). Loading variables that contributed to treatment differences along PC1 or PC2 were identified by their significant PC loadings ( $P \le 0.05$ ). In 2007, PC1 received high positive loadings for soil Ca, K, and ammonium concentrations, and high negative loadings for cover crop biomass, SOM, and marketable fruit number and weight. Marketable fruit weight was directly related to SOM and cover crop biomass showcasing benefits of additions of organic amendment. There was an inverse relationship between marketable fruit weight and Ca, Mg, and ammonium concentration in the soil but this relationship was reversed on PC2. Analysis of 2008 data showed high positive loadings and a direct relationship between soil nutrient status and plant growth characteristics on PC1. Increased soil nutrient status contributed towards better plant growth and development. Yield characteristics were significant on PC2 and were inversely related to soil Ca and ammonium concentration indicating that higher Ca and ammonium, originating due to repeated compost applications were affecting crop yield. In 2009, PC1 explained most of the variation in the data (46%) correlating highly with plant growth and yield. Soil Ca and Mg showed inverse relationship with yield characteristics, whereas K was positively related. There is a possibility that with the addition of composts there is significant increase in soil K which has been shown to interfere with crop uptake of other nutrients, such as Mg and Ca (Daliparthy et al., 1994; Stout and Baker, 1981).

Principal component analysis conducted on dataset collected in 2007, 2008, and 2009 yielded differences among all treatments (compost amendment and cropping system); however, in 2007 these differences were not prominent, although, there is some indication that PC1 separated compost and no-compost treatments (Fig. 2A). This separation was mainly brought out by variables such as marketable fruit count and weight, plant dry weight, EC and SOM. Nocompost treatments, especially for intercrop, showed higher values for non-marketable fruit count and weight on PC1 (Fig 2B). All variables had positive values for PC2 except, cover crop biomass, non-marketable fruit count and weight (Fig. 2C). Segregation of treatments based on compost application was clear in 2008 (Fig. 2D). Most of the compost treatments, regardless of cropping system, fall in the right quadrant of PC1. This separation along PC1 is brought out by variables representing higher plant growth and soil nutrient characteristics (Fig. 2E). Distinct clusters of both compost amended and non-amended treatments can be seen on the PCA plot in 2009 (Fig. 2F). Based on PC1, compost treatments fall in a quadrant which signifies higher soil EC, pH, nitrate, and K concentrations. Plant growth characteristics and yield attributes influenced separation of compost and no-compost treatments.

Multivariate analysis such as PCA has been used by number of studies to illustrate the interaction among variables and their effects on crop growth and yield. Clark et al. (1999) used PCA to compare tomatoes grown under conventional and alternative farming systems based on effects of water, nitrogen and weed abundance. A study to explore the relationship between management practices and changes in soil properties based on nutrient applications, methods of irrigation, tillage, and intrinsic soil properties revealed distinct separation among management treatments using multivariate analysis tool (Smukler et al., 2008). In our study PCA, based on soil chemical properties, crop growth and yield characteristics, was able to separate plots based

on compost and no-compost treatments clearly indicating significant changes in the production system. Although variables collected in our study did not contribute towards separation of cropping system (monocrop or intercrop), interesting relationships might exist if variables such as disease dynamics, pest populations, and net revenue are added into the equation.

# Conclusion

A major tenet of sustainable agriculture is to create and maintain diversity. Intercropping could be an effective tool to enhance diversity and efficiently use resources needed for plant growth and development. The study tested the feasibility of intercropping tomato and cucumber adopting sustainable approaches such as use of cover crops and organic amendments. Most evident benefit of a diverse cropping system from this study is the reduced risk of total crop failure. Management of pest and disease outbreaks requires diverse strategies under organic production systems. Intercropping in such instances can provide some cushion against large economic losses. Most of the differences in plant growth characteristics were based on compost rather than cropping system effect. Cropping system did affect yield attributes in 2008 when the pest and disease pressure was highest in cucumber. Plant mortality and damage caused by cucumber beetle and bacterial wilt was lower in cucumber plants intercropped with tomato. As a result, LER was more than 1. Soil organic matter increased in all treatments suggesting positive impact of rye cover crop and compost. Cover crops not only add SOM but help reduce leaching of nutrients, especially nitrates. Improving soil fertility and productivity through the use of animal manures, compost, agricultural wastes, or other organic inputs is an important underpinning of organic production. Our study clearly shows advantages of using compost and its positive effects on various crop growth characteristics and yield. Soil nutrient status was also significantly influenced by compost. Concentration of nutrients such as N, Ca, Mg, and K
generally increased with compost applications. There was no significant impact on soil pH but compost increased soil EC. Both long and short-term application of compost, in addition to building SOM, contributes to enhanced microbial activity and improves soil characteristics such as structure, bulk density, and water holding capacity. By the end of fourth year of the study improved water holding capacity was observed in soils obtained from compost amended plots. Based on this study our study suggests that adoption of intercropping could be a tool which growers can use to design a sustainable production system that has increased productivity and profitability. Such systems can better use available resources, improve crop performance, withstand pest and disease pressure, provide crop insurance, and guarantee environmental and economic benefits to the farming community.

## Acknowledgements

The authors thank Aristarque Djoko, Bill Chase, Buck Counts, Gary Winchell, Jon Dahl, Pamela Nichol, Zack Hayden, and staff of Horticulture Teaching and Research Center and Soil Testing Laboratory at Michigan State University, who contributed to research efforts for this project. Financial support for the project has been provided by USDA organic grant No. 2005-51300-02391.

5	1	L	1			1	1	
Cropping	Soil organic	nH	$P(maka^{-1})$	Cat	ions (mg k	g <sup>-1</sup> )	Cation Exchange Capacity	
system <sup>a</sup>	matter (%)	pm	P (Ing kg ) -	Ca	Mg	Κ	$(\text{meq } 100 \text{ g}^{-1})$	
Monocrop (T)	3.6	6.9	31	2300	425	136	15.8	
Monocrop (C)	3.1	7.9	20	2625	468	99	17.3	

2312

Table 2.1. Baseline soil nutrient analysis of the study site in 2005, before plots were prepared for treatment with compost and cropping systems for tomato and cucumber production. Values represent mean of four composite samples

463

114

16.1

<sup>a</sup> T=Tomato, C=Cucumber

Intercrop (T+C)

3.2

7.1

Table 2.2. Mean monthly and long-term (8-year) air temperature, precipitation, and relative humidity during tomato-cucumber growing season in 2006, 2007, 2008, and 2009 at Horticulture Teaching and Research Center, Michigan State University, Holt MI 48842.

	Month	Monthly average air temperature (°C)				Total monthly precipitation (mm)				Month	Monthly average relative humidity (%)				
Month	2006	2007	2008	2009	8 year average <sup>a</sup>	2006	2007	2008	2009	8 year average	2006	2007	2008	2009	8 year average
May	14.5	16.0	13.0	14.7	14.3	110.9	97.0	29.4	108.9	104.2	69.9	61.6	61.9	62.5	72.1
June	19.1	20.4	20.0	19.2	19.6	70.9	89.1	112.0	126.0	68.8	67.8	89.1	49.6	71.4	73.3
July	22.6	20.8	21.7	19.3	21.4	80.3	12.4	96.0	61.0	78.1	73.4	66.6	72.6	72.7	74.5
August	20.8	21.4	20.6	20.1	20.4	92.5	140.1	17.0	105.0	54.9	75.8	75.5	72.9	77.0	77.6
Total	n/a	n/a	n/a	n/a	n/a	354.6	338.6	254.4	400.9	306.0	n/a	n/a	n/a	n/a	n/a

<sup>a</sup> 8 year average from 1998-2005,

n/a = not applicable

Date Year	Dry matter				%						mg k	g <sup>-1</sup>		
Dute, I cui	$(kg ha^{-1})$	Ν	Р	Κ	Ca	Mg	Na	S	Fe	Zn	Mn	Cu	В	Al
23 May 2006	25,000	0.65	0.36	0.30	1.34	0.40	0.03	0.12	17,418	134	362	25	6	3898
8 May 2007	25,000	2.06	0.68	2.29	3.77	1.09	0.41	0.39	2666	163	310	101	31	684
9 May 2008	25,000	3.05	0.94	2.49	5.89	1.58	0.41	0.49	3860	237	424	166	45	984
20 May 2009	12,500	2.43	0.80	2.71	3.53	0.98	0.48	0.51	2220	219	299	91	34	792

Table 2.3. Dairy compost rate and nutrient composition for 2006-2009.

A	Cover crop biomass (biomass (t ha <sup>-1</sup> )						
Amendment treatment	2006	2007	2008	2009			
	Monocrop						
Cucumber (C)	7.0	4.7	6.8	1.7			
Cucumber (NC)	7.0	3.2	8.3	1.5			
Tomato (C)	7.4	1.9	2.4	6.6			
Tomato (NC)	7.4	1.9	1.8	5.4			
		Inter	crop				
Cucumber + Tomato (C)	7.3	3.0	8.6	7.5			
Cucumber + Tomato (NC)	7.3	2.7	5.2	7.3			
2							

Table 2.4. Cover crop biomass added to each cropping system and compost treatment, 2006-2009. Values represent mean of eight samples.

<sup>a</sup> C=Compost, NC=No compost

_	To	omato	Cucumber			
_	Seeding	Transplanting	Seeding	Transplanting		
2006 <sup>a</sup>	11 April	7 June	7 June	-		
2007	20 April	31 May	26 May	7 June		
2008	15 April	2 June	30 May	11 June		
2009	15 April	5 June	28 May	16 June		

Table 2.5. Seeding and transplanting schedule for tomato and cucumber transplants for 2006-2009 growing seasons.

<sup>a</sup> Cucumber was direct seeded in 2006.

a	Water holding $(15^{-1})$ $(-1^{-1})$ NO N(1 1 $-1^{-1})$ NU N(1 1					Cations (mg kg <sup>-1</sup> )				
Cropping system"	pН	$EC (dS m^{-1})$	capacity (g g <sup>-1</sup> )	$NO_3-N$ (kg ha <sup>-1</sup> )	$NH_4-N$ (kg ha <sup>-1</sup> )	Ca	Mg	K		
	2007 <sup>c</sup>									
Monocrop (C)	ND	0.44 a <sup>b</sup>	0.23 <sup>NS</sup>	31.4 <sup>NS</sup>	4.5 b	2228.8 <sup>NS</sup>	406.5 <sup>NS</sup>	161.8 ab		
Monocrop (NC)	ND	0.35 b	0.20	29.0	6.8 a	2018.5	385.0	100.5 c		
Intercrop (C)	ND	0.34 bc	0.21	34.7	6.5 ab	2086.0	413.8	195.5 a		
Intercrop (NC)	ND	0.28 c	0.22	31.5	7.4 a	2097.8	408.0	135.3 bc		
	2008									
Monocrop (C)	7.1 <sup>NS</sup>	0.57 a	$0.22^{NS}$	54.2 a	7.5 <sup>NS</sup>	2017.0 <sup>NS</sup>	433.8 <sup>NS</sup>	199.5 b		
Monocrop (NC)	7.3	0.31 c	0.23	37.7 b	7.6	2076.8	417.8	120.5 c		
Intercrop (C)	7.2	0.52 ab	0.22	56.8 a	8.3	2291.8	452.8	240.0 a		
Intercrop (NC)	7.3	0.39 bc	0.25	40.9 b	7.7	2156.0	426.3	133.0 c		
				2009						
Monocrop (C)	7.4 ab	0.42 a	0.36 a	9.3 <sup>NS</sup>	1.9 <sup>NS</sup>	2137.0 b	434.0 b	171.2 b		
Monocrop (NC)	7.6 a	0.23 b	0.28 b	5.4	2.4	2149.5 b	420.0 b	114.2 c		
Intercrop (C)	7.4 ab	0.36 a	0.37 a	10.2	2.3	2518.8 a	527.5 a	234.0 a		
Intercrop (NC)	7.1 b	0.22 b	0.27 b	10.0	2.8	2197.3 b	427.2 b	118.0 c		

Table 2.6. Effect of cropping system and compost on soil chemical and physical properties at the end of the growing season within tomato production system. Each value is a mean of four samples.

<sup>a</sup> C=compost, NC=No compost

<sup>b</sup> Mean separation within columns for individual years. Means followed by same letter(s) are not significantly different ( $P \le 0.05$ )

<sup>c</sup> Analysis conducted on soil samples collected at the end of the growing season each year

<sup>NS</sup> Means are not significantly different ( $P \le 0.05$ )

ND = Not determined

Cropping system <sup>a</sup>		Tomate	o growth characteristics					
- FF 8-9	SPAD	Height (cm)	Stem diameter (cm)	Dry weight per plant (g)				
		$2008^c$						
Monocrop (C)	53.4 <sup>NS</sup>	42.5 a <sup>b</sup>	1.6 a	208.0 a				
Monocrop (NC)	53.6	35.4 b	1.3 b	139.3 b				
Intercrop (C)	54.3	44.7 a	1.9 a	156.4 b				
Intercrop (NC)	56.4	34.9 b	1.3 b	139.1 b				
			2009					
Monocrop (C)	43.2 <sup>NS</sup>	56.6 <sup>NS</sup>	1.5 a	183.9 ab				
Monocrop (NC)	42.6	53.8	1.3 b	121.5 ab				
Intercrop (C)	48.5	56.3	1.4 a	185.2 a				
Intercrop (NC)	48.8	52.4	1.3 b	115.6 b				

Table 2.7. Effect of cropping system and compost on tomato growth characteristics and total dry weight, 2008-2009.

<sup>a</sup> C=compost, NC=No compost

<sup>b</sup> Mean separation within columns for individual years. Means followed by same letter(s) are not significantly different ( $P \le 0.05$ ) <sup>c</sup> Data collected on 30 July and 13 August in 2008 and 2009 respectively.

<sup>NS</sup> Means are not significantly different ( $P \le 0.05$ )

Crossing System <sup>a</sup>	Tomata diagona nating b	Cucumber beetle and bacterial wilt infestation <sup>c</sup>					
Cropping System	Tomato disease rating	Plants dead at 4 <sup>th</sup> harvest (%)	Plants dead at 7 <sup>th</sup> harvest (%)				
Monocrop (C)	1.0 b <sup>d</sup>	46.8 a	63.5 a				
Monocrop (NC)	1.3 b	35.0 a	52.3 ab				
Intercrop (C)	3.0 a	14.2 b	34.7 bc				
Intercrop (NC)	3.5 a	12.4 b	30.9 c				

Table 2.8. Tomato disease pressure and cucumber plant mortality under different cropping system and compost treatments.

<sup>a</sup> C=compost, NC=No compost

<sup>b</sup> Early Blight and Septoria leaf spot was collectively rated on a scale of 0 to 10 (1 for disease free and 10 if all plants were affected) at the end of the growing season in 2007

<sup>c</sup> Data reported for 2008

<sup>d</sup> Mean separation within columns for individual years. Means followed by same letter(s) are not significantly different ( $P \le 0.05$ )

Cropping		Tom	ato			Cucun	nber			
a		Yield (from	12 plants)			Yield (from	12 plant)			
system	Mark	ketable	Non-n	narketable	Mar	ketable	Non-n	narketable		
	Number	Weight (kg)	Number	Weight (kg)	Number	Weight (kg)	Number	Weight (kg)		
		200	)6		2006					
Monocrop (C)	$263^{NS}$	60.6 <sup>NS</sup>	17 b <sup>b</sup>	2.5 b	$15^{NS}$	4.5 <sup>NS</sup>	$23^{NS}$	$5.8^{NS}$		
Monocrop (NC)	261	59.6	20 b	2.7 ab	24	7.5	32	8.8		
Intercrop (C)	183	38.9	42 a	4.4 a	12	3.4	17	4.4		
Intercrop (NC)	226	53.7	40 a	4.3 a	30	9.3	19	10.8		
	2007				2007					
Monocrop (C)	176 <sup>NS</sup>	32.9 <sup>NS</sup>	18 b	3.5 b	44 a	16.1 a	$28^{NS}$	6.6 <sup>NS</sup>		
Monocrop (NC)	146	27.9	23 b	4.4 b	33 ab	11.6 ab	24	5.8		
Intercrop (C)	169	34.9	23 b	5.2 ab	28 ab	10.9 ab	30	8.5		
Intercrop (NC)	158	30.6	45 a	8.7 a	23 b	7.4 b	24	5.9		
		200	)8		2008					
Monocrop (C)	183 a	43.1 a	29 b	4.7 c	23 c	8.6 c	$13^{NS}$	3.3 <sup>NS</sup>		
Monocrop (NC)	165 b	28.4 b	31 b	5.1 c	27 bc	10.0 bc	16	4.1		
Intercrop (C)	164 b	39.0 ab	75 a	14.7 a	52 a	19.2 a	20	4.7		
Intercrop (NC)	168 b	32.7 ab	57 a	9.6 b	38 ab	14.1 ab	14	2.7		
		200	)9			200	9	•		
Monocrop (C)	183 a	36.9 a	36 a	6.8 <sup>NS</sup>	31 b	10.5 b	27 b	5.7 b		
Monocrop (NC)	137 b	23.8 b	21 b	3.8	18 b	5.7 b	26 b	5.2 b		
Intercrop (C)	159 a	29.4 b	35 a	5.9	58 a	23.1 a	41 a	8.1 a		
Intercrop (NC)	115 b	21.3 b	25 a	4.6	32 b	10.9 b	22 b	4.2 b		

Table 2.9. Tomato and cucumber yield in response to cropping system and compost treatments from 2006-2009.

<sup>a</sup> C=compost, NC=No compost <sup>b</sup> Mean separation within columns for individual years. Means followed by same letter(s) are not significantly different ( $P \le 0.05$ ) <sup>NS</sup> Means are not significantly different ( $P \le 0.05$ )

Treatment	Land Equivale	ent Ratio (LER)				
Troutmont	Marketable count	Marketable weight				
	2007					
Compost	0.82 <sup>NS</sup>	0.89 <sup>NS</sup>				
No-Compost	0.89	0.87				
	2008					
Compost	1.63 <sup>NS</sup>	1.68 <sup>NS</sup>				
No-Compost	1.27	1.34				
	20	)09				
Compost	1.37 a <sup>a</sup>	1.56 a				
No-Compost	0.70 b	0.70 b				

Table 2.10. Land Equivalent Ratio for cucumber-tomato intercropping system, 2006-2009.

