ABSTRACT

Title of Document: **LOCAL LETTUCE: HEAT TOLERANT ROMAINE CULTIVARS AND VERMICOMPOST SOIL AMENDMENT TO INCREASE SUSTAINABILITY IN THE MID-ATLANTIC.**

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Local production of lettuce in the Mid-Atlantic utilizing heat-tolerant romaine cultivars and vermicompost soil amendment has the potential to significantly increase sustainability of agriculture. Heat tolerant cultivars would facilitate season extension into the summer. Vermicompost, compost produced using earthworms, may increase yield and quality of lettuce crops. This research tested a system incorporating these two practices. Success was assessed on lettuce yield and quality of lettuce across three seasons (spring, summer, and fall) and food safety risk of vermicompost. Several of the heat tolerant cultivars showed marketing potential when grown in the summer. Vermicompost did not significantly increase lettuce performance, but trends indicate that it may help, especially at higher rates. No food safety risk was associated with tested materials.

LOCAL LETTUCE: HEAT TOLERANT ROMAINE CULTIVARS AND VERMICOMPOST SOIL AMENDMENT TO INCREASE SUSTIANABILITY IN THE MID-ATLANTIC

By

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Preface

This thesis describes the work I performed investigating a system with the potential to enhance sustainability of local lettuce production in the Mid-Atlantic region. It is organized into three chapters. Chapter 1 is a comprehensive literature review including sustainable agriculture as it pertains to lettuce production, followed by investigation of two elements used by the system proposed here. These include 1) the potential and limitations of lettuce production in a hot climate and 2) the use vermicompost soil amendment in agriculture to boost yield and quality of crops.

Chapter 2 has been written in the style of a manuscript to be submitted to the peer-reviewed journal *HortTechnology* published by the American Society of Horticultural Science (ASHS). It includes data from one year of experiments performed in 2013. After an additional year of this study (2014), the manuscript will be updated and submitted. You will notice that there are inconsistencies in the system of measurement (metric or standard) used to collect data. This is according to the author guidelines for the journal *HortTechnology.* Reviewers of the journal found that asking authors to change all units to the SI system of measurement led to many errors, and therefore ask that data remain in the original units in which it was collected.

Chapter 3 goes beyond the data and conclusions presented in the manuscript and addresses broader impacts, such as unanswered questions, ongoing research, and educational impact.

I began my master's degree with a rather eclectic background including a Bachelor's degree in general biology, research experience in labs from entomology to field ecology, and work experience involving greenroofs, medicinal plants, and urban agriculture. It was my desire to learn how to perform credible, applied scientific research, while contributing to the advancement of sustainable agriculture for local growers. I believe that this project has accomplished that and that my education has prepared me well for a career where I may continue to do such work.

I am finishing my work at the University of Maryland, perhaps a little pre-maturely, to begin a job with Cornell Cooperative Extension. While I intended to stay long enough to complete two years of data collection, the job demands that I begin before this can happen. I plan to return several times in the next year to complete the second year of data collection and analysis necessary to publish the findings from this research.

Through a largely self-guided graduate education, I have been given the opportunity to learn a tremendous amount about plant science, scientific research, and education. I look forward to using the knowledge and skills I have acquired here to guide my career. I feel well equipped to give back to the scientific and agricultural communities through meaningful research and education.

Acknowledgements

First and foremost, I would like to thank Chris Walsh for his guidance through not only this project but through my entire graduate education. I consulted him on everything from experimental design to job applications. I truly would not be where I am today without his support and encouragement. Convincing me to come to graduate school in the plant sciences has been one of the best things that could have happened to me and has led to a very promising career in horticulture.

Thank you also to Shirley Micallef and Pat Millner for all of their help. Both provided me with information, ideas, and contacts that informed my research, and provided me with resources essential to this study. Their genuine excitement for scientific research and my project was motivating and encouraging. It is inspiring to learn from such strong women in the demanding environment of academic science.

Thank you to Eunhee Park and Yaguang Luo for their tremendous help with the sensory evaluation portion of this study. Use of their facility made for a very professional and accurate evaluation of the lettuce cultivars. This experiment could not have taken place without Eunhee's expertise. She made it easy to learn an effective protocol and smoothly execute it, even under a serious time crunch in a test kitchen stacked full of identical looking plates of lettuce.

Also thank you to the many students and friends who volunteered their valuable time to helping me complete this project. Thank you to Elizabeth Prinkey, Sarah Allard, Donna Pahl, and many more. From hours under the fume hood pipetting dilutions to long days out in the sun, I couldn't have done it without your (wo)man power and moral support.

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Chapter 1: Literature Review

Lettuce (*Lactuca sativa*) is one of the most important vegetables in the U.S., with great potential for local, more sustainable production in the Mid-Atlantic region. To begin, sustainable agriculture will be briefly explored to define the relevance and context of lettuce production in this region. Following is a thorough review of lettuce production and the potential and limitations for its use in season extension through the summer in this region. Then, vermicompost, an organic soil amendment, is reviewed for its potential to increase productivity and sustainability, specifically of lettuce crop. The success of a system incorporating summer lettuce and vermicompost may fuel sustainable agriculture in the Mid-Atlantic by drawing on the local model.

1.1 Lettuce production in the context of sustainable agriculture

Sustainable is one of the most coveted words in agriculture today. 'Sustainable agriculture' is a field that has undergone enormous growth since the 1980s, whose present principles are derived from the original ideology of 'organic agriculture' of the 1960s. These core values include maintaining nature as a model, prioritizing soil health, and exercising an overall anti-materialist philosophy (Youngberg, 2013). According to experts in the field, today "there is a growing consensus in support of the three fundamental prerequisites… 1) Ecological soundness, 2) Economic viability, and 3) Social responsibility" (Ikerd, 1993; Lightfoot, 2001; Lyson, 2004; Neher, 1992). There are many perspectives on which practices satisfy this definition. Regardless, there is an increasing demand for sustainable agriculture and it is coming from a multitude of diverse sources. With this, it is imperative that we continue to develop novel solutions.

While these three criteria and their solutions often overlap, each can be characterized separately. Ecological soundness should be achieved by incorporating high diversity and extensive recycling of materials (Lightfoot, 2001). It can be monitored using selected indicators of nutrient cycling, hydrology, and resource conservation (Neher 1992). This is especially important to do as our population approaches 9 billion, putting significant strain on our food system and the environment. Much of conventional agriculture, especially in the United States, has abandoned ecologically sound practices in favor of high-input, capital-intensive monocultures that maximize short-term profits (Neher, 1992). However, economic viability and ecological soundness are not mutually exclusive. As demonstrated by many case studies, ecological soundness has the power to increase economic growth of productions and environmental resilience of agroecosystems (Lightfoot, 2001).

Despite growing popular interest in agriculture and the food system, economic viability of agriculture as a livelihood seems to be in decline for many farmers. According to EPA surveys, only 45% of farmers claim farming as their primary occupation and the number of farms in the country has been in decline since 1935 (EPA, 2013). The average age of farmers is increasing, indicating that younger generations are seeking employment off the farm. In addition, agriculture in the US relies heavily on government subsidies (Neher, 1992) and increasing regulations, such as the proposed Food Safety Modernization Act, are putting even more financial burden on farmers. This is especially magnified for small farmers as compared to large commercial operations. The USDA offers some support for beginning farmers through programs such as the Beginning Farmers and Ranchers Development Program and the Start2Farm Program

(FSA, 2014). More work needs to be done to find ways to make farming economically viable and attractive to future generations.

With regard to social sustainability, our communities are dependent on an effective food system. Social injustice present in our food system is characterized by chronic illness 'epidemics' (of diabetes, heart disease, and obesity), prevalent hunger, and exploitation of farm workers (Allen, 2008). This is despite efforts of many organizations and individuals to call attention to these problems, and the increasing popular interest in the food system. Michael Pollen's *Omnivore's Dilemma*, Eric Schlosser's *Fast Food Nation*, and Barry Estabrook's *Tomatoland* are just a few examples of top-selling nonfiction that have elucidated some of these injustices. Social injustice can be partly attributed to the convenience and affordability of mass-produced processed foods, a system founded on profit-driven corporate farms, companies, and distributors. Despite growing awareness and concern, benefits of food revolution often affect privileged people who are not victims of social injustice. Influential people, namely academics and researchers, play a key role in making change through educating future generations, identifying key issues, and developing research that tests current ideologies and practices (Allen, 2008). With the correct action, we can develop a socially just food system such that fresh healthy produce is more appealing and accessible, without infringing on basic human rights.

Achieving these three components is especially difficult in agricultural systems for a number of reasons. Most notably, solutions are highly case specific, meaning there is no precise, set formula that can be applied to all situations (Neher, 1992; von-Wiren-Lehr, 2001). Solutions must therefore be 'tailored to specific regions, soil types, topography, and climate' (Lockeretz, 1988). Another difficulty is that agrosystems are complex, dynamic systems of 'manifold interacting parameters' including both anthropogenic and ecological factors (von Wiren-Lehr, 2001). Finally, success of agroecosystems achieving sustainability must be monitored at multiple scales 'ranging below and above individual ecosystems' from population to global systems (Neher, 1992). Fortunately, strategies for monitoring success are available (Neher, 1992; von Wiren-Lehr, 2001), and operation-specific solutions are continuously being developed.

Taking a local approach is one strategy that has been successful in increasing sustainability in many communities. This model has been termed 'civic agriculture' by Lyson (Lyson, 2001, 2004). Contrary to 'commodity agriculture,' which views agriculture primarily as a business, with the goal of maximizing production and minimizing costs (land, labor, capital, and management), 'civic agriculture' is a locally-based, small-scale system sensitive to specific social and economic concerns of a locality (Lyson, 2004). It is a system that tailors its approach to the specific locality in which it is implemented.

Research has shown that local agriculture is effective at satisfying the three basic criteria of sustainability. Examination of characteristics of communities with varying economic models found that "in general, counties dominated by large scale, absentee owned agricultural enterprises have less favorable welfare[1] outcomes" (Lyson, 2001) and that local systems on the other hand, were capable of increasing vitality of the community, financial status and well-being of individuals, and environmental quality of production (Ikerd, 1993; Lyson 2001, 2004). Case studies conducted by Lightfoot and

[1] Welfare was defined as poverty rate, unemployment, low birth weight, and rate of violent crime

Noble demonstrated that smallholder farming systems and systems favoring ecological soundness can improve production and income of operations (Lightfoot 2003). These insights may be used to model locally-based systems that will increase sustainability of the food system in many regions.

In any region, pursuit of local sustainable agriculture should target suitable crops and practices. In the Mid-Atlantic, there is tremendous potential for increasing the sustainability of lettuce production. Many farmers local to the Mid-Atlantic already produce leafy greens in the spring and fall, and could easily market summer leafy greens to their existing customer base at CSAs and farmer's markets. This review will consider the potential for season extension of lettuce through the use of heat tolerant cultivars and the use of vermicompost soil amendment to increase yield and quality of the crop.

1.2 Lettuce as a target crop for sustainability in the Mid-Atlantic

1.2.1 Lettuce: a critical crop in the United States

Lettuce is one of the most important specialty crops produced and distributed in the United States. Currently it is the leading vegetable crop in terms of production value (Jore, 2012). In addition, the U.S. is the leading exporter of lettuce, with 327,268 MT exported in 2010 (Jore, 2012). During the past five years, per capita consumption of romaine lettuce in the United States has increased nearly three-fold that of consumption 20 years ago (Jore, 2012).

The main types of lettuce are heading lettuce (iceberg, butterhead, Boston, and Bibb), romaine (Cos) and leaf lettuces (Jore, 2012). Heading lettuce and romaine varieties, make up the majority of lettuce production. They are typically produced in raised beds, covered with black plastic mulch and provided drip irrigation. A crop usually matures in 65-70 days (Smith, 2009; Wahid, 2007). Growth is dependent on sufficient nitrogen availability. Lettuce typically requires 100-120 lbs N per acre, however, total N uptake in the first 30 days is very low (Smith, 2009). Therefore, best management practices require careful water and nutrient management.

1.2.2 Heat tolerance is a limiting factor for lettuce production

Lettuce is a cool weather crop which is especially sensitive to heat stress and day length—thus limiting the regions and environmental conditions under which it can be grown. Virtually all plant species have a heat-stress threshold, above which they exhibit morphological, anatomical, and phenological effects, as well as physiological responses including changes in water relations, photosynthetic ability, hormones and secondary metabolites (Wahid, 2007). Threshold temperatures and specific responses vary with plant species and developmental stage.

Lettuce seeds are extremely sensitive to heat, typically having a lower heat-threshold than developing plants. Thermoinhibition occurs for seeds imbibed at temperatures greater than 25-33^{0}C (77-91^{0}F) (Argyris, 2008). A common practice used to overcome this, especially in the lettuce-producing Imperial Valley of CA, is to sow seeds during the day and then water in the evening so that early stages of germination take place in soil cooled by evaporation (Janick, 1992). These solutions are not feasible in places with high nighttime temperatures.