<sup>a</sup> Mean separation within columns for individual years. Means followed by different letter(s) are significantly different ( $P \le 0.05$ ) NS Means are not significantly different ( $P \le 0.05$ )

Loading Variable	20	07	20	08	20	09
	PCA1 (31%)	PCA2 (23%)	PCA1 (36%)	PCA2 (32%)	PCA1 (46%)	PCA2 (21%)
Ca	$0.72^{a}$	$0.55^{a}$	$0.57^{a}$	-0.77 <sup>a</sup>	$-0.55^{a}$	0.75 <sup>a</sup>
Κ	0.26	$0.79^{a}$	0.93 <sup>a</sup>	0.19	0.16	$0.97^{a}$
Mg	$0.74^{a}$	$0.55^{a}$	0.39	-0.46	-0.35	0.69 <sup>a</sup>
Nitrate	0.25	0.71 <sup>a</sup>	$0.77^{a}$	0.53 <sup>a</sup>	0.19	0.18
Ammonium	$0.84^{a}$	0.25	$0.60^{a}$	-0.74 <sup>a</sup>	$-0.76^{a}$	-0.20
pН	ND	ND	ND	ND	0.32	0.04
EC	-0.47	0.07	$0.53^{a}$	0.59 <sup>a</sup>	0.32	0.75 <sup>a</sup>
CC	-0.67 <sup>a</sup>	-0.15	0.45	0.45	$0.59^{a}$	-0.08
SOM	$-0.56^{a}$	0.22	$0.77^{a}$	-0.48	-0.36	$0.88^{a}$
Plant Height	ND	ND	$0.80^{a}$	0.35	0.96 <sup>a</sup>	-0.07
Stem diameter	ND	ND	$0.82^{a}$	0.38	$0.90^{a}$	0.12
SPAD	-0.02	0.03	-0.18	0.27	0.20	0.05
Dry wt	-0.45	0.51 <sup>a</sup>	0.08	$0.74^{a}$	$0.89^{a}$	0.19
Mcount	-0.67 <sup>a</sup>	0.56 <sup>a</sup>	-0.24	0.89 <sup>a</sup>	0.93 <sup>a</sup>	0.04
Mwt	-0.63 <sup>a</sup>	$0.58^{a}$	0.25	0.91 <sup>a</sup>	$0.97^{a}$	-0.01
Nmcount	0.43	-0.44	0.69 <sup>a</sup>	-0.28	$0.88^{a}$	0.20
Nmwt	0.45	-0.42	$0.71^{a}$	-0.14	0.92 <sup>a</sup>	0.00

Table 2.11. Principal component loadings (correlation between original loading variable and principal component) as a measure of influence of a loading variable on overall treatment differences.

<sup>a</sup> Factor loadings statistically significant ( $P \le 0.05$ )

CC = Cover crop biomass, SOM = Soil organic matter, Dry wt = Plant dry weight, Mcount = Marketable fruit count, Mwt = Marketable fruit weight, ND = Not determined; Nmcount = Non-marketable fruit count, Nmwt = Non-marketable fruit weight.



Fig. 2.1. Soil organic matter accumulation under cropping system and compost treatments. Samples were collected at the end of each growing season from 2007 to 2009. Compost treatments were maintained in 2010 although no cover crop or cucumber crop was planted in 2009 or 2010, respectively. Soil samples were collected in July for 2010. Error bars showing standard error (n=4). I-C=Intercrop compost, M-C=Monocrop compost, I-NC=Intercrop No-compost, and M-NC=Monocrop No-compost.



Fig. 2.2. PCA of tomato cropping system and compost treatments in 2007. Scatter plot of treatments (A) and variables explaining the variation (B) on PCA1 and PCA 2 axis. CC=Cover crop biomass, Dry wt=Plant dry weight, Mcount=Marketable fruit count, Mwt=Marketable fruit weight, Nmcount=Non-marketable fruit count, Nmwt=Non-marketable fruit weight, SOM=Soil organic matter). For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this dissertation.



Fig. 2.3. PCA of tomato cropping system and compost treatments in 2008. Scatter plot of treatments (A) and variables explaining the variation (B) on PCA1 and PCA 2 axis. CC=Cover crop biomass, Dry wt=Plant dry weight, Mcount=Marketable fruit count, Mwt=Marketable fruit weight, Nmcnt=Non-marketable fruit count, Nmwt=Non-marketable fruit weight, SOM=Soil organic matter).



Fig. 2.4. PCA of tomato cropping system and compost treatments in 2009. Scatter plot of treatments (A) and variables explaining the variation (B) on PCA1 and PCA 2 axis. CC=Cover crop biomass, Dry wt=Plant dry weight, Mcount=Marketable fruit count, Mwt=Marketable fruit weight, Nmcount=Non-marketable fruit count, Nmwt=Non-marketable fruit weight, SOM=Soil organic matter).

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# CHAPTER III

# SOIL MICROBIAL COMMUNITY AND PROPERTIES RESPOND TO WINTER COVER CROPS AND ORGANIC AMENDMENTS IN ORGANIC CUCUMBER PRODUCTION SYSTEM

Soil microbial community and properties respond to winter cover crops and organic amendments in organic cucumber production system

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#### Abstract

Soil microorganisms play a crucial role in mineralization and breakdown of complex organic compounds in soil. Microbial population and functional diversity is greatly influenced by quantity and quality of crop residue and other organic amendments incorporated. This study investigated the effect of incorporation of cover crops [rye (Secale cereale L.) or a mixture of rye and hairy-vetch (Vicia villosa Roth.)] and compost on the soil microflora and microfauna under an organic cucumber production system. Each cover crop treatment was used with or without compost application in a split-plot experimental design. Microbial biomass and respiration, metabolic quotient, nematode population distribution, and microbial functional diversity was measured at the end of the growing season. Metabolic characteristics of the soil microbial community were determined using 31 C substrates on Biolog-Ecoplate<sup>TM</sup>. Community level physiological profile (CLPP) was determined by calculating average well color development (AWCD), richness (S), Shannon-Weaver diversity index (E), and evenness (E). Respiration was affected largely by compost than cover crop treatment with soils receiving compost having higher respiration rates. Highest microbial biomass was found in the soils amended with rye and compost (195-210  $\mu$ g g<sup>-1</sup> dry soil). Regression analysis between microbial biomass and soil organic matter (SOM) showed strong correlation ( $R^2$  value of 0.68 and 0.56) in two out of the three years. Calcium, Mg, and K concentrations in soil also positively correlated with microbial biomass. There were significant differences among soils in numbers of plant parasitic, bacterial, and fungal feeding nematodes during the initial years of the study but the differences were not evident later. Shannon-Weaver diversity index) was significantly affected by cover crop treatment with rye treatments generally exhibiting higher degree of diversity. Average well color development increased with incubation period and differences were usually evident 72 h after

incubation. There minimal differences between cover crop treatment but compost application translated in higher AWCD. Biolog-Ecoplate<sup>TM</sup> assay was sensitive to changes in the short-term. Principal component analysis of the Biolog data allowed the differentiation of treatments but distribution patterns varied from year to year. We conclude that both rye and rye-vetch mixture can affect the functional diversity of soil microbial community but differences in those effects are marginal. Microbial community was more responsive to compost applications than cover crop effects.

# Introduction

A high and increasing demand for sustainably produced fruit and vegetables has encouraged growers to transition to sustainable and organic production systems (Klonsky, 2004). Such ecologically sound systems have the potential to address a number of ongoing issues in mainstream agriculture namely environmental pollution due to chemical fertilizers and pesticides, soil degradation, loss of soil fertility and productivity, and production losses from pest and disease pressure. One of the core philosophies of organic production systems is the development of healthy and productive soils that will provide essential nutrients for plant growth, support a diverse and active soil biotic community, and balance the entire farm ecosystem (Insam, 2001; Mäder et al., 2002). Soil biology is directly linked to agricultural sustainability as it is the driving force behind decomposition processes that break down complex organic molecules and substances and convert them to plant available forms (Friedel et al., 2001). Large, stable, and active soil microbial community is an underpinning for sustaining the productivity of soils under sustainable and organic farming systems. To develop such systems growers adopt strategies such as crop rotations, cover cropping, and incorporation of organic amendments (manures and composts) that significantly increase soil organic matter and improve soil biology and quality (Bending et al., 2002, Buyer et al., 2010)

Rye (Secale cereale L.) and hairy vetch (Vicia villosa Roth.) are among the most common cover crops used in regions with temperate climate because of their winter hardiness, large biomass production, and, in the case of hairy vetch, capacity to fix atmospheric nitrogen (Abdul-Baki et al., 1996). When mowed and incorporated these cover crops add soil organic matter, improve soil structure and increase soil biological activity (Carrera et al., 2007; Lundquist et al., 1999). In a three year study, Buyer et al. (2010) demonstrated that the incorporation of both rye and vetch cover crop increased soil microbial biomass considerably. Along with cover crops, use of composts and manures is perceived as an integral component for organic production as it provides essential plant nutrients, adds soil organic matter, and improves soil quality and structure (Russo and Webber, 2007). Addition of manures and compost has been shown to positively increase the abundance of various components of soil food web (bacteria, fungi, protozoan and nematode density) and affect a number of soil characteristic, including organic matter, and respiration (Carrera et al., 2007; Ferris et al., 2004; Lundquist et al., 1999; Treonis et al., 2010). With increasing number of growers utilizing cover crops and organic amendments in their production systems, it becomes all the more important to better understand the effects of such strategies on soil food webs, and other biotic assemblages responsible for decomposition and generation of soluble nutrients for plant uptake. After incorporation, nutrients available in cover crops and organic amendments have to pass through a decomposition pathway which involves a number of soil microorganisms including, bacteria, fungi, and nematodes. Thus, the quality and quantity of plant residues entering the soil can significantly influence soil microorganisms and soil microbial processes (Govaerts et al., 2007). Both crop residue and SOM

quality have the potential to increase functional diversity of soil microbial community (Bending et al. 2002).

Soil contains enormous number of diverse living organisms that influence various ecosystem processes, including formation of organic matter, recycling of nutrients, modification of soil physical and chemical properties, and suppression pests and diseases (Coleman et al., 1978). Biological characteristics of soil play a vital role in defining soil quality and health. Soil quality is an effective indicator of soil fertility and reflects changes in soil properties which are both inherent and anthropogenic. Soil quality can be estimated and quantified through evaluation of physical, biochemical, or microbial parameters (Glover et al., 2000). A number of soil microbial parameters such as microbial biomass, respiration, metabolic quotient, and community profiles have the potential for use as diagnostic indicators of soil quality. Such indicators have been widely used in discerning changes in soil quality and to make comparisons between different soil types and contrasting management systems (Bending et al., 2004; Schloter et al., 2003). A great deal of effort has gone into the measurement of soil microbial biomass which consists of both dormant and metabolically active microorganisms (smaller than approximately  $10 \,\mu\text{m}$ ) and measured by direct and indirect techniques (fumigation-incubation, substrateinduced respiration, fumigation-extraction, and ATP content).

In this study we compared four organic cucumber production systems which differ based on incorporation of plant residues and organic amendment inputs. The specific aim of this study was to investigate the impact of cover crop and soil organic amendment on cucumber yield, soil chemical and biological characteristics. Under different cover crop and organic amendment treatments we evaluated parameters such as soil microbial biomass, nematode community composition, and microbial diversity of aerobic microbial community, that rapidly respond to

management systems in a short period of time, such as the 3-year study presented in this paper. Another simple approach to measure soil microbial diversity is to examine the number of different C substrates that are metabolized by the culturable microbial community. This approach of substrate utilization pattern can be obtained using the Biolog-EcoPlate<sup>TM</sup> system (Garland and Mills, 1991; Zak et al., 1994). The Biolog-EcoPlate<sup>TM</sup> system assess the ability of inoculated populations to utilize substrates over time and the speed at which the substrates are utilized, as a result generating a community level physiological profile (CLPP) of the aerobic microbial community in the Biolog-EcoPlate<sup>TM</sup> inoculation system (Garland and Mills, 1991). In our study we hypothesized that: 1) addition of compost would increase cucumber yield and positively influence the abundance of soil microbial biomass and affect soil microbial community, and, 2) a cover crop mixture of rye:vetch would enhance microbial biomass, and affect nematode counts and microbial community structure when compared to a rye alone cover crop. Indicators such as soil microbial biomass, nematode community composition, and diversity of microbial communities are valuable tools that can differentiate soils under different management systems.

#### Materials and methods

#### Field preparation and production

This study was conducted from 2005 to 2009 at the Horticulture Teaching and Research Center (HTRC), Michigan State University, Holt, MI. The soil was a Capac loam with 0% to 3% slope. Capac loam is a somewhat poorly drained, moderately to moderately slowly permeable soil formed in loamy glacial till on the low parts of moraines and till plains. The soil at the research site was under transition (starting 2005) from a conventional corn/soybean rotation to an

organic cucumber and tomato production system. Although the study started with the planting of cover crops in the fall of 2005 and vegetable production in 2006, this paper will focus on results from 2007 to 2009 season. Mean monthly and long-term air temperature, precipitation, and relative humidity during the growing season at HTRC are summarized in Table 1. The experimental design was a split-plot design with four replications. The main plot treatment was the cover crop treatment, cereal rye or a mixture of rye and hairy vetch, and the split was the subplot with presence or absence of compost application (compost or no-compost). For rye treatments, cover crop of cereal rve was drilled at a rate of 78 kg  $ha^{-1}$  in the fall on 18 September, 22 September, and 26 September in 2006, 2007 and 2008, respectively. Rye:hairy vetch treatment was also seeded on same dates at 39 kg ha<sup>-1</sup> and 28 kg ha<sup>-1</sup> of rye and hairy vetch respectively. The following spring, dairy compost at the rate of 25 t ha<sup>-1</sup> was hand applied to compost treatments. The rate of compost was reduced to 12.5 t ha<sup>-1</sup> in 2009 due to higher expected availability of nutrients and to avoid phosphorus build up (Table 2). Each year, after the application of compost, rye cover crop at Feekes Growth Stage 5 (Weisz, 2011) was mowed and later incorporated using a chisel plow. Each year, in the spring, before incorporation of cover crop, four biomass subsamples (shoot and root) from individual 0.25 m<sup>2</sup> area were collected and dried at 60 °C until constant weight to determine cover crop dry weight.

Ten day old cucumber seedlings were transplanted after 28 d of cover crop incorporation on raised beds covered with black plastic mulch and drip irrigated. Each bed was 7.6 m long, 0.6 m wide and 0.2 m high with one row of cucumber. Cucumber was harvested seven times in 2007, 2008, and six times in 2009 with a 2-3 d interval between harvests. Fruits were graded as marketable (U.S. Fancy, U.S. Extra #1, U.S. #1, U.S. #1 Small, and U.S. #1 Large) or

nonmarketable grades (deformed, overgrown, damaged by cuts, scars, sunscald, sunburn, dirt, disease, or insects) (USDA, 1958).

#### Soil sampling and nutrient analysis

Soil samples were collected at the end of the growing season (August) each year. Four soil cores (0-15 cm depth) were collected from raised beds between individual plants and composited. Samples were immediately taken to the lab and stored at 4 °C. Later a part of the sample was dried at 38 °C for 3 d, and ground using a flail grinder for chemical analysis. Soil organic matter was determined by loss of weight-on-ignition method (Combs and Nathan, 1998). A 1N neutral ammonium acetate solution was used to extract calcium, potassium, and magnesium. Analysis of potassium and calcium was carried out by flame emission method and a colorimetric method was used for magnesium (Warncke and Brown, 1998).

# Soil microbial biomass and nematode population

Soil samples were removed from the cooler (4  $^{\circ}$ C) and kept at room temperature for 24 h. Soils from each plot were then sieved (2 mm) and visible organic residues and stones were removed. Microbial biomass was determined by Chloroform Fumigation Incubation method based on Jenkinson and Powlson (1976). Six 50 g soil samples from each replication were weighed into beakers. Three of those samples were fumigated with alcohol-free CHCl<sub>3</sub> for 24 h, while the remaining three served as non-fumigated controls. After fumigation, each fumigated sample was inoculated with approximately 1 g of its corresponding non-fumigated sample, thoroughly mixed and brought to 55% water holding capacity. Samples (both fumigated and nonfumigated) were then incubated at 22  $^{\circ}$ C for 10 d in 1 L air-tight mason jar with a rubber septum on the lid. After the incubation period C0<sub>2</sub> measurements were taken using an Infrared Gas Analyzer (Qubit S151 CO<sub>2</sub> analyzer, Qubit System Inc., Kingston, Ontario, Canada). Soil microbial biomass (SMB) was calculated using the following equation:  $1.73*F_{C} - 0.56*NF_{C}$ , where  $F_{C}$  and  $UF_{C}$  are the mineralized carbon from fumigated and non-fumigated soil samples (Horwath et al., 1996).

Nematodes were extracted from 100 g soil using centrifugal floatation technique (Jenkins, 1964). Nematode identification and counting was done by the Plant Diagnostic Laboratory at Michigan State University. Nematodes were separated into food preference groups; plant parasitic (lesion, spiral, and stunt), predatory, bacterial feeding, fungal feeding or predatory based on morphology of the stoma and esophagus.

#### Community level physiological profile (CLPP)

Substrate utilization patterns of culturable soil microbial population were determined using Biolog-EcoPlate<sup>TM</sup> (BIOLOG Inc., CA, USA) by a procedure adapted from Garland and Mills (1991). Soil (10 g field-moist weight) samples were shaken with 90 ml of sterilized saline solution (0.85% NaCl, w/v) for 60 min and then pre-incubated for 18 h. Samples were brought to  $10^3$  final dilution before inoculation. Each Biolog-EcoPlate<sup>TM</sup> (96-well) consists of three replicates, each one comprising 31 sole carbon sources and water blank (control well). A 150 µl aliquot was inoculated into each microplate well. The rate of utilization of C sources is indicated by the reduction of tetrazolium which changes from colorless to purple. The plates were incubated at 25 °C, and color development in each well was recorded as optical density (OD) at 590 nm with a plate reader (Bio-Rad 680, Bio-Rad Laboratories, Hercules, CA, USA) over a 7 d period (24, 48, 72, 96, 120, 144, and 168 h). Readings obtained soon after inoculation (day 0) were subtracted from subsequent readings to eliminate background color of the substrates and the bacterial suspension. In addition, color response of the control well was subtracted from the color response of each of the response wells. The average well color development (AWCD) value was calculated for each sample at each time point by using the following equation:

AWCD = 
$$\sum OD_i = 31$$
,

where OD<sub>i</sub> is the optical density value from each well. Substrate richness (S) was calculated by counting total number of C substrates oxidized by individual treatments on Biolog-EcoPlate<sup>TM</sup> (counting all positive OD readings). Diversity parameters, such as Shannon-Weaver diversity index (H) and Evenness (E) were calculated using the following equations (Shannon and Weaver 1969; Zak et al., 1994):

$$H = -\sum p_i (\ln p_i)$$
 and  $E = H/\log S$ ,

where  $p_i$  is the ratio of the corrected absorbance value of each well to the sum of absorbance value of all wells. Subsequently, well color responses were normalized by dividing the absorbance values by AWCD to reduce bias between samples due to differences in innoculum densities (Garland, 1997).

#### Statistical analysis

Crop yield, soil physical, chemical and biological data were analyzed by PROC MIXED procedure of Statistical Analysis Systems Institute Inc., Version 9.2; Cary NC. Significant differences between treatment means were separated by 'Ismeans' and 'pdiff' statement in SAS  $(P \le 0.05$  level). Color response data, based on substrate utilization, was further analyzed using Principal component analysis (PCA) (R Statistical Software, R Development Core Team, 2008). Substrate utilization assay data were analyzed after substrates were divided into six groups and the average absorbance per category was calculated (Zak et al., 1994). All meaningful loadings (>0.5) were included and considered significant in the interpretation of principal components (Manly, 1994). In most cases principal component 1 (PC1) and principal component 2 (PC2) would account for most of the variance. Biplots were constructed to interpret the analysis, with the original variables drawn as vectors to summarize the correlation between the variable and both illustrated axes.

# Results

# Cover crop biomass and cucumber yield

Amount of biomass produced by cover crops varied each year. Over all biomass produced in 2007 was lower than 2008 and 2009 (Fig. 1). Biomass accumulation was strongly influenced by prevailing weather conditions, late fall and early spring. Differences in biomass between cover crop treatments were largely due to crop type and compost treatments. Rye:vetch:no-compost treatment consistently performed below average in all the years. This is partly due to low seeding rate of rye in rye:vetch treatment, low fertility, and poor establishment of vetch as a result of late planting dates. Rye, coupled with compost applications, produced abundant biomass in 2008 and 2009. Unlike studies (Sainju et al., 2005) that reported higher biomass yield with rye:vetch mixture than their respective monocultures, rye:vetch biomass yields were statistically similar to rye biomass in 2007 and 2008, but were lower in 2009.

There were significant differences present in cucumber marketable yields which were related more to compost application than the cover crop treatment. Compost incorporation adds nutrients, improves soil structure, and increases crop yield. Application of compost has been
shown to increase crop growth and yield (Stofella and Kahn, 2001; Smukler et al. 2008). Compost applications, irrespective of cover crop treatment, significantly increased cucumber yields in 2007 and 2009, however, due to large variability; differences were not statistically significant in 2008. One of the major objectives of incorporating vetch in a cropping system is its ability to fix atmospheric N; however, use of vetch with rye without any additional nutrient source, as the case in this study, is not enough to increase cucumber yields. Biculture of hairy vetch and rye cover crop may increase N supply, summer crop yields, and N-uptake compared with rye (Sainju et al., 2005), however, cover crop performance and its effect on successive crop can greatly vary depending upon climate, geography and length of growing season (Abdul-Baki et al., 1996)

## Soil respiration, microbial biomass, and metabolic quotient

Both cover crop and compost treatment had a significant effect on basal soil respiration (i.e.  $CO_2$  evolution) (Fig. 2A). Soil samples from compost amended plots showed higher respiration rates than non-amended plots. Soil respiration ranged from 126.5 µg  $CO_2$  g dry soil<sup>-1</sup> (rye:vetch:no-compost treatment) to 282.3 µg  $CO_2$  g dry soil<sup>-1</sup>(rye:compost treatment). Differences in respiration rate due to cover crop treatment were not observed in 2007 and 2009, however, in 2008, rye outperformed rye:vetch cover crop treatment.

Microbial biomass significantly differed among soils with different cover crop and amendment treatments (Fig. 2B). In 2007, the only statistically significant difference was found between rye:compost and rye:vetch:no-compost treatment. The greatest microbial biomass was found in the plots treated with rye:compost (195-210  $\mu$ g g dry soil<sup>-1</sup>) in 2008. Microbial biomass

followed trend similar to 2007 in 2009. Metabolic quotient, which is the ratio of soil respiration to microbial biomass, was significantly different among treatments only in 2008. Rye:compost, rye:no-compost, and rye:vetch:compost treatments showed statistically similar values which were higher than rye:vetch:no-compost. Soil microbial biomass constitutes the active pool of soil organic matter (SOM). Soil microbes typically are C-limited (Smith and Paul, 1990) and lower microbial biomass in soils can be explained as a function of reduced organic C in the soil (Fließbach and Mäder, 2000). Regression analysis between microbial biomass and SOM showed strong correlation ( $R^2$  value of 0.68 and 0.56) in two out of the three years (Fig. 3). Microbial biomass increased with increasing SOM content in 2008 and 2009, however, in 2007 the relationship was not strong ( $R^2$ =0.003). Microbial biomass increased with increasing concentration of cations (Fig. 4). During the three growing seasons, microbial biomass was highly correlated to soil magnesium concentration ( $R^2$  of 0.39, 0.67, and 0.52 in 2007, 2008 and 2009, respectively). Calcium and potassium concentrations were also positively correlated. Metabolic quotient (qCO<sub>2</sub>) did not differ among the cover crop/compost treatments, except in 2008, where rye:compost treatment had higher  $qCO_2$  when compared to rye:vetch:no-compost treatment.

#### Nematode community distribution

In 2007, there were no statistically significant differences in plant parasite, omnivore, or predatory nematode counts (Table 3). Populations of bacterivorous nematodes were significantly higher in rye:vetch than rye alone treatments. Between rye:vetch:compost and rye:vetch:no-compost treatment, bacterivore nematodes were higher in the former treatment. Cover crop and

compost treatments did not differ significantly for omnivore, predatory, bacterivorous, or fungivorous nematodes in 2008, however, significantly higher abundance of plant parasitic nematodes was observed in rye:vetch treatments. By the end of the last growing season (2009), there were no statistically significant differences for any kind of nematodes in any of the treatments.

## Community-level physiological profile

Readings from the fifth day of incubation were found most significant and are reported here. The richness (number of substrate utilized) of the bacterial functional communities, denoted by S, was significantly higher in rye:compost, rye:no-compost, and rye:vetch:compost treatments than rye:vetch:no-compost treatment in 2007. That difference, however, was not observed in 2008 and 2009. Soil bacterial functional diversity index (Shannon-Weaver diversity index) was significantly affected by cover crop treatment. Although, rye cover crop treatment showed higher functional diversity than rye:vetch treatment, differences were not statistically significant. Within the two cover crop treatments, there was no difference between compost or no-compost application. Average well color development increased with incubation period. Rye:vetch:no-compost treatment consistently exhibited the lowest AWCD at all sampling periods in 2007. Cover crop treatments without compost had significantly lower AWCD in 2008. Rye:compost treatment had the highest AWCD after 72, 96, 120, and 144 h of incubation, followed by rye:vetch:compost treatment ( $P \leq 0.05$ ). After 168 h of incubation, both rye and rye:vetch compost treatments, had similar AWCD which was higher than their no-compost treatments. In 2009, rye:no-compost treatment constantly had the lowest AWCD throughout the sampling period.

Principal component analysis yielded distinct differences among treatments (cover crops and compost). The proportion of variation explained by PC1 ranged from 33 to 55%. Principal component loadings, comprising of six categories of Biolog-EcoPlate<sup>TM</sup> C substrates, contributed towards the spread of variables along PC1 and PC2. During the 2007 season, microorganisms that utilize amides, carboxylic acids, and polymers influenced the spread of treatments along PC1 axis, while, carbohydrates and carboxylic acids influenced differences along PC2 axis (Table 4). In 2008 utilization of microorganisms that metabolize amino acids and carbohydrates lead to segregation of treatments on PC1 axis, whereas, those that breakdown polymers and miscellaneous compounds influenced the spread of treatments on PC2. Microbial substrate utilization of all substrates, except carbohydrates, was significant in separating treatments on PC1 axis in 2009; however, only amide and carbohydrate metabolizing bacteria significantly influenced treatment difference along PC2 axis. Both PC1 and PC2 separated treatments in all years, but the degree of separation varied (Fig. 7). Similar treatments clustered around each other in 2007. Rye:vetch:compost and rye:vetch:no-compost treatments were widely separated from each other on PC1 axis, however, PC1 could not separate compost or no-compost treatments of rye cover crop treatment. Distinct patterns were visible in 2008, with PC1 separating compost or no-compost treatments, irrespective of cover crop treatment. There was no cover crop effect in either of the compost treatment. The spatial pattern of treatments was mixed in 2009, with rye:no-compost treatment placed far apart from all other treatments. Distribution along PC2 did not reveal distinct patterns in any of the years. Also, the percent of total variance in data set attributed to PC2 was not high and ranged from 23 to 28%.

A side-by-side comparison of principal component scores and loading variables is shown in Fig. 7. Along the PC1 axis, in 2007, compost treatments were positively correlated with two loading variables - carboxylic acids and the miscellaneous group (phosphates and ester) (Fig. 7 A-B). This pattern was consistent in 2008 where majority of compost treatments positively correlated with the miscellaneous loading variable. By the end of the fourth year of the study in 2009, all treatments except rye:no-compost, positively correlated with all loading variables. Along the PC2 axis, in 2007, soil microorganisms that utilize carbohydrates and amino acids were positively correlated with RC and negatively correlated with rye:vetch:compost treatment. On the contrary such an association could not be established in 2009. It is difficult to draw any logical inference from PC2 in 2010 as the distribution of treatments (cover crops/compost) does not follow any set pattern or trend.

## Discussion

The objective of this study was to understand changes in below ground biology brought out by two very commonly used organic amendments, cover crops and compost, under sustainable production systems. We focused on rye and a rye:vetch mixture, as it is a widely accepted cover crop system and suitable to temperate climatic regions. Compost, on the other hand, is an indispensable tool, required to maintain soil fertility and associated with desirable soil properties including increased soil organic matter, CEC, water holding capacity and soil microbial activity (Drinkwater et al., 1995). During the course of our study the amount of cover crop biomass added to the treatments varied. Rye cover crop treatment, in general, produced higher biomass that the mixture. This is primarily due to higher seeding rate in rye only cover crop treatment and sub-optimal establishment of vetch due to severe fall and spring weather conditions. Cucumber yields were significantly higher in compost amended treatments due to higher fertility and nutrient availability. Cucumber yields have been shown to respond positively to compost applications (Nair and Ngouajio, 2010; Roe et al., 1997). We had hypothesized that

rye:vetch mixture would increase cucumber yield over rye only treatment (Teasdale and Abdul-Baki, 1998), but there were no statistically significant differences.

#### Soil respiration, microbial biomass, and metabolic quotient

There was no effect of cover crop on soil respiration rates in 2007 and 2008; however, compost had a significant impact. Respiration rates were higher in soils in which compost was added, suggesting that largest activity of soil microorganisms in those soils. Other studies have also shown higher respiration rates in soils as a result of addition of manure or compost (Gunapala and Scow, 1998; Treonis et al., 2010; Tu et al., 2006). Soil microbes typically are C-limited (Smith and Paul 1990), thus, when compost is added there is a rapid increase in microbial activity and soil respiration due to increased carbon reserves. Fließbach et al. (2007) reported higher basal respiration rates in organically managed production systems when compared to unfertilized control plots. Respiration rate was highest in rye:compost treatment in 2008 which could be attributed to higher cover crop biomass added to that system (Fig. 1).

Stable and sustainable soils are defined as those with high level of biological activity and diversity, and capability to release nutrients from soil organic matter (Friedel et al., 2001, Smith and Paul, 1990). Differences in microbial biomass and activity may have implications for nutrient availability to crops. Thus soil microbial biomass can be used as an effective indicator to predict overall fertility and productivity of cropping systems (Bending et al., 2004). A number of studies have compared soil respiration, microbial biomass, and other microbial properties among contrasting management systems and reported significant differences (Bending et al., 2004; Bulluck et al., 2002; Lundquist et al., 1999). In our study, little variation and only minor differences, especially for microbial biomass, were found in 2007 and 2009, primarily due to the

overall organic framework of our study with every treatment having a cover crop component. Undoubtedly, differences would still exist, largely due to quantity and quality of plant residue incorporated into the soil. Noticeably, rye:compost treatment had significantly higher microbial biomass in 2008 which can be traced back to high amount of biomass entering that system. Increase in the quantity of organic inputs often result in high microbial biomass (Fließach and Mäder, 2000). Organic farming systems with compost applications had 34% higher microbial biomass than treatments which did not get any manure application (Fließbach et al., 2007). Our results support those findings, as we also, in general, observed lower soil respiration rates and microbial biomass in rye:vetch:no-compost treatment which corresponded with low cover crop biomass production in that system, partly due to low fertility status (no-compost). Our results indicate a positive correlation between microbial biomass and soil organic matter for all years, except 2007. With the addition of cover crops and compost more organic matter is added which facilitates rapid microbial population growth. Addition of such amendments not only adds C, but contributes towards increased calcium, potassium, and magnesium concentrations in soil (Bulluck et al., 2002). Clark et al. (1998) also reported greater concentrations of calcium, potassium, and magnesium in soils receiving cover crop and manure applications. Since the establishment of our study in 2005, cover crops and composts have been continuously applied for 5 and 4 years respectively. Regression analysis between microbial biomass and cation concentration (Ca, K, and Mg), revealed significant correlations. Microbial biomass increased with increasing soil cation concentration.

An understanding of microbial processes is important for the management of farming systems, particularly those that rely on organic inputs of nutrients (Smith and Paul, 1990). One of the factors of interest to understand microbial processes is qCO<sub>2</sub>. Metabolic quotient is an

indicator of microbial C utilization efficiency and increase in its value indicates reduced microbial efficiency. There were clear differences in  $qCO_2$  between rye:compost and rye:vetch:no-compost treatment. Higher organic matter input in rye:compost resulted in increased  $qCO_2$ . Other studies have also reported increase in  $qCO_2$  with organic matter additions (Lundquist et al., 1999). Moreover, effects of fertilizer and manure applications on  $qCO_2$  depend on soil nutrient status, with applications, reducing  $qCO_2$  under nutrient stress conditions, and vice versa (Wardle and Ghani, 1995). Metabolic quotient is often associated with stress; however, the validity of such a relationship is still questionable and needs further understanding (Wardle and Ghani, 1995).

#### Nematode community distribution

Organic amendments, including cover crops and compost, contribute to improved soil health by enhancing the activity and abundance of decomposer organisms in the soil. Such amendments have a positive impact on increasing soil microbial biomass and abundance of bacterivorous and fungivorous nematodes (Bulluck et al., 2002, Ferris et al., 1999; Gunapala and Scow, 1998). We did observe a positive effect of rye:vetch cover crop mixture on population of bacterivorous and fungivorous nematodes in 2007. Increases in the population of free-living nematodes (both bacterivorous and fungivorous) are affected by plant residue types (McSorley and Fredrick, 1999). Incorporation of sunhemp (*Crotalaria juncea*), a plant belonging to the same family as hairy vetch, has been shown to increase bacterivorous and fungivorous nematode populations in soil (Wang et al., 2001). In the rye:vetch cover crop treatment, addition of compost significantly increased the count of bacterivorous nematodes primarily due to rapid

increase in bacterial population resulting from compost application (Ferris et al., 1996). Fungal feeding nematodes have been shown to be in higher numbers when the residue incorporated had high C:N ratio (Villenave et al., 2010). In contrast, we found higher fungivorous nematode counts in rye:vetch treatment than rye alone, but this difference was not observed in subsequent years.

With lower counts of plant parasitic nematodes, clearly, the effect of rye was substantial. Rye has been shown to be a poor host to a number of key nematode pests (McSorley and Gallaher, 1992). In a study conducted on testing the effect of winter cover crops on nematode population levels, Wang et al. (2004), found that cereal cover crops, including rye, decreased numbers of a key nematode pest, *Meloidogyne incognita*, better than the leguminous cover crops during the winter season. Short-chain fatty acids along with other organic acids, found in decomposing rye residues, are found to be toxic to plant parasitic nematodes (Patrick et al., 1965). Cyclic hydroxamic acids found in the family Poaceae have also been implicated in the nematicidal properties of rye. In our study, despite the presence of rye, rye:vetch mixture could not reduce the plant parasitic nematode population to the levels obtained by using rye alone as a cover crop. The reason could be the low seeding rate of rye in rye:vetch mixture, which was half of what was used for rye alone treatment. Surprisingly, we did not see a positive effect of compost in suppressing plant parasitic nematodes. Incorporation of animal manure or compost is generally considered to increase the number of nematode antagonistic microorganisms thereby providing nematode suppression, however, a clear relationship between animal manure, antagonistic microorganisms, and nematode suppression has not been demonstrated (Oka, 2010). The abundance of omnivore and predator nematodes was fairly low in all years. Our research plots comprised of soils with higher clay content, thereby lesser pore space, which limited the

growth of omnivore and predator nematode population. Omnivore and predator nematodes are generally larger than other nematode types.

## Community level physiological profile

Residue quantity and quality, soil type, environmental conditions, and their complex interactions significantly influence soil microbial community structure (Garbeva et al., 2004). Biolog-EcoPlate<sup>TM</sup>, which contains 31 ecologically relevant simple C substrates, characterizes substrate utilization pattern of culturable microorganisms to produce CLPP (Garland and Mills, 1991). Such profiles can then be used to derive information on functional or metabolic diversity and in turn effects of factors including cover crops and compost, on soil microbial community structure. Our data shows that the functional diversity as indicated by C substrate utilization pattern was influenced by cover crops in one of the years (2007). Lower level of substrate utilization pattern by rye:vetch:no-compost treatment correlates with low microbial biomass in that system. Shannon-Weaver diversity varied from year to year. Compost applications did not increase Shannon-Weaver diversity within cover crop treatments in 2007, however in 2008 compost application produced higher diversity in rye:vetch:compost than rye:vetch:no-compost treatment. The structure and metabolic diversity of soil microorganisms has been shown to be affected by soil management practices (Bending et al., 2002). Gomez et al. (2006) reported higher microbial diversity in soils amended with two types of vermicomposts or chicken manure than a control soil (without amendment). Although, our treatments contain differences in residue quality (rye vs. rye:vetch) and presence or absence of compost, in general, our results show that after four years of cover crop/compost treatments, CLPP, based on substrate utilization, Shannon-Weaver diversity index, and Evenness index, had a converging trend. By the end of

fourth year, there was no difference in either of those indices. Similarly, Bending et al. (2002), found significant convergence in microbial community functioning during decomposition of residues of widely different quality. In soils with higher soil organic matter, in our case ranging from 3 to 4.5%, differences in CLPP between high and low quality crop residue types tend to converge as residue decomposition progresses (Bending et al. 2002).

The AWCD is a diversity index which reflects microbial density on Biolog-EcoPlate<sup>TM</sup>. In 2008, the application of compost in both cover crop treatments significantly increased AWCD. Biolog substrate utilization assay detects the copiotrophic, or the "*r*" selected bacteria which would be rapidly affected by the high level of organic carbon and nitrogen due to the addition of compost. Compost applications have been previously reported to increase microbial diversity indices possibly as a result of growth and development of diverse microorganisms (Flieβach and Mäder, 1997). The AWCD response in 2009 showed signs of treatment convergence, except for rye:vetch:no-compost, which had significantly lower AWCD. This could be attributed to the low amount of C entering that system due to reduced amount of cover erop biomass incorporated and lack of compost.

In 2007, PCA was not able to provide a clear interpretation of how microorganisms were affected by our treatments based on the spatial orientation of treatments on a scatter plot, although similar treatments did clustered around each other. Based on the separation by PC1, the only treatment which clearly separated out from others was rye:vetch:no-compost. However, in 2008, PC1 accounting for 45% of total variation, produced a greater degree of separation between compost and no-compost treatments, regardless of cover crop used (rye or rye:vetch). The PCA separation suggests that compost application has an important influence on microbial

community activity, but the influence of cover crop is less when compost is included as a soil management practice. An on-farm research revealed that annual or biennial applications of dairy manure resulted in soil microbial communities distinguishable from soil that received no manure or occasional applications (Bucher and Lanyon, 2005). In our case, we found that compost treatments on PC1 were positively correlated with two loading variables (or class of substrates on Biolog-EcoPlate<sup>TM</sup>), carbohydrates and miscellaneous. Miscellaneous class of substrates on Biolog-EcoPlate<sup>TM</sup> comprised of compounds that contain esters and phosphates. It has been established that application of dairy compost, can significantly contribute to higher levels of phosphorus in the soils. Thus repeated application of dairy compost in our study could have favored the growth of microorganisms that actively metabolize phosphorus rich compounds. Surprisingly, PC1, accounting for 55% of total variation, could not separate rye:vetch:nocompost treatment from other compost treatments in 2009. This reflects shifts in microbial community structure with similarities emerging between rye:vetch:no-compost and rye:vetch:compost treatment. This was accounted, to a certain extent by PC2, which separated rye alone treatments from rye:vetch treatments. Crop diversity can impact microbial communities as each crop influences soil microflora differently primarily due to differences in the quality and quantity of C compounds incorporated into the soil.