The production of many hormones play a role in germination, including abscisic acid, gibberellins, and ethylene. These are affected by temperature (Argyris, 2008). High levels of ABA in particular, specifically whose biosynthesis and metabolism are controlled in part by LsZEP1, LsNCED4, and SDR1, lead to thermoinhibition (Argyris, 2008; Huo, 2013). Ethylene, on the other hand, may help overcome thermoinhibition. Exogenous ethylene application increased activity of endo-β-mannanase leading to increased germination (Nascimento, 2004). This was attributed to weakening of the endosperm, thus facilitating emergence of the radical.

Maturation of lettuce is controlled by heat and photoperiod. Transplants grown under these types of stress will flower (bolt) prematurely or form "loose, fluffy heads" preventing the harvest of a high quality crop (Smith, 2011). Ideal daytime and nighttime temperatures are 17-28^{o}C (63-83^{o}F) and 3-12^{o}C (37-53^{o}F) respectively (Smith, 2011). Rappaport reported that night air temperatures above 65^{0}F as well as air and soil temperatures above 65^{0}F caused accelerated stalk development (Rappaport, 1956). In addition, long days induce flowering response (Ryder, 2005; Waycott, 1995).

Flowering time is a trait dependent on polygenetic inheritance (Ryder, 2005; Silva, 1995). At least six genes are involved in controlling early flowering response in lettuce. Ryder identified the first genes *Ef-1* and *Ef-2* (Ryder, 1988). Subsequently, Ryder and Kim, and later Ryder and Milligan identified a number of other implicated genes (Ryder, 2005).

Manipulation of these genes may be used in the future to create heat-tolerant lettuce cultivars. Genetic factors were shown to have greater impact than environmental factors on both broad and narrow sense heritability of flowering time (Silva, 1999). In conventional breeding programs, many crosses between cultivars of *Lactuca sativa* as well as interspecific crosses with *L. serriola*, prickly lettuce and *L. sativa*'s closest wild relative, have yielded improved cultivars. Ryder and Milligan produced new cultivars by crossing 'Salinas' and other cultivars with *L. serriola* (Ryder, 1988, 1989). Other breeding efforts added an estimated 8.7 and 10.2 days to flowering from their crosses between contrasting lettuce cultivars Vitoria x Brasil-303 and Baba x Elisa (Silva, 1999).

Variety trials in various places around the world have evaluated days to flowering and identified suitable cultivars for heat tolerance and other climactic challenges (de Souza, 2008; Dufault, 2006, 2009; Simmone, 2002). Dufault *et al.* evaluated approximately eight planting dates for seven lettuce cultivars to be used in long term commercial lettuce production in South Carolina (Dufault, 2006, 2009). Simonne *et al.* tested seventeen cultivars for production in the Southeastern United States, identifying suitable cultivars based on earliness to flower and bolting, consumer perception, and antioxidant content (Simonne, 2002). However, these same researchers explain that variation in quality 'are numerous and are a result of complex genetic, physiological, and environmental influences,' making it extremely difficult to know how specific cultivars will perform under climatic conditions of different regions and growing seasons (Dufault, 2006). Suitable cultivars and planting dates are therefore dependent on local conditions.

Bolting plants tend to taste bitter due to the buildup of certain chemical constituents. Total phenolics and sesquiterpene lactones are generally considered the primary components responsible for bitterness (Bunning, 2010). In one study, lactucin glycosides were identified as the principle sesquiterpene lactone conferring consumer perception of bitterness in colored lettuce and chicory (Price, 1990). Analysis of sesquiterpene lactones in ten green and red lettuce cultivars identified lactucopicrin as the most significant contributor to bitterness due to its higher concentration and lower bitterness threshold (Seo, 2009). Commercially available lettuces were found to have very low levels or were devoid of the three main sesquiterpene lactones present in their wild parents *L. saligna* and *L. virosa* (Tamaki, 1995). The major components of the milky exudate produced by *Lactuca* species are novel 15-oxalyl and 8-sulfate conjugates of the guaianolide sesquiterpene lactones, lactucin, deoxylactucin, and lactucopicrin (Sessa, 2000).

These bitter components create a product undesirable to consumers (Drewnowski, 2000). Bitterness is commonly used as a selection criterion for both breeding and identifying suitable cultivars for specific regions (Bunning, 2010; Ernest, 2012; Simonne, 2002). Thresholds have been established for detection of bitter constituents, and are useful in evaluating consumer acceptability (Van Beek, 1990).

Genetics may be the presiding factor for the bitterness perceived in bolting plants. Evaluation of sensory attributes and phenolic concentrations of diverse lettuce cultivars in response to growing season, found both sensory and chemical characteristics to be better correlated with cultivar than with environmental factors (Bunning, 2010). This demonstrates potential for the selection of existing cultivars and breeding of new cultivars for use in diverse climates. In any climate, limiting production of these constituents would create a superior cultivar. Therefore, bitterness should always be taken into consideration when evaluating consumer acceptance. Luckily, the concentration of these compounds and thus the perception of bitterness can be controlled by a mixture of culture and genetics.

1.2.3 Challenges and prospects for the Mid-Atlantic

Growers in warmer climates are limited by the tendency of current lettuce cultivars to flower early (bolt) and taste bitter, as described above. As a result, over 90% of lettuce produced in the U.S. is grown in only two states: California or Arizona (Jore, 2012). Growers in these areas shift their operations between AZ and CA to facilitate year-round production in a mild climate. In the Mid-Atlantic region, an area infamous for its hot, wet summers, a large scale lettuce production industry does not currently exist and small growers are typically unable to produce lettuce in the summer season. This is mainly due to a lack of suitable heat-tolerant cultivars. A variety trial at the University of Delaware in 2012 of 44 cultivars of butterhead, iceberg, leaf, and romaine lettuce for use in summer season only recommended five cultivars: one butterhead, one leaf, one romaine, and two iceberg cultivars. Ten other cultivars showed some heat tolerance, resistance to bolting, and reduced bitterness (Ernest, 2012).

In a preliminary planting in the same year, nine cultivars of heat-resistant iceberg and romaine heading lettuce were grown at the Wye Research and Education Center on the Eastern Shore of Maryland. Cultural practices were also implemented to increase time to flower and bolting, including reflective plastic and evaporative cooling with the goal of reducing soil and air temperatures. A number of these California cultivars showed great potential and are being evaluated in current research.

There is great demand in the Mid-Atlantic for local, heat tolerant lettuce cultivars. Successful production of lettuce in this climate would justify the establishment of a large-scale lettuce production industry. Reducing miles travelled by refrigerated vehicles is much more economical for farmers and reduces consumption of fossil fuels. The belt of farmers in the Mid-Atlantic region already producing summer leafy greens—mostly spinach and kale—demonstrates that there is land available for farming. In addition, a new production industry necessitates the establishment of new processing plants which will provide more jobs, economic benefits to the area, and reduced cost to processors and distributors. Warm climate lettuce production also has the potential to benefit small scale productions by providing growers with another marketable summer crop. In light of the growing local food movement, consumers will be happy to see another local vegetable in their markets and CSA baskets. However, all this is contingent on identifying cultivars suitable for the Mid-Atlantic summer climate.

1.3 Vermicompost soil amendment for use in lettuce production

1.3.1 What is vermicompost?

1.3.1.1 Definition

Vermicomposts are highly fragmented, soil-like, organic materials with exceptional physical, chemical, and biological properties (Brown, 2000; Edwards, 1998; Orozco, 1996). They are produced using a mesophilic bio-oxidative process in which earthworms, primarily epigeic or litter-feeding species such as *Eisenia foetida* and *Lumbricus rubellus,* and associated microorganisms and other soil decomposers interact to break down organic wastes (Dominguez, 2011a). Vermicomposting is an attractive solution to managing industrial, urban, and agricultural wastes including biosolids, paper waste, cattle manure, and vegetable refuse, because it accelerates decomposition time and requires significantly less labor input than traditional composting (i.e. windrows) (Edwards, 1998). Additionally, the production of vermicompost emits less greenhouse gas than comparable processes (Lleo, 2013). These materials have demonstrated benefits to a diverse array of plants (Edwards, 1998; Orozco, 1993).

1.3.1.1 The role of earthworms in vermicomposting

During vermicomposting, earthworms interact with organic wastes and associated flora and fauna both superficially and internally to create physical, chemical, and biological changes (Brown, 2000). The sphere of influence of earthworms in the environment, including the earthworm populations, as well as the entire soil volume, microbial and invertebrate populations affected by the earthworms has been termed the 'drilosphere' (Brown, 2000; Lavelle, 1988). External influences include the excretion of mucus and physical contact with soil particles, leading to movement of soil particles during the shaping of burrows and the stimulation of microorganisms by the secreted mucus and associated enzymes (Brown, 2000).

Material ingested by earthworms is subjected to the earthworm digestive system. Organic material enters the earthworm through the mouth or buccal chamber of the earthworm. It then passes through the pharynx, where the pharyngeal gland secretes an acid mucus that coats particles and aids in decomposition (Edwards and Bohlen, 1996). This mucus plays a major role in the decomposition of organic material, stabilizing pH, providing available nutrients for endogenous microorganisms, and coating of material excreted by the earthworm. Material then travels through the esophagus into the crop and gizzard. In this extremely muscular segment, material is mechanically broken down into small fragments (Edwards and Bohlen, 1996).

Next, material enters the intestine, the final portion of the digestive tract, and the majority of the length of the earthworm in which enzymes are secreted and nutrients are absorbed. Here, decomposition is accomplished primarily by gut-associated microorganisms capable of secreting extracellular enzymes which degrade cellulose and phenolic compounds (Dominguez, 2011a; Jack, 2011). Digestive enzymes found in the gut of different species of earthworm include chitinase, protesase, phosphatase, cellulase, and many other glucosidic enzymes (Brown, 2000). While there is some evidence that earthworms produce certain digestive enzymes and may derive nutrients directly from leaves, earthworms mostly rely on detritivorous organisms for available forms of nutrients (Edwards and Bohlen, 1996; Jack, 2011). In return, the mucus secreted by the earthworm digestive tract provides microorganisms with an available carbon source. It is still unclear whether these organisms are specific endosymbionts of earthworms or if they are ingested with other organic material (Jack, 2011).

Decomposition processes that occur within the earthworm gut can be classified as Gut Associated Processes or GAPs (Dominguez, 2011a). After material is excreted it undergoes a maturation-like phase, similar to the maturation phase of windrow compost, during which materials undergo cast associated processes or CAPs. CAPs are indirectly caused by earthworm activities and direct effects of microorganisms colonizing the material, and physical modifications (Dominguez, 2011a). During maturation, vermicomposts reach their optimum in terms of biological properties, but it is still unclear what this optimum is and what may be the expiration date (Dominguez, 2011a).

The final product of vermicomposting is a finely divided, organic material with improved physical, chemical, and biological properties. The following sections will explore specific properties of vermicompost in more depth and the resulting effect of vermicompost on crops.

1.3.1.3 Physical properties

Epigeic earthworms, the earthworms used most often in vermicomposting are primarily litter dwellers, limited to the top few centimeters of soil. In forest ecosystems this corresponds to the LFH or A_o horizon: mostly recognizable, decaying organic matter. In vermicomposts, earthworms will typically be present in the top few centimeters of material, moving upward in a pile if fresh material is added or pile is turned.

Through vermicomposting, earthworms physically rotate and aerate the pile contributing to communition or physical grinding of soil particles into smaller particles, incorporation of oxygen and moisture, and dispersal of materials and associated microorganisms (Brown, 1995). By burrowing and casting, earthworms contribute physically to porosity, aggregation, pedogenesis, and litter breakdown (Brown, 1995). Naturally, these properties are overlapping with chemical and biological properties that are further described below.

The result is a material with overall improved physical characteristics. Excrement or ‘castings’ are mostly in the form of mucus which acts as lubricant, binds soil particles to form burrow walls, and gives particles aggregative, soil-like consistency (Edwards and Bohlen, 1996). It has been well documented that these materials exhibit increased aeration, porosity, and drainage (Edwards, 1988; Hidalgo, 2006). Vermicompost is highly fragmented, giving it a greater surface area than related materials such as feedstock or windrowed compost (Garg, 2006; Pereira, 2014), contributing more sites for adsorption of nutrients and plant growth compounds, and microbial colonization.

1.3.1.4 Chemical properties: nutrient dynamics, heavy metals, humic substances, and plant growth regulators

Vermicomposting has various and often seemingly conflicting effects on the chemical characteristics of organic matter. This includes changes in nutrient dynamics [carbon (C), nitrogen (N), and phosphorous (P) as well as many micronutrients], humic substances, heavy metals, and plant growth promoting compounds such as plant growth hormones.

Activity of earthworms through vermicomposting both consumes and conserves C. Vermicompost is typically much lower in organic C than the original material because organic C is metabolized by the worms and gut-associated microorganisms. C is used as an energy source for these organisms and is lost to the atmosphere as CO_2 in respiration. However, in the long term, C is also protected by mucus produced by the earthworm gut, which coats the materials forming soil-like aggregates which line the walls of earthworm burrows (Brown, 2000; Lavelle *et al.*, 1988).

Vermicomposting also contributes to both the stabilization and leaching of other nutrients. Increases in plant-available forms of N, P, and K in vermicomposts compared to starting materials have all been reported (Arancon, 2011; Orozco 1996). This has been attributed to increased microbial activity which converts organic forms of nitrogen to inorganic or mineral forms through ammonification and nitrification. Increased nutrient availability has also been attributed to increased surface area of the material and increased humic acids, which create more places for mineral forms of nutrients to be adsorbed (Dominguez, 2011b).