#### Conclusion

Overall our results demonstrate that soil management practices, such as incorporation of cover crops and compost can enhance soil biological activity. Soil biological properties such as respiration, microbial biomass, nematode population, and microbial community structure can be used an indicator of management induced changes to soil quality. For most soil biological

properties evaluated, use of rye or rye:vetch mixture did not lead to major differences, however, the use of compost significantly altered various biological parameters within cover crop treatments. Compost application increased microbial biomass and had a positive impact on soil microbial community. The results highlight higher microbial activity and diversity in soils receiving yearly compost applications. Except in 2008, effects of compost, based on CLLP patterns derived from Biolog-EcoPlate<sup>TM</sup>, were not consistent to distinguish between compost and no-compost treatments. It may be that we need some more time to see those effects as soil microbial community can be relatively robust towards short-term effects, thus mainly indicating long term effects (Flieβack et al., 2007).

## Acknowledgements

We thank Aristarque Djoko, Buck Counts, Pamela Nichol, and the support staff at Horticulture Teaching and Research Center at Michigan State University for their assistance with composting, crop planting, irrigation, and harvesting operations.

	Monthly average air temperature (°C)				Total	monthly pr	recipitation	n (mm)	Monthly average relative humidity (%)				
Month	2007	2008	2009	8 year average <sup>a</sup>	2007	2008	2009	8 year average	2007	2008	2009	8 year average	
May	16.0	13.0	14.7	14.3	97.0	29.4	108.9	104.2	61.6	61.9	62.5	72.1	
June	20.4	20.0	19.2	19.6	89.1	112.0	126.0	68.8	89.1	49.6	71.4	73.3	
July	20.8	21.7	19.3	21.4	12.4	96.0	61.0	78.1	66.6	72.6	72.7	74.5	
August	21.4	20.6	20.1	20.4	140.1	17.0	105.0	54.9	75.5	72.9	77.0	77.6	
Total	n/a	n/a	n/a	n/a	338.6	254.4	400.9	306.0	n/a	n/a	n/a	n/a	

Table 3.1. Mean monthly and long-term (8-year) air temperature, precipitation and relative humidity during tomato-cucumber growing season in 2007, 2008, and 2009 at Horticulture Teaching and Research Center, Michigan State University, Holt MI 48842.

<sup>a</sup> 8 year average from 1998-2005,

n/a = not applicable

Date, Year	Dry matter		0/0					mg kg <sup>-1</sup>						
	$(t ha^{-1})$	Ν	Р	Κ	Ca	Mg	Na	S	Fe	Zn	Mn	Cu	В	Al
8 May 2007	25.0	2.06	0.68	2.29	3.77	1.09	0.41	0.39	2666	163	310	101	31	684
9 May 2008	25.0	3.05	0.94	2.49	5.89	1.58	0.41	0.49	3860	237	424	166	45	984
20 May 2009	12.5	2.43	0.80	2.71	3.53	0.98	0.48	0.51	2220	219	299	91	34	792

Table 3.2. Rates of dairy compost and associated nutrient concentration, applied annually to compost treatments, 2007-2009.

Treatment <sup>X</sup>	Nematode counts <sup>y</sup>							
Treatment	Plant parasites	Omnivorous	Predatory	Bacterivorous	Fungivorous			
		_	2007					
RC	69 <sup>NS</sup>	$4^{NS}$	$2^{NS}$	83c	18b			
RNC	78	4	3	28c	23b			
RVC	67	5	2	456a	105a			
RVNC	67	4	2	211b	97a			
Significance								
Cover crop	0.69	0.42	0.47	* * *	*			
Compost	0.87	0.82	0.76	***	0.94			
Cover crop x Compost	0.87	0.82	0.76	***	0.78			
			2008					
RC	140b	$15^{NS}$	$10^{NS}$	45 <sup>NS</sup>	$28^{NS}$			
RNC	160b	10	8	35	47			
RVC	222a	8	5	40	30			
RVNC	255a	18	15	28	30			
Significance								
Cover crop	*	0.88	0.90	0.42	0.69			
Compost	0.69	0.68	0.43	0.13	0.27			
Cover crop x Compost	0.92	0.26	0.29	0.89	0.27			
			<u>2009</u>					
RC	170 <sup>NS</sup>	$5^{NS}$	0	$78^{NS}$	58 <sup>NS</sup>			
RNC	78	8	0	138	65			
RVC	225	10	0	273	70			
RVNC	43	13	0	172	30			
Significance								
Cover crop	0.86	0.62	-	0.29	0.80			
Compost	0.16	0.29	-	0.80	0.65			
Cover crop x Compost	0.35	1.00	-	0.38	0.52			

Table 3.3. Nematode population distribution, affected by cover crop and compost treatment, in  $100 \text{ cm}^3$  soil samples collected at the end of growing season from 2007-2009.

<sup>x</sup> RC= Rye:Compost, RNC= Rye:No-compost, RVC= Rye:Vetch:Compost, RVNC=Rye:Vetch:No-compost

<sup>y</sup> Data collected from three replications of 100 cm<sup>3</sup> soil each

<sup>z</sup> Mean separation within columns for individual years. Means followed by same letter(s) are not significantly different

<sup>NS</sup>Means are not significantly different ( $P \le 0.05$ )

\*, \*\*, \*\*\* represent significance at  $P \le 0.05$ , 0.01, 0.001, respectively.

Loading Variable	20	08	20	09	2010		
	PCA1 (33%) <sup>a</sup>	PCA2 (26%)	PCA1 (45%)	PCA2 (23%)	PCA1 (55%)	PCA2 (28%)	
Amides	0.85 <sup>b</sup>	-0.38	-0.29	-0.15	0.67 <sup>b</sup>	-0.57 <sup>b</sup>	
Amino acids	0.15	0.22	$-0.50^{b}$	-0.12	$0.89^{b}$	-0.32	
Carbohydrate	0.02	0.93 <sup>b</sup>	$0.54^{b}$	-0.32	0.32	$0.94^{b}$	
Carboxylic acids	-0.67 <sup>b</sup>	-0.53 <sup>b</sup>	-0.45	0.15	$0.88^{b}$	0.41	
Polymers	$0.85^{b}$	-0.23	-0.10	$0.74^{b}$	0.93 <sup>b</sup>	0.26	
Miscellaneous	-0.28	-0.41	0.40	0.53 <sup>b</sup>	$0.58^{b}$	-0.39	

Table 3.4. Principal component loadings (correlation between original loading variable and principal component) as a measure of influence of a loading variable on overall treatment differences

<sup>a</sup> Percent of total variance in data set, including all cover crop/compost treatments, attributed to principal component 1 (PC1) and 2 (PC2)

<sup>b</sup> Factor loadings statistically significant ( $P \le 0.05$ ). A high positive or negative correlation indicates higher degree of influence of loading variable on differences among treatments determined for a principal component.



Fig 3.1. Cover crop biomass (A) and cucumber marketable yield (B) for 2007-2009 growing season. Error bars indicate standard error (n=4). RC= Rye:Compost, RNC= Rye:No-compost, RVC= Rye:Vetch:Compost, RVC=Rye:Vetch:No-compost



Fig. 3.2. Soil respiration, microbial biomass, and metabolic quotient in soils amended with Rye:Compost (RC), Rye:No-compost (RNC), Rye:Vetch:Compost (RVC), and Rye:Vetch:No-compost (RVNC) in 2007, 2008, and 2009. Bars with different letters within a year are significantly different ( $P \le 0.05$ ).



Fig. 3.3. Regression analysis between soil microbial biomass and soil organic matter from soils collected at the end of the growing season (2007-2009).



Fig.3. 4. Correlation between soil microbial biomass and soil concentrations of calcium, magnesium and potassium. Soil samples were collected at the end of each growing season in 2007 (A), 2008 (B), and 2009 (C).



Fig 3.5. Average well color development (AWCD) obtained by Biolog-EcoPlate<sup>TM</sup> incubation of all treatments. Treatments: RC=Rye:Compost, RNC=Rye:no-compost, RVC=Rye:Vetch:compost, and RVNC=Rye:Vetch:no-compost. Error bars represent standard error.



Fig. 3.6. Bacterial richness (A), functional diversity (B) and evenness (C) indices of soils under cover crop and compost treatments based on community level physiological profile (CLPP). Treatments: RC=Rye:Compost, RNC=Rye:no-compost, RVC=Rye:Vetch:compost, and RVNC=Rye:Vetch:no-compost. Bars with different letters within a year are significantly different ( $P \le 0.05$ ). NS=Means are not significantly different.



Fig. 3.7. Loading variables of Biolog-Ecoplate<sup>TM</sup> carbon substrates (A) explaining variation of cover crop and compost treatments in 2007. Miscellaneous group of substrate comprise of phosphates and esters. PCA scatter plot of cover crop and compost treatments on PC1 and PC2 axis. Treatments included rye:compost ( $RC \bullet$ ), rye:no-compost ( $RNC \blacktriangle$ ), rye:vetch compost ( $RVC \bullet$ ), and rye:vetch:no-compost ( $RVNC \blacksquare$ )



Fig. 3.8. Loading variables of Biolog-Ecoplate<sup>TM</sup> carbon substrates (A) explaining variation of cover crop and compost treatments in 2008. Miscellaneous group of substrate comprise of phosphates and esters. PCA scatter plot of cover crop and compost treatments on PC1 and PC2 axis. Treatments included rye:compost (RC $\bullet$ ), rye:no-compost (RNC $\bullet$ ), rye:vetch compost (RVC $\bullet$ ), and rye:vetch:no-compost (RVNC $\blacksquare$ )



Fig. 3.9. Loading variables of Biolog-Ecoplate<sup>TM</sup> carbon substrates (A) explaining variation of cover crop and compost treatments in 2009. Miscellaneous group of substrate comprise of phosphates and esters. PCA scatter plot of cover crop and compost treatments on PC1 and PC2 axis. Treatments included rye:compost (RC♦), rye:no-compost (RNC▲), rye:vetch compost (RVC●), and rye:vetch:no-compost (RVNC■)

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CHAPTER IV

# IMPACT OF COVER CROPS AND ORGANIC AMENDMENTS ON MARKETABLE QUALITY AND FUNCTIONAL FOOD VALUE OF TOMATO

Impact of cover crops and organic amendments on marketable quality and functional food value of tomato

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#### Abstract

In recent years, there has been a growing demand for sustainably produced food and food products. This has lead to growers adopting strategies that make their production system ecologically sound and environmentally stable. Cover crops and organic amendments such as composts and manures have become critical components of sustainable cropping systems. Studies on effects of such inputs on soil nutrient and biological characteristics, erosion, weed suppression, plant insect and disease interactions, and crop performance are ongoing, but their impact on postharvest fruit quality and functional food qualities have not been thoroughly investigated. This three-year study investigates effects of two cover crops, rye (Secale cereale L.) and hairy vetch (Vicia villosa Roth.) with or without an organic amendment (dairy compost) on tomato postharvest marketable qualities, and health beneficial properties. Tomatoes were grown in four cropping systems, rye-compost, rye-no-compost, rye-vetch-compost, or rye-vetchno-compost. There was minimal effect of cover crops, but, compost addition significantly increased marketable fruit quality (proportion of marketable fruit and average fruit weight). Fruit quality aspects such as density, firmness, and total soluble solids did not differ among cover crop and compost treatments, although, in one of the years internal fruit firmness was significantly enhanced by rye-vetch cover crop treatment. Percentage antioxidant activity varied but was higher in tomatoes grown in rye-vetch-compost treatment. Functional food quality of the tomato extracts, with respect to the ability to inhibit cyclooxygenase (COX) enzyme activity was affected by cover crops and compost. Tomatoes grown in rye-vetch-compost treatment showed highest inhibition of COX enzyme. This interdisciplinary research showcases how sustainable production practices can influence marketable quality, and postharvest functional food properties of tomato fruit. With increasing consumer awareness and attention towards sustainable and

organic food produce, more research is needed to better understand effects of organic and sustainable production practices on food quality and health beneficial traits.

#### Introduction

Demand for organically produced fruits and vegetables with health-beneficial traits have led to a widespread interest in organic farming. The global sales of organic food and drink reached 50.9 billion US dollars in 2008 (Sahota, 2010). Sales of organic food and beverages in United States grew from 1 billion in 1990 to 24.8 billion US dollars in 2009. With this growing demand, the global market for organic food and drink was estimated to generate revenues close to 60 billion US dollars in 2010 (Organic Monitor, 2010). In view of the renewed interest in organic farming, triggered by the high demand for organic products, growers are eager to transition to organic production practices (Klonsky, 2004). To transition and to sustain their organic production enterprise, growers adopt strategies and techniques such as crop rotations, cover cropping, integrated pest management, and incorporation of organic amendments (manures and composts) to manage crop health and nutrient requirements. Organic amendments, crop rotations, and cover crops are practices that enhance soil quality, help conserve carbon and soil organic matter, and protect soil from erosion. Various cover crops and their combinations can contribute to efficient nutrient cycling, soil quality improvement, weed suppression, and improved water infiltration.

Organic systems emphasize the accumulation of soil organic matter and fertility over time through the use of cover crops, manures, and composts and rely on the activity of a diverse soil ecosystem to make nitrogen (N) and other nutrients available to plants. Rye (*Secale cereal* L.) and hairy vetch (*Vicia villosa* Roth.) are among the most common cover crops used in organic systems in regions with a temperate climate because of their winter hardiness, large
biomass production, and, in the case of hairy vetch, capacity to fix atmospheric nitrogen (Abdul-Baki et al., 1996). Along with cover crops, use of composts and manures is perceived as an important underpinning for organic production as it provides essential plant nutrients, adds soil organic matter, and improves soil structure and quality (Russo and Webber, 2007). In the recent past, large numbers of studies have reported benefits of using cover crops and composts on soil physical, chemical and biological properties, weed and disease suppression capabilities, soil conservation, and crop growth and yield (Creamer et al., 1997; Sainju et al., 2002; Snapp et al., 2005). Although a lot of agronomic advantages of using cover crops and organic amendments have been demonstrated, not much work has been done to understand their impact on produce quality. Given the significant increase in consumer interest in organic food products, there is a need to determine to what extent sustainable production practices including cover cropping and compost applications are affecting food quality. Quality parameters of fruit and vegetables are not only governed by their genetic factors but also by environmental conditions and production practices (Dumas et al., 2003). Very few studies have investigated the impact of cover crops and composts on food quality attributes such as marketable quality, average fruit weight, firmness, and total soluble solids. Since environmental conditions affect plant growth and development, it is likely that soil biology and fertility may have a significant impact on the quality of fruit and vegetables.

In recent years there has been a growing effort aimed at understanding relationships between crop management and functional food quality, especially the antioxidant activity of fruits and vegetables as these foods are the primary sources of flavonoids and other health promoting compounds (Brandt and Molgaard, 2001; Toor et al., 2006). This study focused on tomatoes because of its high per capita consumption, second only to potato (Lucier et al., 2000),

and its ability to provide antioxidant compounds such as lutein, phytoene, vitamin C, vitamin E, and lycopene (Dumas et al., 2003). Lycopene, which gives tomato the characteristic red color, comprises more than 80% of carotenoids in a fully ripe tomato fruit (Nguyen and Schwartz, 1999). Several studies have demonstrated that lycopene in tomatoes exhibits antioxidant activity, suppress cell proliferation, and interfere with the growth of cancer cells (Giovannucci et al., 2002; Levy et al., 1995). The antioxidant content of tomatoes depend on a number of factors, including genotype (cultivar), environment (temperature, light, water), and production conditions (pest and disease incidence, soil condition, nutrient availability, pesticide application, and harvesting stage) (Dumas et al., 2003; Howard et al. 2002). Fertilization regime also plays a pivotal role as carotenoid content in fruit and vegetables tends to increase with higher nitrogen fertilization (Mozafar, 1993). Researchers have also demonstrated that soil conditions, irrigation and cultivation practices significantly affect phytochemical content in crops (Lester and Eichen, 1996; Wang et al. 2002). A large number of studies investigated differences in fruit quality and nutritional value between organically and conventionally grown food crops (Bourn and Prescott, 2002; Worthington, 2001), however, more in depth study within organic cropping systems is lacking. With increasing number of growers utilizing cover crops and organic amendments in their production systems, it becomes all the more important to better understand the effects of such organic inputs on food quality and health promoting properties of food produced under such systems.

Cyclooxygenase enzymes (COX-1 and COX-2) catalyze reactions that produce compounds responsible for inflammation in the body (Laneuville et al., 1994). A complete inhibition of COX-1 is not preferred as it causes gastric ulceration and other side effects in the body. It has been determined that inhibition of COX-2, which is present in inflamed tissues,

reduces inflammation in cells with minimal side effects (Laneuville et al., 1994). Therefore, compounds that selectively inhibit the COX-2 enzyme are better anti-inflammatory products. Tomato extracts, containing lycopene, have been shown to inhibit both COX-1 and COX-2 enzyme activity (Reddy et al., 2005). Researchers have studied effects of tomato cultivars (Aldrich et al., 2010; Lenucci et al, 2006), fertilizer types (Toor et al., 2006), postharvest storage conditions (Javanmardi and Kubota, 2006) on tomato antioxidant properties but studies focusing on cover crop effects are lacking.

This 3-year study focused on quality aspects of organically produced tomatoes giving emphasis to cover crop and compost. The aim of the present study was to compare marketing quality of tomatoes grown under two cover crop systems with or without compost and then to assess whether cover crops and compost treatments affect antioxidant and anti-inflammatory properties of those tomatoes.

### Materials and methods

## Cover crops and compost

This study was conducted from 2006 to 2009 at the Horticulture Teaching and Research Center (HTRC), Michigan State University, Holt, MI. The soil was a Capac loam with 0% to 3% slope. The soil at the research plot was under transition (starting 2005) from a conventional corn/soybean rotation to an organic tomato production system. Mean monthly and long-term air temperature, precipitation, and relative humidity during the growing season at HTRC are summarized in Table 1. The experimental design was a split-plot design with four replications. The first split was the main plot with two treatments based on cover crops (rye alone or combination of rye and hairy vetch) and the second split was the subplot with two treatments (compost or no-compost). Main plot contained eight beds with 14 plants each. Each subplot contained four beds with two middle beds for data collection and one outer bed on each side serving as a guard row. In addition to guard rows, each bed had two guard plants (one plant on either end of the bed). For rye alone treatment, cover crop of cereal rye was drilled at a rate of 78 kg ha<sup>-1</sup> in the fall on 18 September, 22 September, and 26 September in 2006, 2007 and 2008, respectively. Rye and hairy vetch combination treatment was also seeded on same dates at 39 kg ha<sup>-1</sup> and 28 kg ha<sup>-1</sup> of rye and hairy vetch respectively. The following spring, dairy compost at the rate of 25 Mg ha<sup>-1</sup> in 2009 due to higher expected availability of nutrients and to avoid phosphorus build up. Each year, after the application of compost, the cover crops were mowed and later incorporated using a chisel plow.