Vermicomposts are extremely high in humic substances (humines, humic acids, fulvic acids), visually apparent from the characteristic rich black color (Arancon, 2006; Pereira, 2014). The high concentration of hydrophilic groups in these materials coupled with high surface area provide a plethora of binding sites for essential nutrients (Arancon, 2006; Pereira, 2014). Therefore, nutrients are often in higher concentration (de Souza, 2008; Hait, 2012; Hidalgo, 2006; Manivannan, 2009; Pereira, 2014). Gas chromatography mass spectrometry has been used to show that these humic substances bind with plant growth regulators (PGRs), such as auxin, which may influence plant growth (Canellas, 2002).

These binding sites also make vermicompost suitable for bioremediation of heavy metals (Pereira, 2014; Hait, 2012). Heavy metals are adsorbed onto soil particles instead of leaching into the surrounding environment; this material can then be collected. However, the potential for vermicomposts to retain high levels of heavy metals presents concern for phytotoxicity when used as a soil amendment for crops. Vermicompost enriched with Cu, Ni, or Zn decreased lettuce yields compared to natural vermicompost materials applied at rates $\geq$50 t ha^{-1} (Jordao, 2006; 2013). Contrasting research found that vermicomposting reduced the availability of heavy metals although it did not affect availability of Fe and Mn (Hait, 2012). Current research findings are inconclusive about whether the ability to retain heavy metals has a negative impact on crops.

1.3.1.5 Biological properties: Microbial activity in vermicompost

Decomposition of organic matter in the soil is mediated by a diverse food web of microorganisms, protozoa, and invertebrates, whose complex interactions include mutualism, predation, competition, and facilitation (Dominguez, 2011a). Microorganisms, primarily bacteria and fungi, are the most abundant of these decomposers. Earthworms have a profound effect on the composition of microorganism communities. As they move through organic matter or soil, they interact with the material and the associated organisms both superficially and internally, thus affecting the density, diversity, structure, and activity of the microbial community (Brown, 1995, 2000). Casts produced during this process are colonized by microorganisms considered beneficial for plant growth (Dominguez, 2011a; Jack, 2011).

The effect of the earthworms on microbial activity is mostly attributed to the intestinal mucus secreted in the gut and gut-associated enzymes, $CaCO_3$, and antimicrobicidal substances (Brown, 1995). The combination creates a favorable environment for many microorganisms by providing an available C source, neutralizing pH, and reducing competition of certain microorganisms. This environment has the effect of stimulating previously dormant microorganisms, an effect termed the 'Sleeping Beauty Paradox' by Brown et al (2000). The microorganisms (sleeping beauties) are reawakened by the kiss (mucus) of prince charming (the earthworm).

A number of studies have documented an increase in overall microbial activity and biomass in vermicomposts. Arancon *et al.* reported significant increase in both microbial biomass-N and dehydrogenase activity in field soils amended with vermicompost, two "excellent indices of overall microbial activity" (Arancon *et al.*, 2006). Other research found microbial number (CFU), microbial biomass-C and respiration rate also to be significantly increased in compost- and vermicompost-amended soils (Chaoui, 2003; Manivannan, 2009). In corn trials, microbial biomass-C and enzyme activity (dehydrogenase, urease, β-glucosidase, phosphatase, arylsulfatase) were significantly higher in vermicompost-amended soils than compost-amended soils by the end of a three year period (Tejada, 2011). On the other hand, phosopholipid fatty acid (PLFA) analysis of organic matter from experiments using vermicompost 'mesocosms' showed an overall decrease in microbial biomass (Dominguez, 2011a). Mesocosms consisted of 2 L jars containing pig slurry feedstock that were colonized by earthworms (*Eisenia andrei*) for 1 month. Carbon availability and competition with earthworms were cited as possible limiting factors for microbial growth. However, samples from these mesocosms were analyzed immediately after sampling. Subsequent maturation period in combination with high surface area of vermicompost product may lead to an increase in microbial activity and biomass over time, similar to results of other experiments.

Research has shown that earthworms also have a significant impact on the structure of the microbial community. In the mesocosm experiments described above, specific PLFAs were used as biomarkers to characterize microbial communities by determining presence or absence of specific microbial groups. Results indicated that earthworm species had a significant effect on microbial community regardless of feedstock material (Dominguez, 2011a). Similar experiments analyzing fatty acid methyl esters (FAMEs) profiles yielded similar results (Lores *et al*., 2006). These studies demonstrate the key role earthworms play in shaping the microbial composition of vermicomposts. Other research has found that earthworms selectively stimulate certain actinomycetes (*Nocardia, Oerskovia,* and *Streptomycetes*) and bacteria (*Vibrio* sp.) (Brown, 1995). This selective stimulation has been attributed less to selective feeding, and more to production of antibiotics by actinomycetes in the gut.

Other work has attempted to characterize the specific microbial communities found in vermicomposts. Using scanning and transmission electron microscopy (SEM and TEM), Jolly *et al*. (1993) were able to describe physical characteristics of flora of the gut wall of two earthworm species. They identified multiple physical attachments of bacteria species that allowed them to persist longer in the earthworm gut. Fracchia *et al*. (2006) used amplified 16S rDNA to characterize differences in microbial communities in compost and vermicompost that was 1, 2, and 12 years old. They were able to describe two very different communities that had little variation between replicates: phyla Firmicutes and Actinobacteria were the primary actors in compost while Chlorflexi, Bacteroidetes, and Gemmatimonadetes were predominant in vermicompost (Fracchia *et al.*, 2006).

Gopal *et al.* (2009) investigated the effects of vermicomposting two different feedstock materials using *Eudrilis* sp. on microbial communities, with a concentration on beneficial bacteria. Population densities of 15 microbial groups were amplified including general microflora (aerobic heterotrophic bacteria, filamentous actinomycetes, fungi, and spore forming bacteria) and plant beneficial bacteria (free-living N_2 fixers, *Azotobacter*, *Azospirillum*, autotrophic *Nitrosomonas*, autotrophic *Nitrobacter*, ammonifying bacteria, fluorescent pseudomonads, phosphate solubilizers, cellulose degraders, silicate solubilizers, and *Trichoderma* spp.). Densities of groups were found to be different in substrate, earthworm gut, and vermicompost, and dependent on starting material.

Overall, 9 of the 15 groups were amplified in a substrate consisting of coconut leaves and 10% cow manure, while 5 groups were amplified in cow manure only. Differences were attributed to nutritional interactions and requirements of earthworms, feed quality, and survival of different microorganisms through the earthworm gut (Gopal *et al.*, 2009). For example, *Azotobacter* and *Trichoderma* were not found in vermicompost product of substrate lacking cow manure, possibly due to negative influence of earthworm gut activity. Also, *Nitrosomonas*, P-solubilizers, and silicate solubilizers decreased in the earthworm gut and then increased in vermicompost, possibly due to the increased availability of O_2 once expelled. The influence of nutrient availability provided by vermicompost is examined further below.

In general, earthworms produce an environment that selectively stimulates microorganisms. In return, these microorganisms aid the earthworm by decomposing organic material such as lignin and cellulose. This mutualistic relationship leads to the accelerated decomposition of organic matter accomplished by vermicomposting, and the improved physical, chemical and biological properties of final products.

1.3.1.6 Quality of vermicompost material is dependent on multiple factors

Quality of vermicompost is dependent on many variables ranging from composting environment to feedstock material. Unfortunately, most studies have focused on demonstrating the feasibility of one suitable production system—usually motivated by the need to recycle one form of organic waste associated with a region or industry. Some studies, however, have examined differences in materials produced under different conditions. Finished vermicomposts produced from varying feedstock materials had differing amounts of total Kjeldahl Nitrogen. N was highest in textile sludge, followed by textile fiber, institutional wastes, agro-residues, and kitchen waste respectively (Garg, 2007). In addition, reduction of total organic carbon was different for the different feedstocks (Garg, 2007). Studies comparing the effects of vermicompost produced from animal manure, vegetable waste, and paper wastes and found differing properties and different effects on crop performance, with animal manures typically performing better (Arancon, 2006; Tejada, 2011). In these experiments, total N applied to the field was balanced using synthetic fertilizers to meet nutrient recommendations; this eliminated N concentration as an explanatory factor for differential performance.

Liu and Price compared three methods of vermicomposting spent coffee grounds: an enclosed stainless steel vessel, aerated static piles, and vermicompost bins (Liu, 2011). They found changes in temperature and nutrient concentration during composting to be very different between the different methods. Nutrient levels and earthworm mortality also differed in composts of different feedstocks: spent coffee grounds with cardboard supported higher populations than just spent coffee grounds or coffee grounds with coffee filters (Liu, 2011). Suthar compared different sized vermi-reactors with the goal of identifying differences between small scale (experimental size) and pilot-scale (larger) operations. Clear differences were found between the different operations: in pilot-scale operations, mineralization rates were lower and total available N and P higher (Suthar, 2010). This was attributed to different microclimatic conditions as well as different growth and reproduction patterns of earthworms in these systems (Suthar, 2010).

In contrast, different earthworm species do not seem to produce vermicompost of differing quality. Khwairakpam and Bhargava examined the effects of three different species of earthworms commonly used in vermicomposting (*Eisenia foetida*, *Eudrilis eugeniae*, and *Perionyx excavatus*) in monocultures and in combinations. All treatments produced highly stable compost material, but there were no differences in quality with regard to pH, electrical conductivity (EC), total organic carbon (TOC), total phosphorous, total nitrogen, and biological activity (oxygen uptake rate) (Kwairakpam, 2009). Earthworm species does not appear to have an effect on properties of finished vermicompost.

1.3.2 Agricultural use of vermicompost: +/- effects on crops

Extensive research has been conducted evaluating the impact of vermicompost materials on plant growth. In landmark studies performed in the 1980-90s at the Rothamsted Institute in the U.K., plants of over 25 varieties grown in vermicompost outperformed control plants (Edwards, 1992). However, studies since then have shown both positive and negative effects of vermicompost as a soil amendment. Table 1.1 lists studies investigating the effects of vermicompost materials on crops that will be examined in the present review, organized by crop, vermicompost feedstock, and nature of effects on crops.

Table 1.1. Effects of vermicomposts applied to field and greenhouse grown crops

Primary Author	Year	Crop(s)	Environment	Feedstock	Results[z]
Edwards	1992	various	Field	various	+
Arancon	2004	pepper	Greenhouse	food waste	+/-
Peyvast	2008	spinach	Greenhouse	cattle manure	+
Ievinsh	2011	garden bean, pea, beetroot, radish, cabbage, Swedish turnip	Greenhouse	cattle manure	-
Bachman	2008	tomato, marigold, pepper, cornflower	greenhouse, field	pig manure	+/-
Paul	2005	tomato, eggplant, pepper	greenhouse, field	cattle manure	+/-
Arancon	2005	pepper	Field	cattle manure	+
Arancon	2004	strawberry	Field	food and paper	+
Tejada	2011	maize	Field	manure, food waste, cotton gin	+
Atiyeh	2002	tomato, cucumber	greenhouse	pig manure, pig and food waste	+
Arancon	2006	marigold, pepper, strawberry	greenhouse	cattle manure, food waste, paper	+

[z]positive or negative effects on crops

1.3.2.1 Effects of vermicomposts on greenhouse crops

Studies evaluating vermicompost have largely been conducted in controlled greenhouse environments on containerized plants. The majority of these studies looked at a vermicompost product incorporated with commercial potting mix at different rates based on % (v/v) from 0 to 100 in increments of 10. In this way Arancon *et al*. demonstrated positive effects of vermicompost generated from food scraps on peppers (*Capsicum annuum*) (Arancon *et al.*, 2004a). Rates of up to 60% vermicompost increased fruit weights and fruit number, but higher concentrations decreased fruit weight and number indicating a threshold level where plants experience a toxicity to the material (Arancon *et al.*, 2004a). The optimum rate in their work was lower, only 10% vermicompost.

More recently, Ievinsh *et al*. showed that effects of vermicompost may depend on crop (Ievinsh, 2011). Their greenhouse studies used similar methods and materials as previous studies: vermicompost produced from cattle manure was incorporated into seed-starting media at multiple rates. However, evaluation of garden beans (*Phaseolus vulgaris* L. 'Purple King'), peas (*Pisum sativum* L. 'Rani'), beetroot (*Beta vulgaris* L. 'Cylindra'), radish (*Raphanus sativus* L. 'Crimson Giant') and two cultivars of both cabbage (*Brassica oleracea* L. 'Copenhagen Market 2' and 'Golden Acre') and Swedish turnip (*Brassica napus* var. *napobrassica* L. 'Grunkopfige Gelbe Wilhelmsburger' and 'Golden Ball') demonstrated an 'almost linear' decrease in germination rate with application of compost (Ievinsh, 2011). Interestingly, the vermicompost treatment increased chlorophyll content and photosynthetic activity in plants, suggesting that, while vermicompost had a negative effect on germination of these crops, there was a positive effect on other aspects of their physiology (Ievinsh, 2011).

Other studies have evaluated both germination and subsequent plant growth. Bachman and Metzger reported that vermicompost produced from pig manure used as seed starting media amendment for tomato (*Solanum lycopersicum* Mill. ‘Rutgers’), marigold (*Tagetes patula* L. ‘Queen Sophia’), pepper (*Capsicum esculentum* L. ‘California Wonder’), and cornflower (*Centauria cyanus* L. ‘Imperial’) did not significantly affect germination rate, but when seedlings were subsequently transplanted in the field with amended soil, growth rate of shoots and roots was enhanced by vermicompost (Bachman, 2008). Contradictory to these findings, vermicompost produced from cattle manure used in seed starting media for tomato (*Solanum lycopersicum* L.), eggplant (*Solanum melongena* L.), and peppers (*Capsicum annuum* L.) resulted in enhanced seedling growth in the greenhouse, but did not have a significant effect once plants were transplanted into unamended fields (Paul, 2005). Timing and location of application of vermicompost appears to depend on desired results and life stages of plants, and may not carry over to the field when crops are transplanted from the greenhouse.

1.3.2.2 Effects of vermicomposts on field crops

On the other hand, field studies in which vermicompost amendment was added to the soil typically showed positive results. Arancon *et al*. conducted several field studies involving strawberries (*Fragaria x ananassa*) and peppers (*Capsicum annuum* L.) showing that vermicompost produced from cattle manure, food scraps, and paper waste were each more effective than synthetic fertilizer and sometimes windrowed compost at increasing vegetative growth, yield, leaf area, shoot biomass, flower number (Arancon, 2004b, 2005). Vermicompost treatments significantly increased marketable fruit weight and decreased nonmarketable fruit weight. They were able to eliminate nutrient levels as a possible cause by equalizing the NPK in all plots. In the first year of the study, amendments were applied at 10 and 20 t ha^{-1}, but were lowered to 5 and 10 t ha^{-1} in the second year of the study. These lower rates appeared sufficient for improved performance (Arancon, 2004b, 2005).

Tejada *et al*. demonstrated a positive effect of similar vermicompost products on corn (*Zea mays* 'Tundra') (Tejada, 2011). Results showed that vermicomposts produced from cattle manure, food scraps, and cotton gin waste also applied at 5 and 10 t ha^{-1} increased yield, leaf NPK, and pigments. These studies took place over the course of three years of application, demonstrating an increased plant performance with accumulation of vermicompost (Tejada, 2011).

In the majority of these studies, vermicompost amendments had favorable effects on crop germination, growth, and yield when applied at an appropriate rate. All feedstock material appeared to have similar results, however, manure often showed better results, though not necessarily significantly better. However, responses appear to vary with crop and at different life stages, and results depend on crop type, rate, and compost characteristics. The positive results appear to be linked to abiotic and biotic characteristics of vermicompost.

1.3.2.3 Isolated humic substances from vermicomposts

A few sources attributed increased crop performance to humic substances unique to vermicomposts. Several studies extracted humic acids from vermicompost sources and applied them in a rate study on greenhouse tomatoes (*Solanum lycopersicum* L. 'Rutgers') and cucumbers (*Curcumis sativus* L. 'Long Green') or marigold (*Solanum lycopersicum* L. 'Rutgers'), pepper (*Capsicum annuum grossum* 'King Arthur'), and strawberry (*Fragaria x ananassa* L. 'Tribute') respectively (Arancon, 2006; Atiyeh, 2002). These studies reported increased growth and fruit number with increased vermicompost addition up to about 500-1000 mg humate per kg container medium after which plant growth and fruit development dropped off (Arancon, 2006; Atiyeh, 2002). Increased growth were attributed to hormone-like activity of the humic substances or plant hormones adsorbed to the humates.

1.3.3 Nutrient availability provided by vermicompost materials

As previously mentioned, vermicomposts are a good source of plant-available essential nutrients. However, concentration and availability may vary significantly between vermicomposts. Well-established methods have been used to quantify differences in mineralization rates and total extractable forms of nutrients in a variety of organic materials (Honeycutt, 2005; Tyson, 1993). Typically, controlled lab incubation experiments have been used to characterize materials ranging from raw animal manures to stabilized compost (Honeycutt, 2005; Tyson, 1993). In these experiments, samples are taken periodically, then extractions are used to quantify nutrients and generate a curve illustrating nutrient release and transformations (i.e. mineralization rates). Such tools and characterizations are extremely useful for making recommendations for application of organic materials as nutrient supplements.

It is also possible to evaluate nutrient availability in the field. Due to the increased number of variables involved, experiments typically are restricted to reporting on relative difference among treatments. Experiments measuring plant available forms of nutrients in the primary succession ecosystem of Mount St. Helen's after the 1970 eruption used anion-cation exchange resin bags to measure relative differences in nutrient availability (including net N-mineralization) in the top 10cm of soil in various ecosystem patches. A modified resin-core was effective in measuring NH_4^+, NO_3^-, and soluble reactive P in wetland soils (Orozco, 1996) and resin cores and buried resin bags were effective in Costa Rican lowland rainforests (Zou, 1992). Resin bags were also found to be an effective and reliable method of quantifying net N mineralized for in situ agricultural experiments on land under a 3-year no-till crop rotation, although variation tended to be large and required many replicates to detect differences in treatments (Kolberg, 1997). Resin membranes were also used to estimate N mineralized in sugar beet plots during the growing season (Sims, 2006). However, a comparison of in situ methods of measuring net N mineralization of soil amended with organic materials found that resin bags and a new soil-resin trap underestimated long term N mineralization rates. This was because N adsorption efficiency was reduced beyond 45 to 90 days, most likely due to altered water content dynamics within devices and degradation of bags and traps (Hanselman, 2004).

Some work has been done to quantify and compare nutrient availability in vermicomposts. Chaoui studied mineralization rates and nutrient uptake in an incubation experiment and wheat bioassay. Although synthetic fertilizers began with higher levels of total extractable N (ammonium and nitrate), levels decreased steadily over time; organic amendments (vermicompost and compost) on the other hand, saw an increase in total extractable N that peaked at 35 and 43 days respectively (Chaoui, 2003). Total extractable P and K were higher in soils amended with organic composts than in those with synthetic fertilizer (Chaoui, 2003). The results of the study demonstrate that vermicomposts are an effective source of slow-release, readily available nutrients for plants (Chaoui, 2003). Similarly, Manivannan *et al.* found vermicomposts to be effective sources of plant available nutrients. They demonstrated a significant increase in available N, P, K and many micronutrients in soils amended with vermicompost as compared to soils amended with synthetic fertilizer, where both were applied at recommended rates (Manivannan, 2009).

The time scale of nutrient availability is also critical when applying both organic and synthetic fertilizers. For lettuce, as well as many other crops, nitrogen is the limiting essential nutrient. Lettuce typically takes up 100-120 lbs of nitrogen per acre. While a crop takes approximately 65-70 days to mature, N uptake is very low in the first 30 days (Smith, 2009). Therefore, for best management practices, nitrogen availability should be greater after the first half of the growing period.

To accommodate nitrogen needs while minimizing leaching of nutrients, slow release fertilizers (SRFs) or controlled release fertilizers (CRFs) can be used. The purpose of these materials is to provide sufficient nutrient levels to crops while reducing nutrient leaching (Morgan, 2009). They do so by gradually making nutrients available as compared to typical synthetic fertilizers which often provide nutrients in an available form all at once.

Composts, including vermicompost, are considered SRFs. Mineralization occurs gradually in these materials, facilitated by microbial decomposition or chemical hydrolysis (Morgan, 2009). In studies comparing the mineralization and nitrification rates of organic materials over 75 days, different materials exhibited very different patterns of release rates (Chaoui, 2003). Characterizing specific composts based on the time scale of nitrogen release and availability would be helpful for recommending application rates of such materials and matching them to specific crop needs.

1.3.4 Plant disease suppression by vermicompost

In addition to improving physical soil conditions, introducing growth-promoting compounds and beneficial microorganisms, and increasing nutrient availability, vermicompost is used to provide protection from many plant pests. Research has shown that applications of various kinds of compost in both the greenhouse and the field has the potential to suppress many common soil-borne pathogens including damping-off and root rots (*Pythium ultimum*, *Rhizoctonia solani*, *Phytophthora* spp.), and wilts (*Fusarium oxysporum,* and *Verticillium dahliae*) (Noble, 2005). However, there is significant variation in level of disease control between and within studies, and even within treatments of the same study. Variations depend on a breadth of factors including soil treatment, pathogen, inoculum, crop, soil type, application rate and replication, and environment (Jack, 2011; Noble, 2005). As expected, results of field experiments tend to be less consistent than containerized experiments.

Research examining the effects of vermicompost and earthworms on plant disease has also yielded variable results. Earthworm activity was correlated with significant reduction in disease caused by *Gaeumannomyces graminis* var. *tritici* on wheat (Stephens and Davoren, 1995) and caused significant reduction in *Fusarium culmorum* biomass of infected wheat straw applied to the soil surface (Wolfarth, 2011). Earthworms have also been effectively used to spread biocontrol agents such as disease-suppressive microorganisms. *Apporectodea* spp. was able to spread *Pseudomonas corrugata,* an effective biocontrol for *Gaeumannomyces graminis* significantly deeper than in control pots (Stephens *et al.*, 1993).

Action of vermicompost soil amendment in plant disease control is more variable, and less studied than compost. Szczech and Smolinska (2001) showed significant suppression of *Phytophthora nicotianae* (Breda de Haan) of tomato seedlings by addition of vermicomposted animal manure. However, results were not always positive. Root and stem rot of cucumber (*Fusarium oxysporum* f.sp. *radicis-cucumerinum* Owen) while suppressed by vermicomposted cattle manure, was not suppressed by vermicompost of the same feedstock (Kannangara *et al.*, 2000).

Increased rate and number of applications of both compost and vermicompost tend to increase suppression of disease (Jack, 2011; Noble, 2005). However, higher rates may be detrimental to plant health due to factors such as increased compaction and salt stress. Clearly, multiple factors need to be taken into account when using composts in suppression of plant diseases.

Suppressive action of composts and vermicomposts on plant diseases may be explained by a number of different mechanisms. Effects have mostly been attributed to biological factors, especially introduction of beneficial microorganisms. Postulated mechanisms include competition, antagonism, and antibiosis between such microorganisms and pathogenic organisms, stimulation of innate systemic response (ISR) in plants, increased plant vigor, metabolizing of plant exudates by beneficial microorganisms, and improved soil properties (Noble, 2005). Possible antagonist species present in compost amended substrates include a broad range of organisms including *Bacillus* spp., *Enterobacter* spp., *Flavobacterium balustinum* Harrison, *Pseudomonas* spp. (one of the most important groups of plant growth promoting rhizobacteria PGPR), and *Streptomyces* spp.; as well as *Penicillium* spp., several *Trichoderma* spp., *Gliocladium virens* Miller, Giddens & Foster, among others (Hoitink, 1986).

Suppression of plant pathogens by vermicompost materials does seem likely. However degree of suppression and control is highly variable due to the complex nature of soil systems and the numerous effects these materials have on the physical, chemical, and biological condition of the soil. More research is necessary to determine specific mechanisms of disease suppression, especially through control of the numerous variables involved.

1.3.5 Food safety considerations of vermicompost

Outbreaks of food-borne illness—most commonly linked to the human bacterial pathogens *Salmonella enterica* and *Escherichia coli*—have become much more frequent in the past twenty years, especially on fresh produce. Outbreaks have been attributed to both pre- and post-harvest handling procedures, and have likely become more common due to increase in consumer consumption of fresh produce and its centralized preparation and distribution (Fonseca, 2007). The most common cause of outbreaks is cross-contamination between the produce and another tainted material, typically raw meat and dairy in the kitchen and water or manure in the field (Fonseca, 2007). This is an especially important concern for vermicompost usage as its production is not a thermophilic process. Earthworms require an environment between 15-25^{0}C (59-77^{0}F) which are insufficient temperatures to kill human pathogens, leading to possible contamination of fresh produce.

A few studies have investigated the impact of earthworms on human pathogens. Experiments were conducted in which windrows of biosolids were inoculated with four human pathogen indicators (*Salmonella*, fecal coliforms, enteric viruses, and helmintha ova). Activity of *Eisenia foetida* was demonstrated to decrease all of the pathogens within 144 hours when compared to control rows (Eastman, 2001). Reductions in fecal coliforms were 6.4-log reduction for windrows with earthworms as compared to 1.6-log reduction in control rows. Reductions of all indicator organisms were significant enough for vermicomposting to be considered as an effective means of stabilizing these materials. In another study that examined vermicompost as a means of stabilizing sewage waste, earthworms were capable of eliminating fecal coliforms and *E. coli* while traditional composting methods were not (Sinha, 2010). The number of total coliforms was reduced on average by over 99% for vermicompost reactors using three types of earthworms (Khwairakpam, 2009). The ability of earthworms to sterilize feedstock material during vermicomposting may be attributed to the microflora of the earthworm gut, which is colonized by a diverse collection of bacteria and fungi, including some such as *Penicillium* spp. and *Aspergillus* spp. that produce antibiotics capable of killing human pathogens (Singleton, 2003; Sinha, 2010).