## Planting and crop management

Non-treated tomato (*Solanum Lycopersicum* L. 'Big Beef', Seedway, Hall, New York) seeds were seeded in 98-celled flats with organic growing medium. Medium comprised of peat (Sunshine Professional Grade, Sun Gro Horticulture Ltd., British Columbia), dairy compost, and No. 2 vermiculite (Michigan Growers Products, Galesburg, Michigan) in a ratio 2:1:1 (by volume). Flats were then moved into a heated greenhouse (22 °C). Seedlings were hardened before transplanting, by moving them out of the greenhouse and placing them inside a lath house for 5 d. Seedlings were transplanted to the field on raised beds covered with black plastic mulch and drip irrigated. Each bed was 7.6 m long, 0.6 m wide and 0.2 m high with one row of cucumber or tomato. Distance between transplants within the bed was 45 cm and between beds was 167 cm (center to center).

Crops were drip irrigated as needed. Weeds near the plant were hand weeded while weeds growing in the aisles were managed by hoeing. Tomatoes were staked, tied and all cultural operations undertaken according to standard production protocol. Tobacco hornworms, the major insect pests of tomato in the region, were controlled by two applications of Dipel<sup>®</sup> (Bt formulation; Valent Biosciences Corp., California, USA). In 2007, incidence of tomato early blight (*Alternaria solani*) and septoria leaf spot (*Septoria lycopersici*) was observed late in the season and was managed using a biofungicide (Sonata<sup>®</sup>, Agraquest Inc., California, USA).

#### Fruit harvest and sampling

For the calculation of marketable quality, tomatoes were harvested five times each year from 2007-2009. Fruits were graded as marketable (U.S. #1, U.S. Combination, U.S. #2, and U.S. #3) or nonmarketable (deformed, small, cracked, or damaged by cuts, scars, disease, or insects) (USDA, 1991). The ratio of marketable to non-marketable fruit weights and numbers was calculated and used an index of marketable quality measurement. To obtain samples for fruit density, firmness, total soluble solute determination three uniformly sized and colored tomato fruits per replicate plot were randomly selected from the bulked harvest. Before harvesting for yield, to determine antioxidant and anti-inflammatory properties, three fruits were randomly harvested at the pink stage from each treatment. To avoid confounding effects that can arise due to location of the fruit on the plant, fruits were harvested from the second truss from the bottom of each plant. Fruits were stored at room temperature until they were uniformly red. Antioxidant and anti-inflammatory assays were carried out only for tomato samples collected in 2009 since three years of cover crop and compost treatments would have sufficiently conditioned the soil for cover crop and compost effects.

#### Fruit density, firmness, and total soluble solids

Three uniformly sized and colored tomato fruits per replicate treatment were used to determine fruit density. Fruit volume was estimated using the water displacement method (Jenni et al., 1997). Fruit density was calculated by dividing fruit weight by the volume of water displaced. Firmness measurements were made on three individual fruit per replicate treatment. After determination of density, fruit were wiped clean and used for firmness measurements. Equatorial slices, 1 mm thick, were cut and pressure was determined using a pressure gauge (Imada Digital Force Gauge, Imada Inc. Northbrook, Illinois). Four pressure readings were taken near the periphery and four in the center of the slice (Fig. 1). The method was adapted from puncture test studies conducted by Lana et al. (2007) on fresh cut tomato slices. Total soluble solid (Brix) is an index of soluble solids concentration in fruit. After firmness determination, fruit slices were homogenized in a blender for 10 seconds. Homogenate sample was filtered through Whatman No. 1 filter paper using vacuum and the total soluble solid of the filtrate was determined (in %) using a digital refractometer (Atago PR-32, Atago USA Inc., Kirkland, Wisconsin).

# Extract preparation

Three uniformly ripe fruit were used for preparation of water, methanol, and ethyl acetate extracts. A pooled sample (300 g) comprising of one quarter section of each fruit was obtained. The pooled sample was then blended with addition of 100 ml water and centrifuged (10,000 rpm, 10 min.). A 200 ml sample of the supernatant was lyophilized to obtain water extracts. The residue was further extracted with methanol (400 ml 3×, Sigma-Aldrich, St. Louis Missouri) followed by ethyl acetate (400 ml 3×, Mallinckrodt Chemicals, St. Louis Missouri) and the

organic extracts evaporated to dryness under reduced pressure using a rotary evaporator (Buchi Corporation, New Castle, Delaware). Dried water, methanolic, and ethyl acetate extracts were later dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich, St. Louis, Missouri) to prepare 10 mg/ml stock solutions.

### Antioxidant assay

Tomato extracts were tested for antioxidant activity by using 3-[4,5-Dimethylthiazol-2yl]-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich, St. Louis, Missouri) assay (Liu and Nair, 2010). The assay involves the reduction of MTT by test samples to produce water-insoluble purple formazan crystals which, after solubilization, can be measured spectrophotometrically. A 10  $\mu$ l extract stock solution (water, methanol, or ethyl acetate) was added to a 190  $\mu$ l of MTT (5 mg/ml strength) and 200  $\mu$ l DMSO in a glass vial (Fisher Scientific, Pittsburgh, Pennsylvania). The solution was incubated for eight hours at 37 °C. After incubation, 200  $\mu$ l of the solution was transferred into a 96-well microplate (Corning Inc., Corning, New York) and read under a microplate reader (BioTek Instruments, Winooski, Vermont) at 570 nm wavelength. Vitamin C (Sigma-Aldrich, St. Louis, Missouri), a natural antioxidant, was used as a positive control at 25 mg/l strength. Every treatment, including positive control, was run in triplicate. Entire antioxidant assay was repeated three times. Data presented is an average of all nine observations and expressed as percentage of antioxidant activity with respect to 25 mg/l vitamin C.

## Anti-inflammatory assay

The COX-1 enzyme used in the assay was prepared from ram seminal vesicles (Oxford Biomedical Research Inc., Oxford, Michigan). Cyclooxygenase-2 enzyme was prepared using an enzyme preparation of insect cell lysate in our laboratory. Both COX-1 and COX-2 enzymes

were diluted with Tris buffer (pH 7) to give a final concentration of 1.5 mg/ml protein. COX-I and COX-II enzyme inhibitory activities of tomato water, methanol, and ethyl acetate extracts were performed in a microchamber at 37 °C by monitoring the initial rate of oxygen uptake by using an oxygen electrode (Instech Laboratories, Plymouth Meetings, Pennsylvania) attached to a YSI Model 5300 Biological Oxygen Monitor (Yellow Springs Instrument, Inc. Yellow Springs, Ohio) (Jayaprakasam et al., 2006; Liu et al., 2009). Tomato extracts and positive controls were dissolved separately in DMSO. Tomato extracts were tested at 250 µg/ml concentration. The positive controls comprised of non-steroidal anti-inflammatory drugs (NSAID): Celebrex<sup>TM</sup>. Naproxen, Vioxx<sup>TM</sup>, and Ibuprofen tested at 1, 15, 1, and 12 µg/ml or 11, 26, 3.2, and 32 µM strength, respectively. Celebrex<sup>TM</sup> capsules and Vioxx<sup>TM</sup> tablets were physician's professional samples provided by Dr. Subash Gupta, Sparrow Pain Center, Sparrow Hospital, MI. Naproxen and Ibuprofen were purchased from Sigma, St. Louis, Missouri. Each assay mixture contained 0.6 ml of 0.1 M tris buffer (pH=7.0), 1 mM phenol, 85 µg haemoglobin, and 10 µl DMSO or tomato extract (water, methanol, or ethyl acetate). The assay mixture and COX-1 or COX-2 enzyme (20 µl) were injected into the microchamber, and the mixture was allowed to incubate for 2 min. This was followed by the addition of 10 µl of arachidonic acid (1 mg/ml) to initiate the reaction. Data were recorded using Quick-Log for windows data acquisition and control software (Strawberry Tree, Inc., Sunnyvale, CA). Each tomato extract sample was tested for its COX activity in duplicate. Separation of compounds in the ethyl acetate extracts was also conducted using Thin Layer Chromatography (TLC) with silica gel as the stationary phase and a mixture of hexane, ether and chloroform (2:1:1 by volume) as the liquid phase (moving phase).

## Statistical analysis

Analysis of variance was performed using the PROC MIXED procedure of SAS (Statistical Analysis Systems Institute Inc., Version 9.2; Cary NC). Significant differences between treatment means were separated by 'Ismeans' and 'pdiff' statement in SAS ( $P \le 0.05$  level).

# **Results and discussions**

#### *Marketable quality*

Cover crop and compost significantly influenced both marketable and non-marketable fruit yield (fruit number and weight). As yield is not the focus of this manuscript and is outside the scope of this journal, we evaluated marketable quality with respect to the proportion of marketable fruit count and weight as a way to measure the impact of cover crop and compost on commercial yield quality. There were no significant differences in the proportion of marketable fruit numbers and weights between treatments in 2007 and 2008 (Table 2). In 2007, rye-compost treatment increased the proportion of marketable fruit (count and weight) but rye-vetch-compost treatment performed better in 2008. In, 2009 differences in proportion of marketable fruit counts were discrete and more a function of compost than cover crop. Irrespective of cover crop, treatments which received compost had higher proportion of marketable fruit counts. Addition of compost provides essential nutrients for plant growth and has been shown to increase crop yield and productivity (Roe et al., 1997). Positive effects of the compost used in this study have been previously demonstrated in an organic cucumber cropping system (Nair and Ngouajio, 2010).

The cover crop combination of rye-vetch did not produce expected results under nocompost treatment, however, under the compost treatment; the combination increased the

proportion of marketable fruit numbers. Proportion of marketable fruit weight was highest for the rye-vetch-compost treatment. Use of rye and hairy vetch cover crop mixes have been reported to enhance total marketable yield and average fruit weight in tomato (Abdul-Baki et al., 1996).

Based on the intended end use, fruit weight can be a component of fruit quality that affects the market value of the product (U.S. Dept. of Agriculture, 1994). The heavier the fruit, the more likely it adds value to the marketing value of the product. Average fruit weight was not significantly different among treatments in 2007, but in 2008 and 2009 differences were observed. In 2008, although cover crops did not have any significant effect, application of compost influenced average fruit weight under both cover crop treatments. Compost treatments produced larger fruits as compared to no-compost treatments. Addition of compost not only provides essential nutrients for plant and soil microorganisms, but increases crop productivity and fruit quality. Consistently compost treatments produced higher average fruit weights in 2009. Cover crop treatment was also significant with differences between no-compost treatments of rye and rye-vetch. Rye-vetch-no-compost treatment produced fruits with smallest average fruit weights, thereby lowering fruit quality.

One of the major objectives of incorporating vetch in a cropping system is its ability to fix atmospheric N; however, use of vetch with rye without any additional nutrient source, as the case in this study, is not enough to produce a good quality crop. Use of vetch alone as a cover crop has been shown to improve tomato production (Campiglia et al., 2010; Sainju et al., 2002), however, its use in combination with other cover crops, needs further investigation. Another important aspect is the cover crop growing condition, which is influenced by climate and geography (Abdul-Baki et al., 1996). Cover crop performance and its effect on successive crop can greatly vary depending upon length of the growing season, weather conditions, seeding rate,

soil conditions, and pest and disease interactions. Combinations and mixes that work in one geographical location might not work for others.

### Fruit density, firmness, and total soluble solids

Fruit density was not significantly different among treatments in all three seasons. Neither the cover crop nor compost treatment produced any significant effect on fruit density (Table 3). Similar results were observed for fruit firmness except in 2008, when fruit firmness in the center of the fruit differed among treatments. Rye-vetch-no-compost treatment produced fruit with greater degree of firmness as compared to rye cover crop treatments. However, there was no difference between cover crop treatments when compost was used. Importance of indices such as fruit firmness has major implications on post-harvest handling of fruits. A number of factors such as shipping, storage, processing, and retail value are directly linked to fruit firmness and other quality attributes. In this study, in general, fruit firmness did not vary much between cover crop and compost amendment treatments. Other studies conducted to compare tomato fruit quality between organic and conventional production system have reported similar results (Riahi et al., 2009).

Total soluble solids ranged from 4.5% to 4.7%, 4.6% to 4.9%, and 4.7% to 5.5% in 2007, 2008, and 2009 respectively; however, there were no differences among treatments (Table 3). Studies have reported increase in tomato TSS values with the use of compost and other organic amendments (Barrett et al., 2007; Gutierrez-Miceli et al., 2007). In contrast, Toor et al. (2006) did not find any difference in TSS values when tomato plants were grown in three synthetic and two organic fertilizer treatments. Effect of nutrition management on crop quality can vary substantially among regions based on climatic and growing conditions, soil characteristics, and production practices. In our study use of cover crops and compost did impact fruit marketability

characteristics but its impact on intrinsic fruit qualities such as density, firmness, and total soluble solute concentration was not prominent. Further studies are needed to evaluate fruit quality parameters such as firmness and TSS as these indices typically exhibit large variations between individual pieces, and even within the different tissues in the same individual piece (Lesage and Destain 1996).

## Antioxidant activity

Tomatoes contain a number of antioxidant compounds such as vitamin C, phenolics, and carotenoids (Lenucci et al., 2006). Compounds present in tomato show strong antioxidant properties as they terminate the oxidation chain reactions by removing free radical intermediates and inhibit other oxidation reactions by oxidizing themselves. There are number of assays, such as 2, 2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), the ferric reducing antioxidant power (FRAP), and the oxygen radical absorption capacity (ORAC) assays that have been used frequently to determine antioxidant capacity of variety of compounds. However, these assays have limitations due to overall cost and issues due to solubility of test compounds (Liu and Nair, 2010). We used MTT method as it is cost effective and has been successfully used in the evaluation of plant extracts (Muraina et al., 2009). Further, Liu et al. (2010) validated the use of MTT assay by comparing antioxidant activity (from MTT assay) of a wide variety of natural product extracts and pure compounds with lipid peroxidation inhibitory (LPO) activity of those extracts and compounds.

In this study, vitamin C, a natural antioxidant tested at 20 mg/l concentration, was used as a check to ensure proper functioning of MTT assay and the antioxidant activity of extracts was expressed as a percentage of vitamin C antioxidant activity. Hydrophilic (water) extracts showed lower antioxidant activity as compared to methanolic and ethyl acetate extracts. The antioxidant

activity in water extracts, although low, is due to a mixture of compounds such as sugars and polysacchaides, ascorbic acid, proteins, and glycosides of phenolics including flavanoids. Highest antioxidant activity was showed by rye-vetch-no-compost treatment while there were no significant differences among other treatments (Fig. 2). Since formation of phenolic substances, for example kaempferol and quercetin in tomatoes, need light, it is not surprising that these substances are found mainly in the skins of fruit (Dumas et al., 2003). In this context, the amount of light received by fruits could significantly influence the accumulation of these phenolics. In our study, plants growing under rye-vetch-no-compost treatment had reduced plant foliage, primarily due to inadequate nutrition, which would have led to higher sunlight interception by fruits. This could have increased the concentration of phenolics and thereby antioxidant activity of water extracts of fruits grown under rye-vetch-no-compost treatment.

In the case of methanolic extracts, cover crop treatment was significant with rye-vetch treatment showing higher antioxidant activity when compared to rye alone. Addition of compost did not affect antioxidant activity in rye-vetch treatments. Within the rye treatment, fruits harvested from rye-no-compost treatment showed higher antioxidant activity. Carotenoids and vitamin E are the major antioxidants present in ethyl acetate extract of tomato. At fully ripe stage, lycopene constitutes of more than 80% of the total tomato carotenoids, and gives the fruit its characteristic color (Nguyen and Schwartz, 1999). Among the three extracts, ethyl acetate extract was deep red in color, methanolic extract light yellow and water extract was almost colorless with a pinkish tinge. The ethyl acetate extracts of rye-vetch-compost treatment exhibited the highest antioxidant activity which could be due to higher lycopene content in the fruit grown in that system. Meanwhile, it is also important to acknowledge that high lycopene concentration does not always correlate with high antioxidant activity (Cox et al., 2003). Higher

lycopene content in fruit grown in rye-vetch-compost system can be explained by two factors, first, optimal conditions for plant growth as a result of higher nutrient content in soil brought out by N fixation by vetch and the addition of compost. Fertilization regime plays a critical role in over all plant development. It has also been shown that carotenoid content in fruit and vegetables tend to increase with higher nitrogen fertilization (Mozafar, 1993). Secondly, the growing conditions, in which, fruit in the rye-vetch-compost treatment were growing under dense foliage protected against direct sun exposure which can otherwise inhibit lycopene production (Leoni, 1992). Direct sunlight may favor the accumulation of phenolics in the fruit, but lycopene may develop more readily in fruit protected by crop foliage (Dumas et al., 2003). The positive effects of adding compost were not observed in rye alone treatment where both compost and no-compost treatments showed similar antioxidant activity.

This study confirms the antioxidant potential of tomatoes like many other studies (Cox et al., 2003; Javanmardi and Kubota, 2006; Lenucci et al., 2006) but also demonstrates the impact of cover crops and compost on antioxidant potential of tomato fruits. Consumption of tomatoes can make a significant contribution to the daily requirements of compounds in the human diet that have antioxidant properties such as carotenoids (particularly lycopene), phenolics, and vitamin C (Lenucci et al., 2006). In this study antioxidant activity exhibited by individual extracts is not solely due to a particular compound; in fact it is plausible that antioxidant activity depends upon synergistic effects among all antioxidant compounds and their interaction with other constituents in the extract. Cover crops and organic amendment applications can modify crop growing conditions and thereby influence antioxidant properties of the final produce.

#### Inhibition of Cyclooxygenase enzymes

Cyclooxygenase enzymes (COX-1 and COX-2) play a significant role in mediating pathways that lead to inflammation in the body. Studies have shown that its selective inhibition is correlated with delayed onset or reduced progression of diseases (Lipsky, 1999). There are a variety of fruit and vegetables such as apples, beets, berries, carrots, cherries, lettuce, etc. that possess compounds that inhibit COX enzyme activity (Mulabagal et al., 2007, 2010; Reddy et al., 2005; Seeram et al., 2001). Tomatoes have also shown to be effective in inhibiting COX enzyme activity, as they contain the carotenoid pigment lycopene (Reddy et al., 2005). In this study percentage COX inhibition varied among the three tomato extracts. Degree of inhibition was higher in ethyl acetate extracts followed by methanolic extracts and the lowest in water extracts. The COX-1 enzyme inhibitory activity for water extract was very low ranging from 0%to 2% (Fig. 3A). Such levels of inhibitions are insignificant from a practical standpoint. Methanolic extracts had slightly higher inhibition rates ranging from a mean of 2% to 18% but statistically there were no differences among treatments (Fig. 3B). Similarly, means of ethyl acetate extracts did not differ statistically (Fig. 3C). We observe an increase in COX-1 inhibition rate from water to ethyl acetate extracts primarily due to change in extract composition. Tomato water extracts largely contain sugars and polysaccharides, and water soluble flavanoids where as ethyl acetate extracts contain vitamin E and carotenoids, particularly lycopene, which contributes towards greater degree of COX-1 inhibition. Reddy et al. (2005) demonstrated that pure lycopene suppressed the activity of COX-1 enzyme by 69%. In our study the highest rate of COX-1 inhibition obtained was 32% (by rye-compost treatment). This difference is because we used crude tomato extract, a mixture of a various other compounds along with lycopene, and not

the pure compound. As mentioned earlier, activity of an extract is not solely governed by a single compound, but by a number of compounds and their interaction with each other.