It is also possible that earthworms may not completely neutralize pathogens in a material and instead act as a vector for pathogenic organisms. An investigation of the spatial and temporal survival of *E. coli* O157:H7 inoculum in materials vermicomposted with *Dendrobaena veneta* found that movement of *E. coli* both vertically and laterally could be completely attributed to earthworm movement (Williams, 2006). During the initial stages of composting, *E. coli* O157:H7 concentration was significantly higher in vermicomposts than in inoculated soil, but concentrations were statistically similar after 21 days (Williams, 2006). The results demonstrate that some earthworms are capable of acting as vectors and temporarily increasing concentrations of human pathogens.

It is still unclear what impact earthworms have on human pathogens under field conditions. Characteristics seem to vary based on inputs and treatment of materials. Therefore vermicompost may still pose a food safety risk and is worthy of investigation.

1.4 Summary of future research

Change in lettuce production in the United States favoring a more local approach clearly has the potential to have a positive impact on sustainability. Despite the popularity of lettuce, its production is limited by climate. Identifying cultivars adapted to hot summer climates through breeding and potentially genetic modification should be a target goal. Efforts should be concentrated on elucidating genes and molecular pathways responsible for bitterness and bolting. Because vegetable quality is the result of a complex interaction between genetic, physiological, and environmental factors, extensive variety trials evaluating yield, quality, and sensory characteristics will need to be conducted in specific regions to identify suitable cultivars for that location.

Incorporation of vermicompost into lettuce production seems likely to have a favorable effect on yield and quality of the crop. Soils amended with vermicompost are generally reported to have improved physical, chemical, and biological characteristics, resulting in improved crop performance. However, effects are dependent on nature of the material and the production.

The literature is somewhat lacking in studies that systematically compare specific vermicomposts to each other and to similar materials. It would be useful for research to compare the effects of a wide variety of feedstock, vermicomposting methods, crops, and production methods. However, due to the nature of vermicompost—generally produced locally, in an effort to recycle organic wastes from a particular industry or farm—it may be unfeasible to execute such a broad comparison. Therefore, characterization of specific materials and operations may be the best approach.

Such studies should attempt to quantify physical, chemical, and biological characteristics of the materials and amended soils, as well as the effects on crops. One area of focus should be nutrient availability and release rates under different conditions, especially variations in temperature and moisture. Although many studies have quantified biological activity in the form of microbial respiration and biomass, this is unspecific and yields limited information. Instead, studies might focus on characterization of bacterial communities using Next-Generation sequencing technology.

Food safety risk evaluation is another critical issue that should be examined. The role of earthworms in the spread or control of human pathogens should be carefully evaluated. Various vermicomposting methods and their influence on food safety risk should be further explored.

Chapter 2: Manuscript

2.1 Introduction

Sustainable agriculture is a growing industry, with increasing demand stemming from environmental concerns, social equity, food security, and financial viability (Ikerd, 1990; Lyson, 2004). Farmers are continuously seeking innovative ways to satisfy these demands. The local model is one approach recognized for effectively increasing sustainability. Communities that have adopted a local or "civic agriculture" approach in full or in part often have reduced environmental impact, experience greater welfare for the people in their communities, and provide farmers with a unique niche in the market (Lyson, 2004). Thus, local agriculture is capable of satisfying the three criteria of sustainable philosophy: environmental, social, and economical sustainability (Lyson, 2001). Numerous agricultural practices exist facilitating this model. However, each situation requires unique and dynamic solutions. It is essential to continue developing new solutions ranging from novel crops to sustainable inputs.

Lettuce is an ideal target for increasing sustainability. It is one of the most important vegetable crops in the United States (Jore, 2012). It is a cool-weather crop with a heat-threshold of approximately 27^{0}C (80^{0}F) (Smith, 2011). Exposure to high temperatures and long days triggers premature flowering, the formation of loose heads (Ryder, 2005; Smith, 2011; Waycott, 1995), and buildup of bitter constituents unacceptable to consumers (Bunning, 2010; Drewnowski, 2000; Price, 1990). Due to its climatic limitations, heading lettuce is grown almost exclusively in cool districts in

Arizona and California. A tremendous amount of fossil-fuel resources and food miles are expended to distribute the fresh product across the U.S.

Season extension is one solution to sustainably producing lettuce. Identification of heat-tolerant cultivars with delayed bolting or flowering that could be grown into the summer season would significantly increase local production in warmer climates. Several genes responsible for early flowering have been identified (Ryder, 1988, 1989; Silva, 1999; Waycott, 1995). Crossing existing cultivars, crossing *L. sativa* with wild relatives, and subsequent variety trials have been used to produce and identify cultivars suitable for warmer climates (Silva, 1999, Ryder, 2005, Dufault, 2006, Dufault, 2009, Simmone, 2002, de Souza, 2008).

Vegetable quality is influenced by complex physiological, genetic, and environmental factors highly dependent on the specific region (Dufault, 2006). In the Mid-Atlantic, a large-scale commercial lettuce industry does not exist and small local growers have difficulty growing a crop beyond the spring and fall seasons. However, there is an established market in this area of local growers with dedicated customers, offering the potential for such an industry. Identification of suitable cultivars would facilitate growth of these industries, increasing the sustainability of lettuce production for the area.

Vermicomposting is another possible solution for increasing sustainability using the local model and can be incorporated into sustainable lettuce production. Vermicomposts are soil amendments with improved physical, chemical, and biological properties produced using worms to decompose and recycle organic wastes from industrial, urban, and agricultural operations. Research has demonstrated that these materials have beneficial effects on yield and quality of a variety of crops (Arancon, 2004a, 2004b, 2005, Atiyeh, 2002, Edwards, 1988, Edwards 1992, Peyvast, 2008, Tejada, 2011). Increased performance has been attributed to improved physical and chemical properties of the soil (Edwards, 1992, Edwards, 1998, Orozco, 1996, Garg, 2006, Hidalgo, 2006, Pereira, 2014) quantity and availability of nutrients (Hait, 2012, Pereira, 2014, Hidalgo, 2006, Manivannan, 2009, Chaoui, 2003), and beneficial microbial activity (Arancon, 2006, Chaoui, 2003, Manivannan, 2009, Tejada, 2011, Fracchia, 2006).

Vermicomposts are typically produced locally on a fairly small scale and their production is usually motivated by the need to recycle organic waste from a specific industrial or agricultural operation. Due to the nature of its production, characteristics of materials from different sources will vary. Quality is contingent on factors such as feedstock, production size and method, earthworm behavior, and nature and rate of application (Garg, 2006, Liu, 2011, Suthar, 2010).

As a result, the effects of these materials as soil amendments on yield and performance vary with vermicompost material and nature of application. Many studies have demonstrated increased growth and yield, most notably on pepper (*Capsicum annum* L.) (Arancon, 2004a, 2005, 2006), strawberry (*Fragaria* x *ananassa*) (Arancon, 2004b, 2006), spinach (*Spinacea oleracea*) (Peyvast, 2008), maize (*Zea mays*) (Tejada, 2011), and cucumber (*Cucumis sativus*) (Atiyeh, 2002). Increased rate of application tends to be beneficial to most crops, but only to a certain extent. Potting mixes that were above 60% vermicompost had detrimental effects on pepper growth and yield, attributed to high soluble salt concentration, poor aeration, and heavy metal toxicity. (Arancon, 2006, Atiyeh, 2002).

In other research, vermicompost had detrimental or mixed effects on crops in both greenhouse and field settings. Garden beans (*Phaseolus vulgaris* L. 'Purple King'), peas (*Pisum sativum* L. 'Rani'), beetroot (*Beta vulgaris* L. 'Cylindra'), radish (*Raphanus sativus* L. 'Crimson Giant') and two cultivars of both cabbage (*Brassica oleracea* L. 'Copenhagen Market 2' and 'Golden Acre') and Swedish turnip (*Brassica napus* var. *napobrassica* L. 'Grunkopfige Gelbe Wilhelmsburger' and 'Golden Ball') experienced an almost linear decrease in germination with increased vermicompost application (Ievinsh, 2011). Germination was also decreased for tomato (*Solanum lycopersicum* Mill. 'Rutgers'), marigold (*Tagetes patula* L. 'Queen Sophia'), pepper (*Capsicum esculentum* L. 'California Wonder'), and cornflower (*Centauria cyanus* L. 'Imperial') in a potting mix amended with vermicompost produced from pig manure. The subsequent performance of these plants in field-amended soil was improved (Bachman, 2008). In other research, germination of tomato (*Solanum lycopersicum* L.), eggplant (*Solanum melongena* L.), and pepper (*Capsicum annuum* L.) in potting mix amended with vermicompost produced from cattle manure was enhanced, while the growth and yield of transplants in the field were unaffected (Paul, 2005). Therefore, studies appear to show variable responses of crops to vermicompost.

While yield and quality of crop can be used to assess effectiveness of vermicompost materials, it is difficult to generalize these results. A number of factors including feedstock, rate of application, crop, and life stage contribute to the effectiveness of vermicompost. In addition, physiological requirements, time of year and length of growing season are highly variable for different crops. Determining mineralization rates—the rate at which organic N is converted to mineral N, and thus becomes available to plants—is one way to remedy this. Studies evaluating mineralization and nitrification rates have been used to create tools and make recommendations for application of other organic amendments as a nutrient source including manures and other composts (Tyson, 1993, Honeycutt, 2005). However, little of this has been done for vermicompost materials, especially in comparison to comparable windrowed composts.

An additional concern about using vermicompost is food safety. Unlike standard windrow composting which typically reaches 54-66^{0}C (130-150^{0}F), vermicomposting is not a thermophilic process. Earthworms require an environment between 4.4 and 26.7^{0}C (40 and 80^{0}F) which fall within the growing temperature range of human pathogens including *Salmonella enterica* and *Escherichia coli*. Feedstock materials are often manures or other materials suspected of harboring such pathogens. With the rise in outbreaks of food-borne illness in the past twenty years, government regulation and public concern have become more sensitive to possible contamination of fresh produce (Fonseca, 2007). It is important to identify the extent of the food safety risk associated with particular vermicompost materials. Fecal indicator organisms (fecal coliforms and *E. coli*) are the standard indicators for food safety risk of manures and compost in current regulations and proposed regulations of the Food and Drug Administration's Food Safety Modernization Act (FSMA); total coliforms are a broader group of bacteria, that have also been used to evaluate food safety on produce.

Heat tolerant lettuce cultivars and vermicomposts have the potential to increase sustainability of agriculture in the Mid-Atlantic. This research considered a novel approach to lettuce production integrating these two innovations. Four lettuce cultivars and two vermicompost soil amendments were evaluated across three seasonal plantings (spring, summer, and fall). Lettuce cultivars were compared to commercially available lettuce in the area. Vermicomposts were compared to windrowed compost and a control with no added amendment; organic materials were applied at two rates. Success was evaluated on yield and quality of crop, mineralization rates, consumer perception of sensory attributes, and food safety risk.

Objectives

1) *Evaluate four heat-tolerant romaine lettuce cultivars in the Mid-Atlantic for summer production*
2) *Evaluate the effects of two local vermicompost soil amendments on lettuce performance*

2.2 Materials and methods

2.2.1 Lettuce cultivars

Four cultivars of romaine (Cos) lettuce bred for heat tolerance were grown at the Wye Research and Education Center (WREC) on the Eastern Shore of Maryland during the spring, summer, and fall growing seasons in 2013 (Table 2.1 and Fig. 2.1). Cultivars 'Solid King,' 'Sunbelt,' and 'Green Forest' were obtained from Central Valley Seeds from a breeding project in California; cultivar 'Dov' was obtained from Seedway, as the only recommended romaine cultivar from a variety trial conducted under similar conditions at the University of Delaware in 2012. Daily precipitation and temperature highs and lows were recorded by a weather station about 1 km (0.6 miles) from the field (Table A.1).

Seeds were started in the University of Maryland (UMD) greenhouse research complex approximately 35 days before transplanting, and harvested at maturity, approximately 50 days later (Table A.2). Lettuce was planted in a replicated complete block design (RCBD) with four blocks. Six heads of lettuce were planted per plot. Lettuce was transplanted in two staggered rows into shaped beds covered with 101cm (40") black plastic mulch (Fig. 2.2). Spacing and nutrition was applied according to the recommendations given by the *Maryland Commercial Vegetable Production Recommendations for 2013* following soil analyses performed by A&L Eastern Laboratory in Richmond, VA (Veg Guide 2013).

Table 2.1. Romaine lettuce cultivars and sources

Cultivar	Label	Source
Solid King (SK)	A	Central Valley Seeds – Salinas, CA
Sunbelt (SB)	B	Central Valley Seeds – Salinas, CA
Green Forest (GF)	C	Central Valley Seeds – Salinas, CA
Dov	D	UDel Variety Trial 2012

Figure 2.1. Romaine lettuce cultivars evaluated in the present study; A) Solid King, B) Sunbelt, C) Green Forest, and D) Dov


Figure 2.2. Field plot of lettuce at the Wye Research and Education Center in Queenstown, MD. Lettuce was grown in two staggered rows in shaped beds covered with 101cm (40”) black plastic mulch

2.2.1.1 Yield and quality

Four representative heads of lettuce were harvested from each block for analysis. Lettuce was transported back to the laboratory at UMD, approximately 1 hour from the field. Vehicles were air-conditioned to minimize water loss and wilting. Lettuce was first evaluated for head size (weight, height, and diameter) and stem size (diameter, length, and percent of total height). Color was measured using a Konica Minolta color difference meter (BC-10). This meter measures L*a*b* color space, a three dimensional representation of color. L* dimension measures lightness to darkness, a* and b* dimensions measure red to green and yellow to blue color continuum respectively (Fig. 2.3). Cleaned heads were stored at 4^{0}C and within 72 hours they were subjected to a sensory evaluation by 9 trained panelists at the USDA sensory evaluation kitchen in Beltsville, MD (Fig. 2.4). Panelists were trained approximately one month prior to sensory evaluation. They learned to rate intensity of flavor on a continuous scale from 1-100 by tasting solutions of varying concentrations and then rating many varieties with attributes spanning the intensity scales. Attributes rated by panelists in the sensory evaluation are listed in Table 2.2.

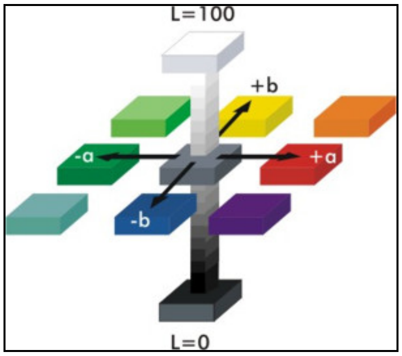


Figure 2.3. L*a*b* color space. Three-dimensional measure of color. L* measures lightness to darkness (100-0); a* dimension measures green to red, more negative values indicating more green, more positive values indicating more red; b* dimension measures blue to yellow, more negative values indicating more blue, more positive values indicating more yellow


Figure 2.4. Sensory evaluation kitchen at USDA facility in Beltsville, MD

Table 2.2. Sensory characteristics evaluated in sensory evaluation of lettuce

Intensity of	Acceptability of
Texture	Flavor
Aroma	Texture
Sweetness	Quality
Sourness	
Bitterness	
Astringency	
Aftertaste	

2.2.1.2 Sensory evaluation

In each season, panelists were asked to evaluate sensory attributes of chopped lettuce leaf pieces of each cultivar (SK, SB, GF, and Dov) and two mixed grocery store varieties. Attributes were divided into two groups. Intensity attributes were rated on the degree of strength of an attribute on a scale from ‘none’ to ‘strong.’ Acceptability attributes were rated on the degree of like or dislike of the sample on a scale from ‘bad’ to ‘good.’ Sweetness, sourness, bitterness, astringency, texture, aroma, and aftertaste were rated on intensity; flavor, texture, and overall quality were rated on acceptability. All cultivars were evaluated at two separate times in one day by each panelist. Intensity attributes were used to compare cultivars; acceptability attributes were used to gauge consumers’ opinions of the lettuce and determine cultivars’ potential marketability.

2.2.2 Vermicompost

The effects of two locally produced vermicomposts on lettuce yield and quality were compared to comparable windrowed compost and a control treatment with no added organic amendment (Table 2.3). Vermicompost 1 (V1) was made by a local vermicompost producer using dairy manure. Vermicompost 2 (V2) was produced by a local urban farm; food waste collected from the DC metropolitan area was fermented using the Bokashi method, thermophilically composted with added leaves and woodchips to reach a C:N ratio of approximately 25:1, and then vermicomposted in 4x4x4ft bins for approximately 3 months. The top 6” containing the earthworms was removed and finished material was screened. A conventional windrowed compost (W) was produced by the USDA composting facility in Beltsville, MD using dairy manure and associated solids (i.e. bedding). Materials were considered finished composts according to the USDA requirements for vermicompost and compost composition, production, and use (USDA, 2011). During the thermophilic portion of composting piles were managed and turned to promote aerobic conditions and reached at least 55°C (131°F) for at least 3 days. During vermicomposting, materials were managed to maintain aerobic conditions and a moisture content between 70 and 90%. Samples of organic amendments were characterized prior to field trials by standard compost analyses at the Penn State Agricultural Analytical Services Laboratory in University Park, PA (Table 2.4).

Organic amendments were applied at two rates, 5 and 10 mt ha^{-1} (11.1-22.2 short ton A^{-1}) wet weight (rate a and b respectively). Treatments included a complete factorial of the three amendments and the two rates, plus a control (no added amendment) for a total of seven treatment combinations. Treatment combinations were applied in an RCBD with four blocks (Fig. 2.5). Organic amendments were applied in addition to nutrition applications recommended by *Maryland Commercial Vegetable Production Recommendations for 2013* after soil analyses performed at A&L Laboratories in Richmond, VA (UMD, 2013). The initial characteristics of amended plots were determined by analysis of soil samples by A&L Eastern Laboratories (Table C.2).

Field plantings were carried out at the same location and times as lettuce cultivar trials. Only the cultivar 'Solid King,' the highest yielding cultivar in a preliminary planting in 2012, was used to evaluate soil treatments. Beds were prepared 1 to 5 days before transplanting. Raised beds were shaped on six foot centers, amendments were raked evenly into the top 3-4 inches of soil, and beds were covered with 40" black plastic mulch. To avoid cross-contamination, 3' spaces were left between plots. Lettuce seedlings were transplanted into prepared beds in two staggered rows at 1' spacing. Soil samples were taken at transplant from each plot and analyzed by A&L Eastern Labs in Richmond, VA for nutrients and soil properties. Analysis of soil by NuMan Pro 3.2 Software confirmed that all nutritional requirements were met.

Table 2.3. Soil amendments tested in vermicompost experiments

Amendment	Feedstock and supplier
V1 – Vermicompost	Dairy manure solids (Full Circle)
V2 – Vermicompost	Food scraps (Eco City Farms)
W - Windrowed compost	Dairy manure and bedding (USDA)
C - Control, no amendment	N/A

Table 2.4. Properties of organic amendments; analysis by Penn State laboratory

Material	pH	Soluble Salts (1:5 w:w mmhos/cm)	Solids (%)	Moisture (%)	Organic Matter (%)	Total N (% as is)	C (%)	C:N Ratio (%)	Nitrate-N (mg/kg)
W	7.5	5.7	63	37	74	1.19	12.4	10.4	475.7
V1	7.1	21.4	41.5	58.5	29.6	1.56	15.7	10	2411.5
V2	6.7	3.68	44.7	55.3	18.7	.89	10	11.3	401.8

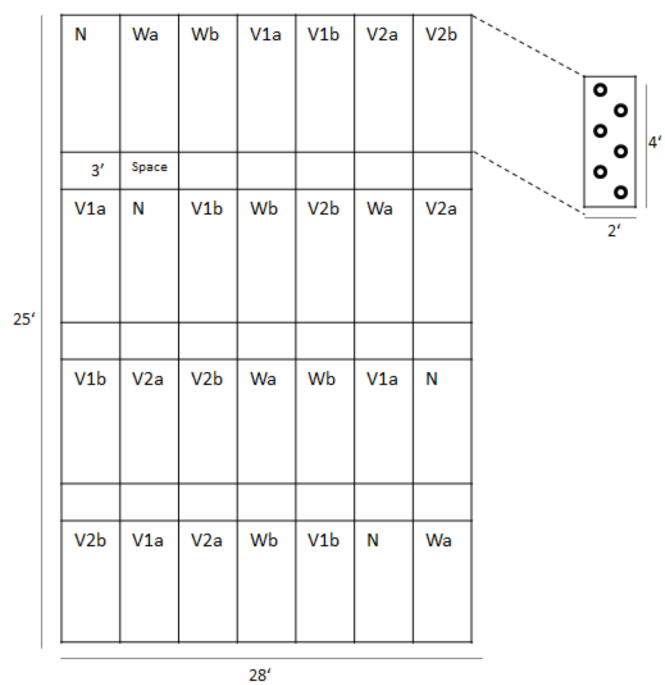


Figure 2.5. Field map of soil treatment plots. Soil treatments (N, Wa, Wb, V1a, V1b, V2a, and V2b) were applied in a randomized complete block design (RCBD) with four blocks. Each plot was 4'x 2' with 2' spacing between rows. 3' was left between plots within rows to avoid cross contamination of soil treatments

2.2.2.1 Lettuce Yield and Quality

Lettuce was harvested at maturity, at about 50 days after transplanting. Three representative heads of lettuce and their respective roots were harvested from each plot and transported to UMD for analysis. Lettuce was evaluated on weight, height, diameter, stem diameter, stem length, color, and root dry weight. Weight, height, stem and color were evaluated on the same day as harvest. Roots were refrigerated until the time of processing, which occurred within 48 hours. After removing leaf and shoot tissue, the roots were soaked in warm water for 30-40 minutes and soil was gently removed. Clean roots were blotted with paper towels and their fresh weight was measured. Roots were subsequently dried at 40^0C (104^0F) until they reached a constant weight (approximately 48hrs) and dry weight was taken.

Chlorophyll concentration was quantified following the protocol from Knudson *et al* (1977). One 5cm square was cut from the top portion of an outer leaf from two representative heads of lettuce from each plot. Leaves were rolled and placed in 20ml vials that were filled with 100% ethanol. The liquid was decanted and refilled every 24 hours for 3 days, until leaves were completely white. The extracted volume was adjusted to 100ml in volumetric flasks. An approximate 2ml aliquot of extract was used to measure absorbance at 665 and 649 nm (A_{665} and A_{649}) spectrophotometrically. Leaf tissue was dried in a drying oven until weight changes were undetectable (approximately 48hrs) and dry weight was recorded. Equations derived by Knudson *et al*. were used to quantify chlorophyll concentration (Knudson, 1977).

2.2.2.2 Food Safety

A leaf sample was taken from each plot using sterile gloves and scissors, sanitized between each plot. Each sample included a total of two inner and two outer leaves from at least two heads of lettuce, avoiding wrapper leaves (which would be removed before sale and not consumed). Samples were placed in sterile Whirlpak® (Nasco, Fort Atkinson, Wisconsin) bags. Soil samples were taken from each plot at three locations between lettuce heads. Samples were taken with a sterile scoop, from under the plastic mulch and placed into a sterile bag. Leaf and soil samples were transported back to the laboratory in insulated coolers filled with chipped ice for analysis.

Samples were stored at 4^0C (40^0F) and processed within 24 hours of harvest. Approximately 25 g of leaf tissue was washed with 225 ml 0.01% sterile peptone water and homogenized in a Seward Stomacher® 400 Circulator at 250 rpm for 2 minutes. For soil samples, 10 g of soil was mixed with 90 ml 0.01% sterile peptone water and shaken, then allowed to settle for 3 minutes. From both leaf washes and soil mixtures, 1 ml of 10^{-1}, 10^{-2}, and 10^{-3} dilutions were plated on 3M (St. Paul, MN) total coliform (TC) and *E. coli* petrifilms. Petrifilms were incubated at 36^0C; TCs and *E. coli* were counted at 24±2 and 48±2 hours respectively. TC and *E. coli* load are reported as the log of colony forming units (CFU) per ml of original (undiluted) solution. Petrifilms have a lower threshold of sensitivity that depends on dilution. Therefore, petrifilms with no visible colonies were not reported as zero, instead they were reported according to 3M recommendations. Log transformation of undiluted concentration was used in the subsequent statistical analysis.

2.2.3 Statistical Analysis

Data were analyzed using SAS (SAS, Version 9.2 for Windows; SAS Institute Inc., Cary, NC). Analysis of variances (ANOVA) were performed using the PROC MIXED procedure. If main effects were significant, means were separated using the TUKEY statement. Significance was measured as $p<0.05$.

Yield, quality, and sensory attributes of lettuce cultivars were assessed using a 2-way analysis of variance (ANOVA) comparing the main effects of cultivar and season, and the cultivar by season interaction. Effects of soil treatments on yield and quality of lettuce crop were also analyzed using ANOVA. To account for the incomplete factorial of treatments (the control was applied at only 1 rate while amendments were applied at two rates), data were analyzed two ways. All seven soil treatment combinations (C, V1a, V1b, V2a, V2b, Wa, Wb) were compared across seasons (1, 2, 3) using a 2-way ANOVA. Also, soil treatments (V1, V2, W) and rates (a, b) were compared within each season using a 2-way ANOVA. Since chlorophyll data were only collected for the summer planting, they were separately analyzed using a 2-way ANOVA within that season. Correlation between soil and leaf total coliform counts was analyzed using PROC MIXED (omitting the CLASS statement) within each season to determine if soil coliform load was a good indicator of leaf coliform load.

2.3 Results

2.3.1 Lettuce cultivars

2.3.1.1 Yield and Quality

Means for the yield and quality characteristics of lettuce cultivars and their statistical significance levels are displayed in Table 2.5. The interaction between cultivar and season was not significant for most variables, meaning the cultivars responded similarly throughout the year. Significant interactions were seen in stem measurements only. These measurements are important because they are indicators of bolting tendency. Both stem length ($p<0.0001$) and percent of total height ($p<0.0001$) showed significant interaction between season and cultivar. Within the spring and fall plantings, none of the cultivars had significantly different stem length or stem percent. All cultivars had significantly greater stem length and percent in the summer season than the spring and fall. Within the summer season, ‘Green Forest’ had significantly greater stem length and percent than the other cultivars indicating that it was more likely to bolt in the summer heat. See Fig. 2.6.