Treatment means of COX-2 inhibition of water extracts were statistically non-significant. Percentage inhibition of COX-2 ranged from 2% (rye-vetch-compost) to 9.5% (rye-compost) (Fig. 3A). In methanolic extracts, inhibition of COX-2 enzyme differed among treatments. The highest inhibition was exhibited by rye-vetch-compost (31%) and the lowest by rye-vetch-nocompost (0%) (Fig. 3B). In both cover crop treatments, rye and rye-vetch, addition of compost enhanced COX-2 inhibition capacity. In addition, a significant difference between rye-compost and rye-vetch-compost indicates a positive effect of vetch on COX-2 inhibition. Surprisingly, no COX-2 inhibitiory activity was observed for rye-vetch-no-compost treatment. The effect of cover crop and compost treatment was more pronounced in ethyl acetate extracts. Rye-vetch-compost treatment was able to inhibit COX-2 enzyme activity by almost 40% (Fig. 3C). This is comparable to the COX-2 inhibition levels of Ibuprofen, a non-steroidal anti-inflammatory drug (Fig. 3D). Similar to methanolic extract, an increase in COX-2 enzyme inhibition was observed, with the addition of compost, in both cover crop treatments.

Comparison between rye-compost and rye-vetch-compost or rye-no-compost and ryevetch-no-compost demonstrates how the addition of vetch cover crop increases the COX-2 inhibitiory activity of the ethyl acetate extract. This means that vetch as a cover crop is improving tomato fruit quality by promoting biosynthesis of compounds that are capable of inhibiting COX enzyme activity, especially COX-2. Our results are supported by a recent finding that hairy vetch mulch activates genes that control production of phytochemicals in tomatoes (Neelam et al., 2008). The researchers found that non-transgenic tomatoes, grown on raised beds with vetch residue, were able to trigger signal pathways that were similar to those regulated by

higher polyamines in a transgenic line. This transgenic line (developed following transformation with yeast *S*-adenosylmethionine decarboxylase gene, *Spe2*, fused to a ripening-specific E8 promoter) accumulates higher polyamines and is richer in juice and nutritional quality. The study further reported higher concentrations of amino acid aspargine, glutamine, isoleucine, phenylalanine, threonine, and valine in tomatoes grown on hairy vetch mulch as compared to those grown on black plastic mulch. Two unidentified compounds, compound A (NMR spectral pattern identical to that of citrate) and compound B (an unidentified multiplet) were significantly higher in pink and red stages of fruits grown on hairy vetch mulch as compared to black plastic mulch. In our study examination of TLC plates of ethyl acetate extracts did not point towards presence of any new component in the rye-vetch ethyl acetate mixture, but the concentration of some compounds was enhanced in the rye-vetch extracts (Fig. 4).

Environmental cues play an important role on gene expression and significantly influence the production of plant metabolites. The study conducted by Neelam et al. (2008), validates the fact that vetch cover crop can activate tomato genes revealing environment-dependent changes in tomato fruit metabolism. Similarly, higher inhibition rates of COX-2 enzymes by ethyl acetate extracts of tomatoes grown on rye-vetch cover crop corroborate the fact that vetch as a cover crop is influencing tomato phytonutrients and fruit quality. There are number of reports that support changes in specific metabolic pathways and activation of genes in tomato plants in response to vetch mulch (Kumar et al., 2004; Mattoo and Abdul-Baki, 2006).

## Conclusion

Fruit quality encompasses many aspects and includes not only flavor, color, and shape, but also postharvest aspects of firmness, TSS content, titratable acidity, and nutritional quality.

Complex interaction of environmental effects, growing conditions and method, and cultivar choices exist which influence crop performance, marketable quality, fruit composition, carpometric characteristics, and antioxidant properties. Thus production systems that adopt sustainable practices such as cover cropping, manure and compost application may significantly influence final produce quality and nutritional value. Results from this study show that cover crops (rye and vetch) and compost directly affect marketable fruit quality. Although fruit carpometric characteristics such as density, firmness, and TSS were altered to a lesser extent, antioxidant and anti-inflammatory properties were affected by cover crop and compost treatments. In the wake of a steady growing demand for sustainably grown food and food products, a large number of growers are transitioning and adopting sustainable production practices. A better understanding on how such production practices are impacting our food quality is fundamental to producing high-quality and nutritionally rich food. Cover crops and compost have been primarily used for nutrient management, weed suppression, erosion control, N scavenging, organic matter addition, pest suppression, and stimulation of soil microorganisms. Their use as a tool for enhancing food quality and nutritional value could benefit a large and growing community of local, sustainable, and organic growers who target consumers seeking high quality nutritious produce.

## Acknowledgements

The authors thank Aristarque Djoko, Bill Chase, Buck Counts, Gary Winchell, Jon Dahl, Pamela Nichol, Zack Hayden, and staff of Horticulture Teaching and Research Center at Michigan State University, who contributed to research efforts for this project. Financial support for the project has been provided partially by USDA organic grant No. 2005-51300-02391.

Month	Monthly average air temperature (°C)				Total monthly precipitation (mm)				Monthly average relative humidity (%)			
	2007	2008	2009	8 year average <sup>z</sup>	2007	2008	2009	8 year average	2007	2008	2009	8 year average
May	16.0	13.0	14.7	14.3	97.0	29.4	108.9	104.2	61.6	61.9	62.5	72.1
June	20.4	20.0	19.2	19.6	89.1	112.0	126.0	68.8	89.1	49.6	71.4	73.3
July	20.8	21.7	19.3	21.4	12.4	96.0	61.0	78.1	66.6	72.6	72.7	74.5
August	21.4	20.6	20.1	20.4	140.1	17.0	105.0	54.9	75.5	72.9	77.0	77.6
Total	n/a	n/a	n/a	n/a	338.6	254.4	400.9	306.0	n/a	n/a	n/a	n/a

Table 4.1. Mean monthly and long-term (8-year) air temperature, precipitation, and relative humidity during tomato-cucumber growing season in 2007, 2008, and 2009 at Horticulture Teaching and Research Center, Michigan State University, Holt MI 48842.

Z 8 year average from 1998-2005

n/a = not applicable

	Marketable quality <sup>x</sup>					
Ireatments	Proportion of market	Average fruit				
	Count	Weight	weight (kg)			
		2007				
Rye-Compost	$78.0^{NS}$	76.9 <sup>NS</sup>	$0.18^{NS}$			
Rye-No-compost	71.2	69.9	0.18			
Rye-Vetch-Compost	76.3	76.0	0.17			
Rye-Vetch-No-compost	75.4	74.3	0.18			
Rye-Compost	80.6 <sup>NS</sup>	81.2 <sup>NS</sup>	0.24a			
Rye-No-compost	82.6	83.8	0.19b			
Rye-Vetch-Compost	84.0	84.8	0.25a			
Rye-Vetch-No-compost	78.4	80.1	0.18b			
		2009				
Rye-Compost	78.6a <sup>y</sup>	77.5ab	0.17a			
Rye-No-compost	71.0b	70.6ab	0.15b			
Rye-Vetch-Compost	83.7a	83.0a	0.16a			
Rye-Vetch-No-compost	66.5b	57.0b	0.11c			

Table 4.2. Tomato marketable quality response as affected by cover crop and compost treatment from 2007-2009.

<sup>x</sup> Data reported from 12 plants per treatment replication

<sup>y</sup> Mean separation within columns for individual years. Means followed by same letter(s) are not significantly different ( $P \le 0.05$ ) NS Means are not significantly different ( $P \le 0.05$ )

Treatment	Density	Firmness (	Total soluble solids				
Treatment	(g/ml)	Inside	Outside	(Brix %)			
	2007						
Rye-Compost	$0.98^{ m NS}$	X	$0.17^{NS}$	4.5 <sup>NS</sup>			
Rye-No-compost	0.99	-	0.22	4.6			
Rye-Vetch-Compost	0.99	-	0.20	4.7			
Rye-Vetch-No-compost	1.00	-	0.20	4.6			
	2008						
Rye-Compost	0.99 <sup>NS</sup>	0.27bc <sup>y</sup>	0.21 <sup>NS</sup>	4.6 <sup>NS</sup>			
Rye-No-compost	1.0	0.25c	0.22	4.9			
Rye-Vetch-Compost	0.99	0.39ab	0.22	4.4			
Rye-Vetch-No-compost	0.99	0.45a	0.22	4.9			
	2009						
Rye-Compost	0.99 <sup>NS</sup>	0.59 <sup>NS</sup>	0.34 <sup>NS</sup>	5.5 <sup>NS</sup>			
Rye-No-compost	0.98	0.33	0.32	4.7			
Rye-Vetch-Compost	0.99	0.38	0.34	5.5			
Rye-Vetch-No-compost	0.98	0.39	0.42	5.1			

Table 4.3. Effect of cover crop and compost treatment on tomato fruit density, firmness, and total soluble solute concentration, 2007-2009.

<sup>x</sup> Not determined

<sup>y</sup> Mean separation within columns for individual years. Means followed by same letter(s) are not significantly different ( $P \le 0.05$ ) NS Means are not significantly different ( $P \le 0.05$ )



Fig. 4.1. Measurement of firmness in tomato slices using digital pressure gauge. Circles showing points, where the tissue was punctured to obtain firmness measurements (four readings taken on the rim and four in the center of the slice).



Fig. 4.2. Effect of cover crop and compost on antioxidant activity of tomato water, methanolic, and ethyl acetate extracts. Antioxidant activity of extracts expressed as percentage antioxidant activity of 20 mg/l concentration of vitamin C. Samples were tested at 250 mg/l strength. RC=Rye-compost, RNC=Rye-No-compost, RVC=Rye-Vetch-compost, RVNC=Rye-Vetch-no-compost. Lower case letters represent mean separation for individual extracts. Means followed by same letter are not significantly different ( $P \le 0.05$ ).



Fig. 4.3. Effect of cover crop and compost on COX-I and COX-II enzyme inhibitory activity of tomato water (A), methanol (B) and ethyl acetate extracts (C). Samples were tested at 250 mg/l strength. Commercial NSAIDs Ibuprofen, Naproxen, Celebrex, and Vioxx were used as positive control and tested at 12, 15, 1, and 1 µg/ml concentrations, respectively. Vertical bars represent the standard deviation of each data point (n = 2). RC=Rye-compost, RNC=Rye-No-compost, RVC=Rye-Vetch-compost, RVNC=Rye-Vetch-no-compost. Lower case letters represent mean separation for cover crop and compost treatments for individual enzyme. Means with same letter(s) are not significantly different ( $P \le 0.05$ ).



Fig. 4.4. Thin Layer chromatography plate of ethyl acetate extracts of tomatoes grown under cover crop and compost treatments. 1=Rye-compost, 2=Rye-no-compost, 3=Rye-vetch-compost, 4=Rye-vetch-no-compost, and 5=conventionally grown tomato. Conventionally grown tomatoes were not part of the current study.

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## LITERATURE CITED

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CHAPTER V

INTEGRATING ROW COVERS AND SOIL AMENDMENTS FOR ORGANIC CUCUMBER PRODUCTION: IMPLICATIONS ON CROP GROWTH, YIELD, AND MICROCLIMATE Integrating row covers and soil amendments for organic cucumber production: Implications on crop growth, yield, and microclimate

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This project was funded by a USDA grant No. 2005-51300-02391. The use of company or product name in this publication does not imply any kind of endorsement. This paper is part of a dissertation by the senior author in partial fulfillment of the requirements for the Ph.D. degree. We would like to thank Dr. Renate Snider for a critical review of an early version of this manuscript.

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Manuscript published in HORTSCIENCE 45(4):1-9. 2010.

## Abstract

The area of organic production has registered a steady increase over past recent years. Transitioning to organic production is not straight forward and often includes a steep learning curve. Organic growers have to develop strategies to best manage nutrients, pests, and crop growth and yield. Additionally, in regions with temperate climate like the Great Lakes region, weather (especially temperature and solar radiation) plays an important role in crop productivity. Growers routinely use compost for nutrient provisioning and row covers for insect exclusion and growth enhancement. The objective of this work was to study the combined effect of row covers (with different light transmission) and compost organic cucumber (Cucumis sativus L.) growth and microclimate. Plots were assigned to three row cover treatments (60% light transmission, 85% light transmission and uncovered) and two amendment treatments (compost and nocompost) in split plot factorial design. Data were collected for ambient air and soil temperature, photosynthetically active radiation (PAR), relative humidity, plant growth characteristics and yield. Row covers modified crop microclimate by increasing air and soil temperature and decreasing PAR. There was a marked increase in the growing degree day accumulations under row covers when compared to uncovered treatment. The impact of row covers on plant growth was significant. Use of row covers increased vine length, flower count, leaf area, leaf count, plant biomass and total marketable yield. Use of compost in conjunction with row covers enhanced the row cover effect. With the use of compost, there were not many significant differences in plant growth characteristics between row cover materials, however, as expected, row cover with 60% transmission was able to trap more heat and reduce light transmission when compared to row cover with 85% transmission. This study clearly shows the importance of organic amendments especially compost in organic vegetable production. Applications of

compost enhanced crop growth and also lead to higher marketable yields. Results of this study suggest additive effects of row cover and compost application on organic cucumber production.

### Introduction

For more than a decade, organic agriculture has gained both popularity and attention among consumers and policy makers. Organic agriculture is the fastest growing agricultural sector in the United States with certified organic land present in all 50 states (Dimitri and Greene, 2002). Globally, there has been a constant demand for organically produced food and an increasing tendency to shift towards environmentally sound production practices (Dimitri and Greene, 2002). Growers have shown a keen interest to transition from conventional to organic crop production practices (Giles, 2004). In the United States, organic food sales grew 15.8% in 2008 (OTA, 2009). Croplands under certified organic vegetable acreages have increased from 48,227 acres in 1997 to 98,525 acres in 2007, which is more than a 100 folds increase (USDA-ERS, 2005). Vegetable production without the use of synthetic fertilizers and pesticides could be challenging and requires implementation of new techniques and production practices. Transitioning to organic production often involves adjustments, technical know-how, and tools to better manage issues pertaining to soil fertility, weed, and pest populations (Dabbert and Madden, 1986). However, once a proper balance is established, organic production minimizes the use of external inputs, improves soil quality, and aims at economic viability with no or minimal impact on the environment.

According to the United States National Agricultural Statistics Service, Michigan ranks third in fresh market cucumber (*Cucumis sativus* L.) production after Florida and Georgia (USDA-NASS, 2008). Value of fresh market cucumber has been estimated at \$14 and \$242

million for Michigan and United states, respectively (USDA-NASS, 2008). In the recent past, growers have indicated strong interest in transitioning to organic production methods and practices. One of the biggest challenges associated with organic cucumber production is the striped cucumber beetle (*Acalymma vittatum* F.) (Hoffman, 1998; Diver and Hinman, 2008). Cucumber beetle is an important pest of cucurbit crops that not only causes feeding damage on plant leaves, blossoms and fruits but also transmits bacterial wilt and can increase the incidence of powdery mildew and fusarium wilt (Diver and Hinman, 2008). Additionally, the variable climate and narrow seasonal window for growing vegetables in regions with a temperate climate, like Michigan, demands innovative crop management tools and efficient insect management strategies (Snapp et al., 2005). Unpredictable climatic conditions in the Great Lakes region, such as high rainfall, low temperatures, and humid conditions early in the growing season delay planting and facilitate early and rapid infestation of pest and diseases.

The role of row covers as an effective pest management tool has been increasing because they serve as a barrier against various insect pests including aphids, cucumber beetles, whiteflies, and pathogens these insects transmit (Bextine et al., 2001; Boisclair and Bernard, 2006; Natwick and Laemmlen, 1993). In addition to insect exclusion, one of the most critical effects of row cover on plants is the modification of environmental factors such as light, humidity, soil and air temperature and air movement (Wells and Loy, 1985). All these factors directly impact plant growth and development; however, the most important one is temperature as it is the key component driving the environment's energy status (Lombard and Richardson, 1979). Row covers have been reported to significantly alter air temperature thereby affecting plant growth through changes in leaf characteristics, biomass accumulation and relative growth rate (Soltani et al., 1995). In regions with cooler temperatures and relatively cloudy days, like the Great Lakes,

light transmission of row covers could affect crop growth. Few studies have addressed the impact of light transmission on row cover performance under limiting sunlight conditions. Row cover materials create a specific microclimate around the plant. Understanding the microclimate and its impact on plant growth and morphology is critical for making good use of row cover technology. Our study focuses on the use of spun-bond polypropylene row covers in organic cucumber production and its effects on plant microclimate, growth, and yield. Spun-bond polypropylene row covers are being widely used for vegetable production in various regions of the United States (Lamont, 2005) but their performance and efficacy to suit the agro-climatic conditions in the Midwest need to be further investigated. Not many studies have documented the effect of spun- bond polypropylene row covers on organic production especially under a temperate climate. It has been a challenge for organic growers to identify geographically appropriate and crop specific practices for efficient crop management (Zehnder et al., 2007). Moreover, our study gains further relevance as organic cucumber production, by itself, has not been adequately investigated in our region.

Apart from the use of row covers, research is needed in areas like soil fertility and nutrient management to better understand crop management practices for organic cucumber production in the Midwest. For nutrient management, organic production systems rely heavily on inputs like composts and other organic amendments to build soil organic matter and meet crop nutrient demand (Russo and Webber, 2007). These inputs have a direct impact on plant growth, soil fertility, quality and health. Soil health is critical as it supports microbial communities that perform essential ecosystem services like nutrient cycling, pathogen suppression, and stabilization of soil aggregates (Carrera et al., 2007). Much work has been done on compost for nutrient management under organic systems, but the use of compost in conjunction with row
covers has not been studied in detail. Possible interactions may exist between soil nutrient status and crop performance under row covers. Our objective, thus, is to i) evaluate the impact of row covers with different light transmission levels on cucumber growth and yield, and ii) test the effect of compost in conjunction with row cover treatments on vegetative and reproductive yields of cucumber.

#### **Materials and Methods**

This study was conducted from 2007 to 2009 at the Horticulture Teaching and Research Center (HTRC) at Michigan State University, Holt, MI. The soil was a Capac loam with 0% to 3% slope. Capac loam is somewhat poorly drained, moderately to moderately slowly permeable soil. The soil at the research plot was under transition (starting in 2006) from a conventional corn/soybean rotation to an organic production system. Table 1 summarizes the mean monthly and long-term air temperature, precipitation and relative humidity during cucumber growing season at HTRC. Like most of the organic growers in the region, a cover crop of cereal rye (*Secale cereale* L.) was drilled at the rate of 78 kg·ha<sup>-1</sup> on 22 Sept. and 26 Sept. in 2007 and 2008, respectively. Dairy compost was applied to the compost treatments at the rate of 25 t·ha<sup>-1</sup> on 8 May 2007 and 20 May 2008. In 2008 compost application was delayed due to excessive rains and persistent water logged conditions in the field. In both years, following the application of compost, the rye cover crop was mowed and incorporated using a chisel plow. The movement of the plow was closely monitored to minimize compost carryover to no-compost treatment plots.