There were significant differences between cultivars in terms of head weight, height, and diameter ($p=0.0001$, $p<0.0001$, and $p=0.0043$). ‘Solid King’ tended to produce the largest heads, while ‘Dov’ and ‘Green Forest’ produced the smallest. Cultivars were significantly smaller in the summer planting with regard to weight, height, and diameter ($p<0.0001$ for all). See Fig. 2.7.

There were no significant differences in L* values for lettuce leaves of different cultivars ($p=0.1060$); however, a* and b* values did vary significantly among cultivars ($p=0.0186$ and $p=0.0027$). More negative a* values indicated ‘Dov’ was the most green of the cultivars and less negative values indicated 'Green Forest' the least; b* values indicated that 'Green Forest' was the most blue while 'Dov' was the least. The a* values were higher in the fall than the spring and summer (p<0.0001), indicating that leaves of fall lettuce were not as green.

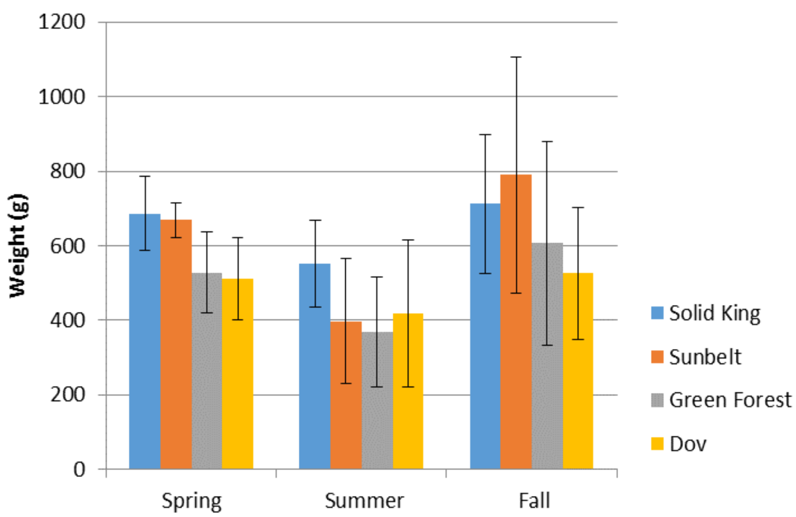


Figure 2.6. Mean head weight of lettuce cultivars in spring, summer, and fall plantings. Cultivars were significantly smaller in summer; Solid King produced significantly larger heads than Green Forest and Dov (*P* = 0.0083). Error bars represent one SD.

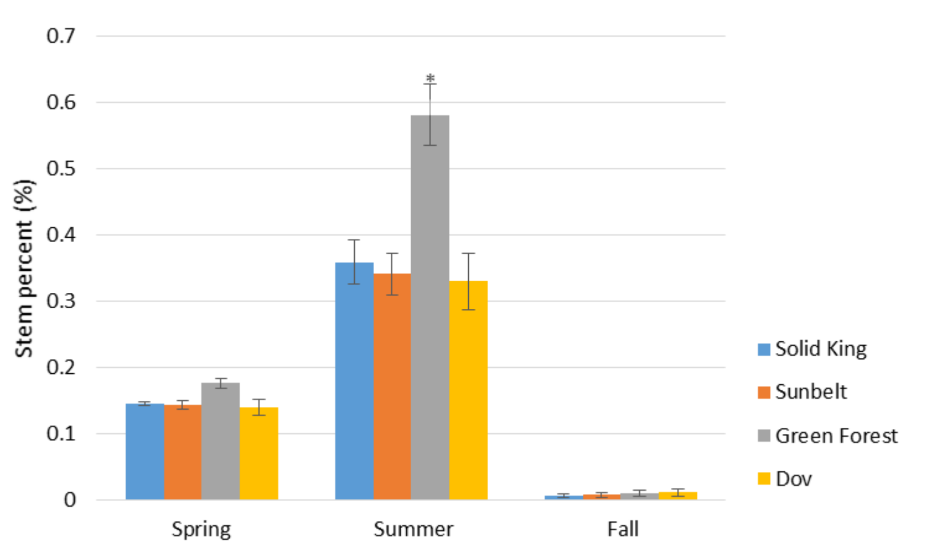


Figure 2.7. Stem percent of total height. Stem is an indicator of bolting; all cultivars were significantly more likely to have greater stem percent in the summer (*P* < 0.0001); cultivar Green Forest had a significantly greater stem percent than other cultivars in the summer (*P* < 0.0001). Error bars represent one SD.

Table 2.5. Yield and quality results; LS means of characteristics of cultivars grown in spring 2013

		Head			Stem			Color	
Independent Variable	Weight (g)	Height (in)	Diameter (in)	Diameter (mm)	Length (cm)	Percent of total height	L*	a*	b*
Cultivar									
SK	870.41^{a}	12.40^{a}	9.37^{ab}	26.87^{a}	6.12^{a}	0.15^{a}	54.44^{a}	-8.49^{ab}	11.66^{a}
SB	838.38^{ab}	12.01^{ab}	9.50^{a}	28.75^{a}	5.81^{a}	0.15^{a}	55.75^{a}	-8.24^{ab}	12.24^{a}
GF	715.53^{b}	12.10^{a}	8.75^{b}	26.45^{a}	8.76^{b}	0.24^{b}	54.67^{a}	-7.99^{a}	11.07^{a}
Dov	704.79^{b}	11.20^{b}	9.16^{ab}	27.36^{a}	5.62^{a}	0.14^{a}	55.64^{a}	-8.78^{b}	13.15^{a}
Season									
Spring	818.52^{a}	12.61^{a}	9.33^{a}	26.70^{a}	4.51^{a}	0.13^{a}	53.31^{a}	-7.18^{a}	12.01^{a}
Summer	650.10^{b}	10.99^{b}	8.75^{b}	24.58^{a}	10.22^{b}	0.39^{b}	55.81^{b}	-7.16^{a}	11.72^{a}
Fall	878.20^{a}	12.19^{a}	9.50^{a}	30.79^{b}	5.00^{a}	-0.01^{c}	56.26^{b}	-10.79^{b}	12.37^{a}
ANOVA									
Cultivar	0.0083**	0.003**	0.0313*	0.0732^{NS}	<.0001**	<.0001**	0.1060^{NS}	0.0186*	0.0027**
Season	0.0001**	0.0001**	0.0043**	<.0001**	<.0001**	<.0001**	<.0001**	<.0001**	0.3804^{NS}
CV*Season	0.5192^{NS}	0.1884^{NS}	0.8968^{NS}	0.7407^{NS}	<.0001**	<.0001**	0.2721^{NS}	0.8926^{NS}	0.8817^{NS}

^{a}= means followed by different letters within a column indicate significant difference between treatments

*=significant (α=0.05), **=significant (α=0.01), ^{NS} =not significant ($p \geq 0.05$)

2.3.1.2 Sensory evaluation

Means of sensory attributes and significance levels are reported in Table 2.6. Differences in sensory attributes between cultivars within each harvest season have been visualized using ‘radar’ or ‘spider-web’ graphs (Fig. 2.8). In these graphs, each spoke or axis represents a unique sensory attribute and each colored line corresponds to one cultivar; the degree of overlap of colored lines indicates the similarity between cultivars.

There were no significant differences in scores for intensity of texture, aroma, sweetness, sourness, astringency, or aftertaste among cultivars. Neither were there significant differences in scores for acceptability of texture or overall quality. There was significant difference in acceptability of flavor ($p=0.0281$). ‘Dov’ and the supermarket control had higher scores of acceptability for flavor than ‘Solid King.’

Intensity of aftertaste was the only attribute for which the difference among seasons was not significant. Texture and sourness were significantly higher in the spring than the fall ($p<0.0001$). Aroma, sweetness, sourness, and astringency were all significantly different ($p=0.0021$, <0.0001, $=0.0089$, and $=0.0043$, respectively). Aroma and sweetness were higher in the spring than both summer and fall ($p<0.01$). Sourness was highest in the fall. Astringency was lowest in the summer. Acceptability of flavor, texture and overall quality were also significantly different with greater acceptability in the spring than in summer and fall ($p<0.0001$).

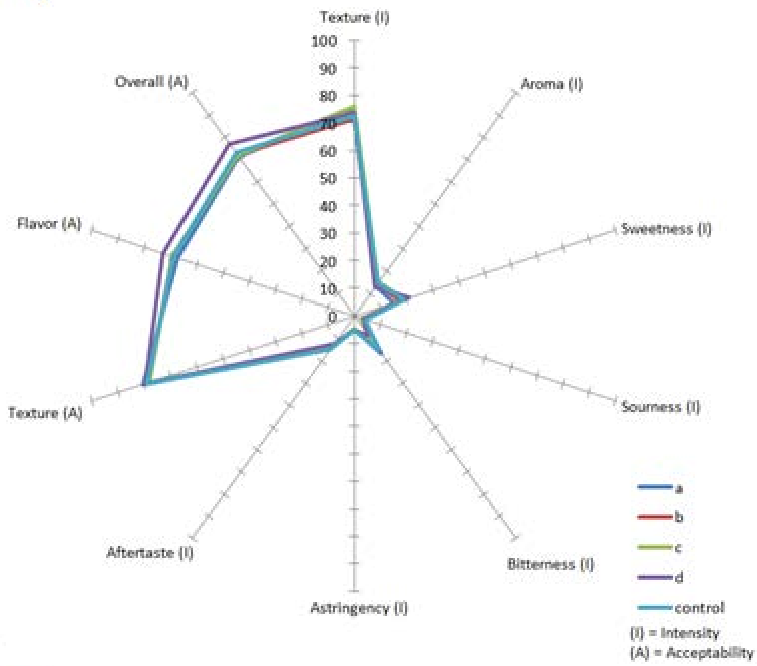
There was a significant interaction between cultivars and seasons for intensity of bitterness, indicating that cultivars reacted differently to the seasons ($p=0.0153$). ‘Solid King’ and ‘Green Forest’ received higher scores for intensity of bitterness in the fall than in the spring, but scores were not different in the summer. The control was more bitter in the spring than in the summer and fall. Bitterness scores of ‘Sunbelt’ and ‘Dov’ were unaffected by season.

Table 2.6. Sensory evaluation results for spring, summer, and fall harvests; LS means of quality characteristics of varieties grown in spring 2013. Characteristics were evaluated by 9 trained sensory evaluation panelists; each response variable was measured on a sliding scale of 1-100 (no units).

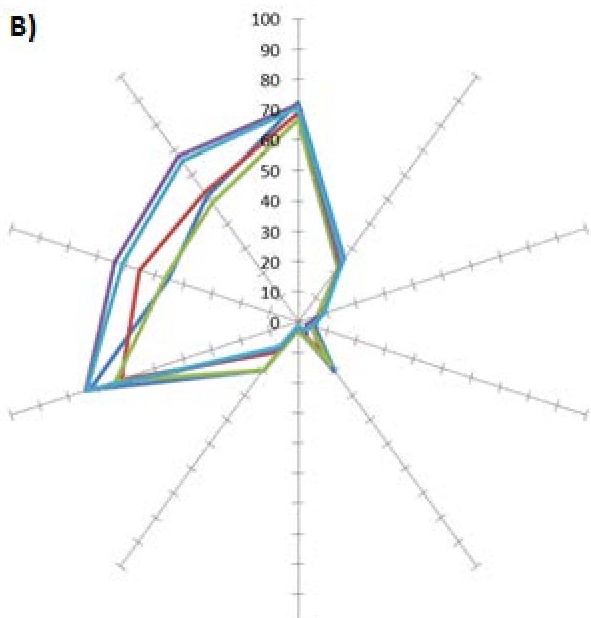
	Intensity of flavor:							Acceptability of:		
	Texture	Aroma	Sweet	Sour	Bitter	Astringent	Aftertaste	Flavor	Texture	Quality
Cultivar										
SK	80.41^{a}	21.80^{a}	3.48^{a}	6.03^{a}	18.96	2.69^{a}	14.84^{a}	57.33^{a}	83.71^{a}	61.92^{a}
SB	78.34^{a}	19.53^{a}	5.33^{a}	3.75^{a}	11.84	1.32^{a}	8.12^{a}	65.12^{ab}	80.41^{a}	65.98^{a}
GF	78.94^{a}	20.59^{a}	5.23^{a}	4.51^{a}	13.91	1.99^{a}	12.86^{a}	61.70^{ab}	81.41^{a}	64.15^{a}
Dov	78.95^{a}	20.09^{a}	7.32^{a}	3.88^{a}	5.79	1.53^{a}	6.79^{a}	70.10^{ab}	85.58^{a}	73.68^{a}
Control	77.43^{a}	21.25^{a}	8.43^{a}	2.71^{a}	5.17	0.33^{a}	6.86^{a}	70.82^{b}	83.32^{a}	73.70^{a}
Season										
Spring	86.01^{a}	14.03^{a}	12.73^{a}	2.49^{a}	9.94	1.92^{a}	9.91^{a}	78.86^{a}	93.23^{a}	81.86^{a}
Summer	79.61^{ab}	23.80^{b}	3.42^{b}	3.74^{ab}	9.45	-0.14^{b}	11.39^{a}	61.77^{b}	80.05^{b}	64.03^{b}
Fall	70.82^{b}	24.13^{b}	1.72^{b}	6.30^{b}	14.02	2.94^{a}	8.38^{a}	54.42^{b}	75.37^{b}	57.77^{b}
ANOVA										
Cultivar	0.9858^{NS}	0.9911^{NS}	0.7046^{NS}	0.4182^{NS}	<.0001**	0.2703^{NS}	0.0457^{NS}	0.0281*	0.7911^{NS}	0.0470^{NS}
Season	0.0002**	0.0021**	<0.0001**	0.0089**	0.0760^{NS}	0.0043**	0.5848^{NS}	<.0001**	<.0001**	<.0001**
CV*Season	0.9981^{NS}	0.9999^{NS}	0.9931^{NS}	0.3891^{NS}	0.0153*	0.4835^{NS}	0.5114^{NS}	0.6148^{NS}	0.8993^{NS}	0.7728^{NS}