Non-treated cucumber seeds (*Cucumis sativus* L. 'Dasher-II'; Seedway, Hall, NY) were seeded into an organic medium, comprised of peat (Sunshine<sup>®</sup> Professional Grade, Sun Gro

Horticulture Ltd., British Columbia, Canada), dairy compost and No. 2 vermiculite (Michigan Growers Products, Galesburg, MI) in the ratio 2:1:1 (by volume) on 30 May and 28 May in 2008 and 2009 respectively, and the flats were placed in a heated greenhouse (22 °C). In order to harden the seedlings before transplanting, they were moved out of the greenhouse and placed inside a lath house for 5 d. Seedlings were transplanted to the field on raised beds covered with black plastic mulch and drip irrigated on 11 June 2008 and 16 June 2009. Each bed was 7.6 m long, 0.6 m wide and 0.2 m high with one cucumber row. Transplants were spaced 45 cm inside the rows, with beds spaced 167 cm from each other (center to center). The experimental design was a split plot design with four replications. Main plot treatments were compost or no-compost treatments. Two row cover treatments and one un-covered control formed the subplots. Row cover treatments consisted of a 60% light transmission spun-bond row cover (RC60; Gro-Guard<sup>®</sup>, Gintec Shade Technologies Inc., Canada), and an 85% light transmission spun-bond row cover (RC85) treatment. Each subplot contained three rows of 14 plants, with the data row in the middle and outer two rows serving as guard rows. In addition to guard rows there were guard plants in each row (one plant on either end of a row). Row covers were installed on appropriate treatment rows 7 d after transplanting, using a galvanized iron hoops, and removed after 3 weeks. Row cover edges and ends were immediately secured with soil after installation. Temperature sensors (WatchDog<sup>®</sup>, Spectrum Technologies, Illinois) and quantum light sensors (PAR Light Sensor, Spectrum Technologies, Illinois) were installed one per treatment, both inside and outside the row covers, to record ambient temperature and PAR. In 2009, temperature sensors were also placed underneath the black plastic mulch at a depth of 2.5 cm. Additionally,

relative humidity sensors (WatchDog<sup>®</sup>, Spectrum Technologies, Illinois) were also installed in 2009.

Row covers were removed on 10 July in 2008 and 17 July in 2009 to facilitate pollination. Soon after the removal of row covers, data were collected on vine length, flower count and leaf chlorophyll content (Minolta SPAD-502 Leaf Chlorophyll Meter, Japan). Vine length was measured from the base of each plant to the growing point of a main vine. Chlorophyll measurements were made on the recently fully expanded leaf and 10 readings were averaged per experimental unit. Vine length and flower count were recorded for 12 plants and averaged. In addition, in 2009, two plants from each treatment were harvested and used for leaf count; leaf area and vine dry weight measurements. Leaf area was measured using LI-3100 Area Meter (Li-Cor Inc., Lincoln, NE). Vines and leaves were subsequently dried at 38 °C for 3 d and weighed. Soon after detecting their presence, in order to control cucumber beetles, Pyganic<sup>®</sup> (McLaughlin Gormley King Company, Minneapolis, MN) was sprayed to uncovered plants and later to the row covered plants once every four days until harvest. Downy mildew was detected later during the season in 2009. Sonata<sup>®</sup> (AgraQuest Inc., Davis, CA) was sprayed once a week to control the spread of downy mildew pathogen. Cucumbers were picked 7 times in 2008 and 6 times in 2009 with an interval of 3 d between harvests. Fruits were graded as marketable (U.S. Fancy, U.S. Extra #1, U.S. #1, U.S. #1 Small, and U.S. #1 Large) or non-marketable (deformed, overgrown, damaged by cuts, scars, sunscald, sunburn, dirt, disease, or insects) grades (USDA, 1958). All data were subjected to analysis of variance (PROC MIXED procedure of Statistical Analysis Systems Institute Inc. version 9.1, Cary NC)

### **Results and Discussion**

## Temperature, relative humidity, and light

In both years mean air temperature under two row covers was higher than the ambient temperature (Fig. 1). In 2008, within the first two-weeks of the 3 week row cover installation, RC60 maintained a slightly higher temperature than RC85, but this temperature difference was not consistent in the last week. Similar results were recorded in 2009. The average difference in air temperature between ambient air and RC60, for 21 day period in 2008 and 23 day period in 2009, was 6.2 °C and 4.4 °C. This temperature difference is largely due to the heat radiation from the soil, black plastic mulch and the plants which is trapped by the spun-bond row covers (Ibarra et al., 2001). Row covers increase air temperature around the crop and their usage has been associated with increased plant growth (Bonanno and Lamont, 1987; Gaye et al., 1992). Many researchers have demonstrated higher air temperatures under row covers and attributed it to row cover permeability and the modified thermal regime inside (Moreno et al., 2002; Motsenbocker and Bonanno, 1989). Although it is desirable to maintain a higher air temperature near the plant canopy, it can also lead to crop injury (Soltani et al, 1995). Higher temperatures under row covers have been correlated with yield loss when temperatures exceed 40  $^{\circ}C$ (Peterson and Taber, 1991). Further, increased temperatures could induce heat stress and affect pollination and fruit set (Gaye et al., 1992; Gerber et al., 1989). None of the row covers used in this experiment allowed the temperature to reach excessive high levels. Temperatures were higher under RC60 than RC85 as the material for RC60 is heavier (40 g.m<sup>-2</sup>) than for RC85 (17) g.m<sup>-2</sup>). Average difference in air temperature between RC60 and RC85 (21 day period in 2008 and 23 day period in 2009) was 1.7 °C and 0.5 °C, in 2008 and 2009 respectively. The fact that

air temperature was higher inside the row cover material with low light transmission (heavy weight) suggests that air movement may play an important role in modulating air temperature inside the row covers. It is likely that air movement was low under the RC60 and thereby maintained high temperature compared to RC85. This type of observation would probably not hold true if polyethylene plastic materials are used.

Soil temperatures under the plastic mulch were collected only for 2009. Soil temperatures fluctuated both in uncovered and row cover treatments. Due to rains during the last week of June, the soils were well saturated and this led to a decrease in soil temperatures. The heavier row cover material (RC60) was able to maintain higher soil temperature than under the uncovered treatments (Fig. 2). During the period when row covers were installed, soil temperatures under RC60 were generally higher than the uncovered treatment. The average difference in soil temperatures between those two treatments were 2.6 °C. Black plastic mulch has been shown to increase mean soil temperatures (Hemphill and Crabtree, 1988; Hemphill and Mansour, 1986) but the effect is more pronounced when it is used in combination with row covers (Soltani et al., 1995). This effect is certainly desirable for growers in temperate regions where soils take longer time to heat up due to prolonged winter and wet springs. Higher soil temperature would enhance root growth; accelerate nutrient uptake, plant growth and overall development. Surprisingly for most of the dates during the period of row cover installation in 2009, relative humidity recorded under the row covers was lower than the ambient air (Fig. 2). Presumably relative humidity values tend to be higher under row covers due to reduced evapotranspiration and condensation of water within the row covers under field conditions (Lamont, 1996; Moreno et al., 2002).

The amount of *PAR* received by plants under each treatment is shown in Fig. 3. As expected, uncovered plants received higher *PAR* when compared to the plants under row covers.

Table 2 summarizes the amount of light received by plants under covered (RC60 or RC85) and uncovered treatments during the period when row covers were installed. In 2008, total amount of *PAR* received by plants under RC60 and RC85 was 26% and 21% lower than the total photon flux received by uncovered plants. Similar pattern was observed in 2009. Row covers reduce the amount of sunlight reaching the plants (Healey and Rickert, 1998) and the reduction depended upon the row cover material. In a study conducted by Moreno et al. (2002), instantaneous solar radiation was reduced by 13% by the use of row covers. They also reported lower cumulative solar radiation by 17% and 16% under perforated polythene and polypropylene floating row covers respectively. Although there was a reduction in the amount of light received by plants under row covers, in our study plants were more vigorous under the row covers. This could primarily be due to increased air and soil temperature, and improved light distribution under the row covers (Jenni et al., 1998; Moreno et al., 2002). Partial shading has been shown to promote plant growth (Lamont, 2005).

# Plant growth and morphology

At the time of row cover removal, plants under row covers were larger than the uncovered plants (Fig. 4). In the compost treatments, cucumber canopy covered most of the bed area when compared to no-compost treatments. There were significant differences in flower counts within treatments in both years. The interaction between row cover treatments and the amendment treatment was also statistically significant. In 2008, the row cover effect was significant as the uncovered plants had the lowest flower count under compost and no-compost treatments (Table 3). Wolfe et al. (1989) had also reported lower flower numbers in cucumber plants grown on black plastic mulch alone when compared to black plastic mulch + spun-bond polypropylene row cover. Differences in flower count between RC60 and RC65 within the

amendment treatments were not statistically significant. However, the amendment effect was significant with compost significantly increasing flower counts in row cover treatments. There was no compost effect on flower count in uncovered treatments. Application of compost adds organic matter and nutrients, improves soil physical properties, and enhances root development and nutrient uptake (Brady and Neil, 2000). As a result, plants have adequate resources for proper vegetative and reproductive development. Similar results were observed in 2009 except that flower counts for row covered and uncovered plants were statistically not significant for no-compost treatment. Our study clearly shows that installation of row covers enhances early flower production in cucumbers which could potentially contribute towards higher early yields. Higher early yields have been reported by a number of researchers in pepper (Gaye et al., 1992), watermelon (Soltani et al., 1995) and cucumber (Ibarra-Jimenez et al., 2004; Wolfe et al., 1989), muskmelon (Motsenbocker and Bonanno, 1989) in response to row cover.

Leaf count and dry weight data were collected only in 2009. There was an interaction effect between cover and amendment treatments for leaf count and leaf dry weights, thus the main effects were analyzed separately. For the compost treatment, plants under RC60 and RC85 had higher number of leaves than uncovered plants; however, there was no difference between the two row cover treatments (Table 4). Under no-compost treatment leaf counts for plants growing with our without row covers were not statistically significant. Effect of compost was highly significant for RC60 and RC85 treatments as the leaf count for compost treatments almost doubled. In the case of uncovered plants, compost had limited effect on leaf counts. Leaf dry weight was also impacted by row covers and compost treatment. Plants under row covers not only had more leaves but accumulated higher leaf biomass. Similar to leaf counts, there were significant differences in leaf dry weights between row cover and uncovered plants. Row covers

significantly increased leaf dry weight when compared to uncovered treatment in both compost and no-compost treatments. Plants under compost treatment accumulated close to 2 times more leaf biomass (dry weight basis) than those grown without compost. Increased leaf number and dry weights reciprocate into increased photosynthetic capacity of the plant, thereby enhancing plant growth and development. In their experiments with muskmelons, Soltani et al. (1995) positively correlated growing degree hours (GDH) with leaf number and leaf dry weight ( $r^2$  of 0.92 and 0.90, respectively) under spun-bond polyester fabric row cover. Thus row covers promote accumulation of higher GDH which in turn increases leaf counts and leaf dry weights.

Unlike other studies, leaf area per plant was similar in all row covers in the absence of compost, thereby stressing the importance of soil fertility on plant growth. Even though, plants under RC60 and RC85 had 20% to 22% higher leaf area than uncovered plants, the differences were statistically not significant (Table 5). Wolfe et al. (1989) demonstrated higher leaf areas in cucumber plants grown on black/clear plastic mulch with row covers (clear plastic/spun-bond) when compared to plants grown on black plastic mulch without row covers. Similar results have been reported in muskmelon (Ibarra et al., 2001) and bell peppers (Jolliffe and Gaye, 1995). All above studies were conducted under conventional production systems where nutrient availability is generally not a limiting factor. The effect of row covers on leaf area was significant in compost treatment. Uncovered plants in compost treatment had lower leaf area when compared to plants under RC60 and RC85. There was no difference in leaf area between plants grown under RC60 and RC85. An interesting observation was that plants grown under RC60 without compost had leaf area statistically similar to uncovered plants grown with compost. This may be due to the microclimate changes brought about by RC60, although the importance of compost cannot be undermined as it has far reaching implications on plant growth and development than

leaf area alone. Specific leaf area which is the ratio of leaf area to leaf dry mass was not affected by the presence of row cover or compost application.

In 2008 cucumber vines were longest for plants under RC60 and RC85 grown with compost (Table 5). Uncovered plants, grown with or without compost, had the shortest vines. Between RC60 and RC85 under no-compost treatment, RC60 produced plants with longer vines. But this difference was not visible in the compost treatment. In 2009 within the no-compost treatment there was no effect of row covers on vine length as RC60, RC85 and uncovered treatments showed similar values. Vine lengths of plants under compost treatment for uncovered and RC85 were statistically similar. Plants under RC60 had the longest vines. In general, compost treatments exhibited longer vines and this could be attributed to the increased nutrients and enhanced microbial activity brought about by the addition of compost. Soil nitrogen in fields under organic production has been positively correlated with soil microbial components (Gunapala and Scow, 1998). Compost treatments in our study produced plants with longer vines and robust growth. Addition of compost did influence vine length, but its effect was insignificant on SPAD readings in 2008 (Table 5). Leaf chlorophyll content was indirectly measured using SPAD meter. In 2009, all row cover treatments with or without compost showed similar SPAD readings and were lower than uncovered + compost treatment. This makes sense as plants under RC60 and RC85 received lower PAR when compared to uncovered plants. Row covers reduce the amount of light reaching the plants but the effect is compensated by increased air and soil temperature, and protection from wind and pests.

Amendment × row cover interaction was significant for plant biomass. Within compost treatment, plants under RC60 and RC85 had higher plant biomass than uncovered plants (Table 6). Higher biomass accumulation under row covers has been previously reported (Wolfe et al.,

1989; Ibbara et al., 2001). Higher biomass has been positively correlated to growing degree day accumulations, which is often used to predict plant biomass and yield (Wolfe et al., 1989). In this study, growing degree day accumulations (during row cover presence) under RC60 and RC85 were higher than outside for both years (Table 7). In 2008 RC60 and RC85 accumulated 82.5% and 68.1% more GDD, respectively when compared to outside. In 2009 row covers increased GDD by 50% when compared to outside. Within no-compost treatment, biomass accumulation was lowest for plants growing uncovered. Unlike compost treatment, plants under RC60 did not produce higher biomass when compared to plants uncovered, probably due to the shading effect of the row cover combined with low availability of nutrients in the no-compost treatment. Use of row covers in systems where nutrient availability and supply is an issue can adversely affect plant growth and ultimately yield. Nutrient management is often the rate limiting factor for efficient and profitable organic vegetable production. Regardless of growing with or without row covers, plants in the compost treatment produced higher biomass. Robust and high quality plants can be positively correlated to healthy and nutrient rich soils. Compost, being a critical component of organic production, supplies nutrients that are released over time and improves soil physical, chemical and biological quality (Bulluck et al., 2002).

#### Yield

In our study the marketable fruit weight did not have any particular trend. In 2008 RC85 + compost treatment produced the highest marketable yield (Table 8). There was no difference in yield between compost and no-compost treatments of RC60 and uncovered plants. Fruit count under compost treatment of row covers were higher than uncovered + compost treatment. In 2009, compost treatments clearly stood apart, both in marketable fruit weight and count. Compost treatments produced highest marketable fruit weight and count. Beneficial effects of

compost on vegetable growth and yield have earlier been reported (Maynard, 1994). Marketable cucumber yields have been shown to respond positively to compost applications (Roe et al., 1997). Correlation between marketable fruit weight and a number of growth parameters were highly significant. Marketable fruit weight was highly correlated with leaf area, followed by plant biomass, leaf number and vine length (Fig. 5). In our study, use of row covers in conjunction with compost improved various plant growth characteristics, indicating a significant contribution made by the vegetative parts towards total marketable yield.

There was no statistical difference in marketable fruit weight or count among plants grown in the compost treatment under RC60, RC85 or without row covers. Similar trend was observed within the no-compost treatment. Thus, in 2009, there was no effect of row covers on marketable crop yield. A number of studies have reported higher yields under row covers but the yield increases are not consistent (Wolfe et al., 1989; Motsenbocker and Bonanno, 1989). Also it is important to note that in most cases higher yields are observed when comparisons are made between plants growing on black plastic mulch + row cover and plants growing on bare soil without row covers (Ibarra et al., 2001; Soltani et al., 1995). Similar yields with or without row covers in our study in 2009 could be explained by lower pest pressure present. No major outbreak of insect (cucumber beetle) or disease damage occurred in 2009 because of which the additional benefit of using row covers as insect barrier was not received. On the other hand, there was moderate insect and disease pressure due to cucumber beetles in 2008. Row covers may not always impact crop yield but it can certainly influence crop microclimate which has a direct impact on plant growth and development (Ibarra et al., 2001; Soltani et al., 1995; Wolfe et al., 1989). The lack of clear yield improvement with the row cover is probably due to the fact that the slow growth in the uncovered plant was compensated late in the season. Unless there are

limiting factors such as adverse weather conditions, fertility issues, insect and disease pressure, etc. at the end of the season, we should not expect major differences in total yield among the row cover treatments.

Interaction between amendment × cover was significant for nonmarketable fruit weight or cull weight in 2008 and 2009 but there was no specific trend. In 2008, within compost treatment, plants under RC85 had higher cull weight than RC60 or uncovered treatment (Table 9). Row cover treatments had higher cull weight than uncovered plants within no-compost treatment. For individual cover treatments, both uncovered plants and plants under RC85 produced higher cull weight with their respective compost treatment. There was no significant difference in cull weight between compost and no-compost treatments for plants under RC60. In 2009, uncovered plants produced the lowest cull weight in the compost treatment, however, there was no difference in cull weight between row covers and uncovered plants in the no-compost treatment. When analyzing the amendment effect, compost treatments of RC60 and RC85 produced higher cull weights while there was no difference in cull weights in the uncovered plants. Higher nonmarketable fruit weight in compost + row cover treatments was due to damage due to pest and diseases. Second and third generation adults of cucumber beetles migrating into the area at mid-season fed on fruits resulting in scarring and decreasing its marketability. In 2009, later during the season, the incidence of downy mildew was found to be more pronounced in row cover treatments. A number of fruits had to be categorized as nonmarketable as they were small in size and misshapen.

Our two year study demonstrates a feasible organic cucumber production system. Yields obtained in our study, if not equal, were comparable to conventional production systems. However, it is important to recognize that organic systems are not straight forward. Every

individual aspect of the system needs to be thoroughly studied and understood for effective crop management (Russo and Webber, 2007). Our experiment focused on studying the impact of row covers on changes in microclimate and plant growth under organic production system. Use of spun-bond row covers influenced microclimate and showed improved plant growth characteristics but not to the extent of a considerable increase in total marketable yield. However, the impact of row covers on air and soil temperature, stem, leaf, and flower characteristics, and plant biomass was remarkable. Several environmental factors influence plant growth and development, with temperature having one of the strongest effects. By influencing air and soil temperature, a crucial factor especially in the northern climates, row covers can increase heat accumulation units and enhance crop growth and development (Jenni et al., 1998; Bonanno and Lamont, 1987). Row covers could also significantly influence plant nutrient status and uptake under field conditions (Moreno et al., 2002). Both row covers tested in this study modified crop microclimate favorably. There were fewer significant differences in terms of plant growth parameters, between RC60 and RC85.

## Conclusion

Most organic systems rely heavily on organic amendments for supply of macro and micro nutrients to meet crop nutrient requirement. Compost serves as an excellent organic amendment that not only adds nutrient but also builds soil organic matter, soil structure, increases soil water holding capacity and stimulates microbial activity. In our study compost application positively affected plant growth and marketable yield. Ecological processes determining yields like nitrogen mineralization potential and microbial and parasitoid diversity and abundance are higher in organic systems (Drinkwater et al., 1995). Organic amendments provide advantages beyond benefits of building soil organic matter and enhancing soil microbial activity since

nutrients that are seldom applied by growers like zinc, manganese, boron, and sulfur, are also supplied. In addition, organic amendments also supplement liming nutrients like Calcium and Magnesium and safeguard potential yield limitations and losses. Use of compost was synergistic to row covers in producing healthy and robust plants. This finding is particularly important for organic production where nutrients are sometimes limiting factors. Row covers not only create a suitable microclimate for plant growth but also act as an insect barrier and greatly influence the turbulent diffusion of carbon dioxide, sensible heat, and water vapor (Mao and Kurata, 1997). In Michigan where weather conditions are cooler during early spring, row covers can provide protected conditions for early planting of cucumber transplants. There are number of practices and techniques a grower can adopt and implement under organic cropping systems like cover cropping, and use of plastic mulch and row covers, but he/she should take into consideration variables like cost of the material, available resources, market, and weather. Weather is by far the most variable and directly affects all ecological processes driving crop growth and development. Use of row covers under organic cucumber production systems could provide some leverage against unpredictable weather conditions and possibly increase farm sustainability and yield.

M d	Monthly average air temperature (°C)		Total monthly precipitation (mm)			Monthly average relative humidity (%)			
Month	2008	2009	10 yr. average <sup>†</sup>	2008	2009	10 yr. average	2008	2009	10 yr. average
June	20.0	19.2	19.6	112	126	71	49.6	71.4	72.2
July	21.7	19.3	21.5	96	61	72	72.6	72.7	73.6
August	20.6	20.1	20.6	17	105	67	72.9	77.0	77.2

Table 5.1. Monthly average air temperature, total precipitation, and relative humidity during the 2008, 2009 growing season and the 10 year average at Horticulture Teaching and Research Center, Michigan State University, Holt, MI 48842.

<sup>†</sup> 10 yr. average from 1998-2007

Table 5.2. Monthly and total photosynthetically active radiation received by cucumber plants uncovered and under RC60 and RC85 in 2008 and 2009 at Horticulture Teaching and Research Center, Michigan State University, Holt, MI 48842.

	$PAR \ (\mu \text{mol.m}^{-2}.\text{d}^{-1})$							
Treatment <sup>z</sup>	2008 <sup>y</sup>		Total	200	2009			
	June	July	received	June	July	received		
RC60	4582.1	4075.3	8657.4	2712.2	6417.8	9130.0		
RC85	4895.4	4400.1	9295.6	2808.0	7303.6	10111.7		
Uncovered	5970.0	5760.4	11730.5	3600.3	9321.9	12922.2		

 $\frac{1}{2}$  RC60= row cover with 60% light transmission, RC85= row cover with 85% light transmission

<sup>y</sup> Rowcovers installed for 21 days in 2008 and 23 days in 2009.

Table 5.3. Cucumber flower counts recorded at the time of row cover removal under row cover and amendment combinations in 2008 and 2009 at Horticulture Teaching and Research Center, Michigan State University, Holt, MI 48842.

	Flower count <sup>Z</sup>							
Amendment	2008				2009			
	RC60 <sup>y</sup>	RC85	Uncovered		RC60	RC85	Uncovered	
Compost	$4.3 a, A^{X}$	4.5 a,A	0.1 b,A	_	10.6 a,A	8.9 a,A	7.1 b,A	
No compost	2.7 a,B	2.3 a,B	0.2 b,A		4.9 a,B	4.8 a,B	4.7 a,A	

 $\overline{z}$  Average number of flowers per plant. Data is the mean of 12 plants per experimental unit.

<sup>y</sup> RC60= row cover with 60% light transmission, RC85= row cover with 85% light transmission

<sup>x</sup> Mean separation for an individual year within columns (uppercase letters) and rows (lowercase letters) with Fisher's protected LSD ( $P \le 0.05$ ).

Table 5.4. Cucumber leaf count and leaf dry weight under row cover and amendment combinations collected at the time of row cover removal in 2009 at Horticulture Teaching and Research Center, Michigan State University, Holt, MI 48842.

	Leaf characteristics (2009)								
Amendment		Leaf count	Z	Leaf dry wt (g)					
	RC60 <sup>y</sup>	RC85	Uncovered	RC60	RC85	Uncovered			
Compost	$85 a, A^{X}$	83 a,A	57 b,A	44.6 $a, A^{W}$	44.9 a,A	31.4 b,A			
No compost	45 a,B	48 a,B	38 a,A	22.3 a,B	23.5 a,B	18.7 a,B			

 $\frac{z}{z}$  Leaves counted from two sample plants harvested at the time of row cover removal.

<sup>y</sup> RC60= row cover with 60% light transmission, RC85= row cover with 85% light transmission

<sup>x</sup> Mean separation for leaf count within columns (uppercase letters) and rows (lowercase letters) with Fisher's protected LSD ( $P \le 0.05$ ).

<sup>w</sup> Mean separation for leaf dry weight within columns (uppercase letters) and rows (lowercase letters) with Fisher's protected LSD ( $P \le 0.05$ ).

Table 5.5. Leaf area, specific leaf area, vine length, and SPAD as affected by amendment and row cover combinations in 2009 at Horticulture Teaching and Research Center, Michigan State University, Holt, MI 48842.

Treatment <sup>Z</sup>	Leaf area $y$ (cm <sup>2</sup> )	SI $\Lambda^{NS}$ (cm <sup>2</sup> .g <sup>-1</sup> )	Vine leng	$gth^{x}(cm)$	$SPAD^{W}$		
Treatment	Leaf area (effi)		2008	2009	2008 <sup>NS</sup>	2009	
Uncovered	3519.6 c <sup>v</sup>	190.2	30.8 d	54.9 c	48.2	49.7 ab	
Uncovered + compost	6323.4 b	202.8	37.5 cd	75.3 b	46.0	51.0 a	
RC60	4389.3 bc	196.8	48.4 b	62.3 c	45.8	44.8 c	
RC60 + compost	9089.1 a	202.1	62.3 a	84.3 a	43.5	46.1 bc	
RC85	4515.5 bc	192.5	45.0 c	56.7 c	47.5	47.5 abc	
RC85 + compost	9223.3 a	203.1	65.8 a	75.9 b	41.6	45.9 bc	

<sup>z</sup> RC60= row cover with 60% light transmission, RC85= row cover with 85% light transmission

<sup>y</sup> Average of total leaf area from two sample plants.

<sup>x</sup> Length of the longest vine. Data are mean of 12 plants/replication.

<sup>w</sup> Mean of SPAD measurements obtained from the first fully opened leaf near the vine tip. Data is the mean of 12 plants.

<sup>V</sup>Mean separation within columns by Fischer's protected LSD ( $P \le 0.05$ ).

NS Non significant

Table 5.6. Cucumber plant biomass under row cover and amendment combinations collected at the time of row cover removal in 2009 at Horticulture Teaching and Research Center, Michigan State University, Holt, MI 48842.

Amondmont	Plant biomass <sup>Z</sup> (g/plant)						
Amenument	RC60 <sup>y</sup>	RC85	Uncovered				
Compost	$64.2 a, A^{X}$	62.5 a,A	42.3 b,A				
No compost	30.9 ab,B	33.0 a,B	24.3 b,B				

 $\overline{z}$  Comprises of above and below ground biomass (dry weights).

<sup>y</sup> RC60= row cover with 60% light transmission, RC85= row cover with 85% light transmission. The row covers were installed 7 d after cucumber transplanting and maintained for 21 and 23 d in 2008 and 2009, respectively

<sup>x</sup> Mean separation within columns (uppercase letters) and rows (lowercase letters) with Fisher's protected LSD ( $P \le 0.05$ ).

	GDD (base temperature 10 $^{\circ}$ C)						
Treatment <sup>z</sup>	2008		Total	200	)9	Total	
	June	July	received <sup>y</sup>	June	July	received	
RC60	219.8	159.9	379.7	122.1	262.5	384.6	
RC85	180.7	169.0	349.7	105.7	273.4	379.1	
Uncovered	112.3	95.7	208.0	78.7	176.6	255.3	

Table 5.7. Monthly growing degree days (GDD) under row cover treatments during cucumber growing season in 2008 and 2009 at Horticulture Teaching and Research Center, Michigan State University, Holt, MI 48842.

 $\overline{^{Z}RC60}$  = row cover with 60% light transmission, RC85= row cover with 85% light transmission

<sup>y</sup> Data represent cumulative of June and July (data recorded for 21day duration in 2008 and 23 days in 2009)

_	Marketable <sup>y</sup> Yield						
Treatment <sup>z</sup>	2008			2009			
	Fruit wt	Fruit count	F	ruit wt	Fruit count		
	(kg/12 plants)	(number/12 plants)	(kg/	12 plants)	(number/12 plants)		
Uncovered	$17.7 \text{ bc}^{\text{x}}$	49 bc	1	l 6.8 b	49 b		
Uncovered + compost	15.9 c	45 c	4	29.6 a	81 a		
RC60	16.4 c	46 bc	1	l 3.7 b	46 b		
RC60 + compost	22.8 bc	65 ab		26.7 a	75 a		
RC85	24.1 b	68 a	1	l 5.8 b	47 b		
RC85 + compost	26.8 a	70 a		29.2 a	81 a		

Table 5.8. Cucumber marketable fruit weight and count under different row cover and amendment combinations in 2008 and 2009 at Horticulture Teaching and Research Center, Michigan State University, Holt, MI 48842.

<sup>*Z*</sup> RC60= row cover with 60% light transmission, RC85= row cover with 85% light transmission

<sup>y</sup> Comprised of fruits of following USDA grades: U.S. Fancy, U.S. Extra #1, U.S. #1, U.S. #1 Small, and U.S. #1 Large.

<sup>x</sup> Mean separation within columns by Fischer's protected LSD ( $P \le 0.05$ ).

	Nonmarketable <sup>z</sup> fruit wt (kg)						
Amendment		2008		2009			
	RC60 <sup>y</sup>	RC85	Uncovered	RC60	RC85	Uncovered	
Compost	$4.1 \text{ b,A}^{\text{X}}$	6.1 a,A	3.5 b,A	7.3 a,A	6.8 a,A	3.3 b,A	
No compost	4.1 a,A	3.7 a,B	2.1 b,B	3.1 a,B	3.6 a,B	2.7 a,A	

Table 5.9. Non-marketable cucumber fruit weight under different row cover and amendment combinations in 2008 and 2009 at Horticulture Teaching and Research Center, Michigan State University, Holt, MI 48842.

 $\overline{z}$  Fruits with defects and diseases were categorized as nonmarketable.

<sup>y</sup> RC60= row cover with 60% light transmission, RC85= row cover with 85% light transmission

<sup>x</sup> Mean separation for an individual year within columns (uppercase letters) and rows (lowercase letters) with Fisher's protected LSD ( $P \le 0.05$ ).



Fig. 5.1. Ambient air temperature outside and inside spun-bond row covers with different light transmission levels. RC6O and RC85 are row covers with 60 and 85% light transmission, respectively. Row covers were installed 7 d after cucumber transplanting and maintained for 21 and 23 d in 2008 and 2009, respectively. The research was conducted at Horticulture Teaching and Research Center, Michigan State University, Holt, MI 48842.



Fig. 5.2. Soil temperature and relative humidity, outside and inside spun-bond row covers with different light transmission levels, recorded in 2009. RC6O and RC85 are row covers with 60 and 85% light transmission, respectively. Row covers were installed 7 d after cucumber transplanting and maintained for 23d. The research was conducted at Horticulture Teaching and Research Center, Michigan State University, Holt, MI 48842.



Fig. 5.3. Amount of *PAR* received outside and inside spun-bond row covers with different light transmission levels. RC6O and RC85 are row covers with 60 and 85% light transmission, respectively. Row covers were installed 7 d after cucumber transplanting and maintained for 21 and 23 d in 2008 and 2009, respectively. The research was conducted at Horticulture Teaching and Research Center, Michigan State University, Holt, MI 48842.



Fig. 5.4. Uncovered and covered plants soon after row cover removal in 2009. A) Uncovered, B) RC60, C) RC85, D) Uncovered + compost, E) RC60 + compost, and F) RC85 + compost. RC6O and RC85 are row covers with 60 and 85% light transmission. Row covers were installed 7 d after cucumber transplanting and maintained for 23 d. The research was conducted at Horticulture Teaching and Research Center, Michigan State University, Holt, MI 48842.



Fig. 5.5. Correlation between cucumber marketable weight versus leaf area, plant biomass, leaf number, and vine length in 2009. Correlations were highly significant ( $P \le 0.001$ ). Data on leaf area, plant biomass, leaf number, and vine length was collected by harvesting two plants from each treatment at the time of row cover removal.

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CHAPTER VI

CONCLUSION AND FUTURE RESEARCH

### Conclusion

The overall objective of this research was to address key issues in different stages of organic vegetable production. Transplant production, the first stage of many vegetable production systems, is critical as good quality transplants are an underpinning, for subsequent field establishment, growth and higher yields. This research project formulated a compost-based growing medium which was supplemented with locally and easily available low-cost organic amendment derived from alfalfa meal. This greenhouse study identified an optimal amendment rate and incubation period to meet transplant nutrient demand and to produce commercially acceptable organic tomato transplants. This information is essential as lack of proper incubation period can lead to poor germination and transplant growth. Linear regression analyses explained trends for different amendment rates and incubation periods on transplant dry weight, height, and chlorophyll content. Cost of production often determines whether the proposed technique or system will be adopted by growers. An economic evaluation of our transplant production system calculated the cost of the growing medium (along with alfalfa-based amendment) to be  $\$77/m^3$ . which is well within the prevailing market value of available commercial organic blends and formulations (\$60-\$197/m<sup>3</sup>).

Studies on intercropping to enhance cropping system biodiversity in tomato production produced interesting results. Effects of intercropping on tomato growth and yield characteristics were less evident; however, cropping system significantly influenced yields in cucumber. Intercropping benefited cucumber crop by substantially reducing cucumber beetle population and bacterial wilt damage. As a result, land equivalent ratio was more than one, demonstrating advantages of intercropping. Regardless of cropping system, compost significantly increased

plant height, stem diameter and dry weight of tomato plants, clearly indicating its positive effects on plant growth.

Studies involving two cover crops (rye and hairy vetch) and their effect on inherent soil biological properties such as respiration, microbial biomass, nematode population distribution, microbial community function, and functional diversity explained changes in soil biology. For most soil biological properties evaluated, use of rye or a mixture of rye and hairy vetch did not lead to major differences; however, the use of compost significantly altered various biological parameters within cover crop treatments. Compost treatments were associated with higher microbial biomass and activity. In 2007, bacterivore nematode population was higher for rye:vetch treatments than rye alone treatments. Multivariate analysis of data based on C utilization assay was helpful in segregating cropping systems based on diversity of microorganisms, but the spatial orientation of systems varied from year to year.

Impact of cover crops on tomato fruit quality aspects such as density, firmness, and total soluble solids did not differ among cover crop and compost treatments, although, in one of the years internal fruit firmness was significantly enhanced by rye-vetch cover crop treatment. Particularly noticeable was the effect of rye-vetch cover crop treatment on functional food properties, antioxidant and anti-inflammatory assay, of tomato fruits. Percentage antioxidant activity varied but was higher in tomatoes grown in rye-vetch-compost treatment. Functional food quality of the tomato extracts, with respect to the ability to inhibit cyclooxygenase (COX) enzyme activity was highest in rye-vetch-compost treatment. This could be attributed to the interaction between vetch cover crop and tomato plant which promotes biosynthesis of compounds in tomato fruit that are capable of inhibiting COX enzyme activity.
The final study of this dissertation evaluated two types of row covers (60% or 85% light transmission) with or without application of compost on microclimate modification and cucumber plant growth. Row covers increased air and soil temperature and decreased photosynthetically active radiation (PAR). Row cover with 60% transmission was able to trap more heat when compared with row cover with 85% transmission. There was a marked increase in the growing degree-day accumulations under row covers when compared with uncovered treatment. As a result of microclimate modification, use of row covers increased vine length, flower count, leaf area, leaf count, plant biomass, and total marketable yield. Use of compost in conjunction with row covers enhanced the row cover effect.

Overall, the scientific knowledge gained through this dissertation will significantly contribute towards understanding of broad ecological implications of crop, pest and soil management techniques among transitioning and organic farming communities in Michigan and other regions with similar climate. This dissertation addressed some critical aspects and problem areas of organic community starting from transplant production to final quality of the produce. Research findings from this dissertation have added to the understanding of the use of cover crops, compost, and other sustainable production strategies such as intercropping in organic production systems and their implication on soil biology, crop growth, fruit quality and overall cropping system productivity.

## **Future research**

Although this research project addressed a wide gamut of issues governing organic vegetable production systems, there are several questions that remain unanswered. Additional research on transplant production and soil management aspects in the field can enhance the

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productivity and sustainability of the cropping enterprise. The following are some areas where future work would be beneficial to the organic and scientific communities:

## Greenhouse studies:

- Understand processes underlying the low germination rate of tomato seeds in growing medium that are not incubated after amendment with alfalfa based products.
- Identify microorganisms that colonize the growing medium soon after the incorporation of alfalfa based organic amendment.
- Determine if seed germination in growing media amended with Bradfield mix and other similar products is affected by biotic factors (microorganisms).
- Study the effect of post emergence application of alfalfa based organic amendment on transplant growth.
- Evaluate other plant and animal based amendments for use under organic production systems.

## Field studies:

- Fine tuning the ratio of rye and vetch cover crop for increased biomass and N-fixation
- Isolation and identification of allelochemicals in plots with repeated incorporation of rye cover crop
- Evaluate other cover crops and combinations that could be incorporated into the production system
- Determine ways for timely seeding of vetch cover crop, especially when it follows a long duration crop like tomato
- Test the effect of repeated compost application on reduction of soil-borne pathogens

• Monitor cucumber beetle population under monocrop and intercrop systems and quantify the impact of intercropping on cucumber beetle infestation

## Fruit quality studies:

- Quantification of total phenolics in tomato fruits grown under different cropping systems
- Identification and quantification of compounds that render COX inhibiting properties to tomatoes grown under rye-vetch cover crop system

These studies will provide additional information to better understand our cropping systems. Organic amendments, such as cover crops and compost, and use of integrated approaches such as row covers can improve plant health and productivity. Growers need to formulate a multi-pronged strategy targeting issues from the start of the production system to the end. A sustainable production system needs a holistic yet simple plan that focuses on production of healthy transplants, preservation of long-term soil productivity, and production of food that has high nutritional and functional value.