^{a}= means followed by different letters within a column indicate significant difference between treatments
*=significant (α=0.05), **=significant (α=0.01), ^{NS}=not significant ($p \geq 0.05$)

A)


Texture (I)
100
90
80
70
60
50
40
30
20
10
0
Aroma (I)
Sweetness (I)
Sourness (I)
Bitterness (I)
Astringency (I)
Aftertaste (I)
Texture (A)
Flavor (A)
Overall (A)
a
b
c
d
control
(I) = Intensity
(A) = Acceptability

B)


100
90
80
70
60
50
40
30
20
10
0

C)

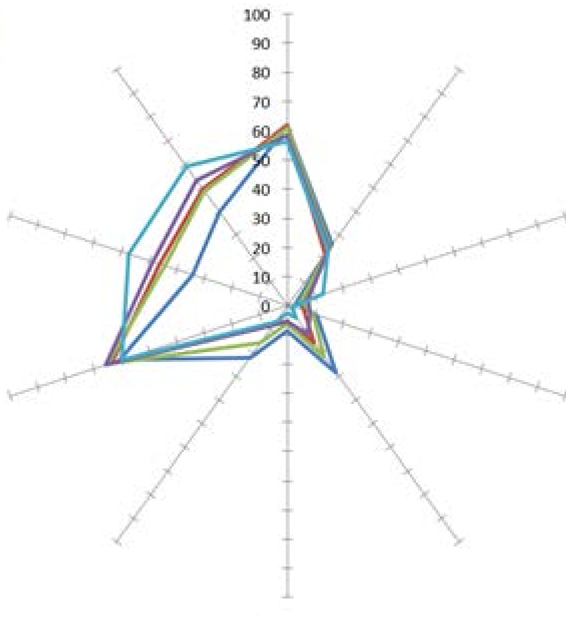


Figure 2.8. Sensory Attributes of Lettuce in A) spring season, B) summer season, and C) fall season. Each colored line represents a lettuce cultivar; ‘a’ Solid King, ‘b’ Sunbelt, ‘c’ Green Forest, ‘d’ Dov, and ‘control’ a commercially available romaine cultivar. Each spoke represents a different sensory attribute. More overlap of colored lines indicates more similarity between cultivars.

2.3.2 Vermicompost

2.3.2.1 Yield and quality

In the 2-way ANOVA evaluating the effects of the seven treatment combinations and three seasons, there were few significant differences between treatment combinations on yield and on quality. Conversely, there were many significant differences between seasons for these parameters. Means for all response variables and significance levels are reported in Table 2.7.

There were significant differences between seasons for head diameter, stem diameter, stem length, stem percent, L* and b* values ($p<0.0001$ for all). Head diameter was smaller in the summer than spring and fall. Stem diameter was largest in the fall and smallest in the spring. Conflicting results were observed for stem measurements. Stem length was greater in the fall than summer, but percent of total head height was greater in the summer than the fall. Spring lettuce had the lowest L* values indicating lighter color. Summer lettuce had the lowest b* values indicating more blue color. There was no effect of season on a* values (green color).

There was a significant interaction between season and treatment for height and weight indicating that soil treatments affected lettuce size differently depending on season ($p=0.0286$, $p=0.0384$). The control heads did not exhibit significant difference in either variable for any of the seasons. For all other treatments, head weight was consistently greater in the fall than in the summer. For the vermicompost treatments, head weight was also greater in the fall than in the spring. See Fig. 2.9. None of the treatments significantly affected head height within harvest seasons. Heads were consistently taller in the fall than in the summer. For V1b, V2a, Wa and Wb, heads were taller in the spring than in the summer.

For the 2-way ANOVA evaluating treatment and rate factorial within each season there were few significant differences. There were no significant interactions between rate and soil treatment. Means and significance levels are reported in Appendix C (Tables C.1, C.2, and C.3).

In the spring season there were significant differences between amendments for weight (p=0.0046), height (p=0.0158), stem diameter (p=0.0328), and root dry weight (p=0.0432). V2 tended to produce smaller heads of lettuce. They were significantly smaller in weight than both V1 and W, smaller in height than V1, smaller in stem diameter than both V1 and W, and smaller in weight than W. There were no significant differences between amendments for head diameter (p=0.8139), color measurements (L*, a*, b*; p=0.6400, 0.8394, and 0.9906), or coliforms (total, *E.* coli; p=0.1288, 0.3252). The higher rate produced heads significantly heavier in weight (p=0.0378) and stem diameter (p=0.0331). Other parameters were not significantly different between rates of application.

In the summer planting, there were significant differences in height between treatments (p=0.0494). Soil amended with V1 produced heads taller than soil amended with W. There was also a significant difference in the a* value for rate of application (p=0.0115). The higher rate of amendment had a less negative a* value, indicating a greener color. Other parameters were not significantly different for treatment or rate.

In the fall planting there was a significant difference in weight between treatments ($p=0.0398$). V1 had significantly heavier heads than W. Other parameters were not significantly different.

Chlorophyll content of leaf tissue was measured only in the summer season. Neither chlorophyll *a* nor chlorophyll *b* content was significantly different between the seven treatment combinations ($p=0.3271$, 0.3269). With regard to soil treatment and rate, there were also no significant differences.

Table 2.7. Effect of vermicompost soil amendments on lettuce grown in the spring, summer, and fall of 2013 (2-Way ANOVA, Season*Trt); LS means of yield and quality characteristics.

	Weight (g)	Head Height (in)	Diameter (in)	Diameter (mm)	Stem Length (mm)	Percent (%)	Color L*	a*	b*	Root Dry weight (g)	Coliforms Log(ltc)^{z}	Log(stc)^{y}
Trt												
N	673.77	11.25	8.35^{a}	24.61^{a}	9.87^{a}	0.0146^{a}	52.28^{a}	-8.32^{a}	11.02^{a}	6.68^{a}	2.05^{a}	1.38^{a}
V1a	767.85	11.62	8.42^{a}	26.68^{a}	10.92^{a}	0.0140^{a}	52.23^{a}	-6.34^{a}	10.79^{a}	6.70^{a}	1.22^{a}	2.37^{a}
V1b	803.38	11.53	8.93^{a}	25.53^{a}	10.04^{a}	0.0134^{a}	51.63^{a}	-8.27^{a}	11.70^{a}	7.74^{a}	0.90^{a}	2.10^{a}
V2a	671.89	11.01	8.46^{a}	24.36^{a}	9.93^{a}	0.0152^{a}	52.19^{a}	-8.59^{a}	11.82^{a}	6.39^{a}	2.16^{a}	1.98^{a}
V2b	745.84	11.32	8.41^{a}	24.98^{a}	10.01^{a}	0.0129^{a}	52.18^{a}	-8.26^{a}	11.50^{a}	6.86^{a}	1.65^{a}	1.81^{a}
Wa	688.37	10.93	8.30^{a}	25.10^{a}	10.02^{a}	0.0156^{a}	51.83^{a}	-6.31^{a}	11.16^{a}	6.92^{a}	1.60^{a}	1.90^{a}
Wb	697.22	11.23	8.38^{a}	25.55^{a}	9.98^{a}	0.0155^{a}	52.58^{a}	-4.76^{a}	11.40^{a}	6.00^{a}	1.74^{a}	1.70^{a}
Season												
1 – Spring	697.83	11.70	8.70^{a}	24.41^{a}	nr	nr	50.31^{a}	-7.41^{a}	12.22^{a}	9.18^{a}	2.99^{a}	2.02^{a}
2 – Summer	530.09	10.07	7.95^{b}	22.19^{b}	8.74^{a}	0.0166^{a}	52.66^{b}	-6.28^{a}	9.61^{b}	4.54^{b}	0.81^{b}	0.89^{b}
3 – Fall	935.64	12.04	8.74^{a}	29.17^{c}	11.49^{b}	0.0123^{b}	53.41^{b}	-8.09^{a}	12.19^{a}	6.54^{c}	1.05^{b}	2.75^{c}
ANOVA												
Trt	0.0539*	0.0466*	0.1942^{NS}	0.1176^{NS}	0.3523^{NS}	0.2323^{NS}	0.9237^{NS}	0.5105^{NS}	0.6397^{NS}	0.6254^{NS}	0.1627^{NS}	0.3375^{NS}
Planting	<.0001**	<.0001**	<.0001**	<.0001**	<.0001**	<.0001**	<.0001**	0.4765^{NS}	<.0001**	<.0001**	<.0001**	<.0001**
Trt*Pl	0.0286*	0.0384*	0.5930^{NS}	0.3956^{NS}	0.7098^{NS}	0.0538^{NS}	0.8667^{NS}	0.4903^{NS}	0.4018^{NS}	0.5868^{NS}	0.0558^{NS}	0.8475^{NS}

^{a}= means followed by different letters within a column indicate significant difference between treatments

^{z}= Log (leaf total coliforms), log of total coliform counts from leaf wash dilutions

^{y} = Log (soil total coliforms), log of total coliform counts from soil sample dilutions

*=significant (α=0.05), **=significant (α=0.01), ^{NS}=not significant ($p \geq 0.05$)

nr=not recorded

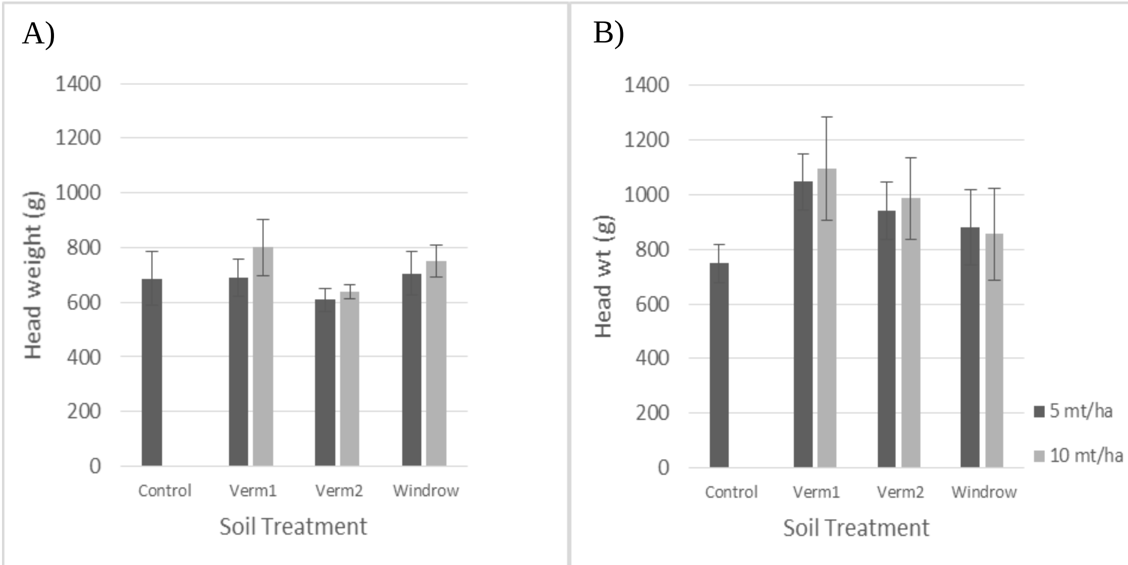


Figure 2.9. Mean head weights for A) spring and B) fall harvests. Different colored bars represent different rates of application; dark grey 5 mt/ha, light grey 10 mt/ha. Mostly heads were not significantly different between treatments ($P > 0.05$); however, trends seem to be that increased rate of application of any of the materials resulted in increased head weight. Vermicompost 1 (V1) tended to produce larger heads of lettuce than other materials.

2.3.2.2 Food safety

E. coli was never detected in lettuce leaf washes. In only two instances, very low concentrations (<10 CFU/225ml) of *E. coli* were detected in the soil samples. These were both in the fall planting in one replication of V2b and Wb each.

TCs were significantly different between seasons for both leaf and soil washes ($p<0.0001$ for both). Leaf TCs were higher in spring than summer and fall; soil TCs were highest in fall and lowest in summer. TCs were not significantly different among the soil treatments (Fig. 2.10). A significant positive correlation was found between soil and leaf TCs measured in the spring season ($R^2=0.3109$, $p=0.0021$). Soil and leaf TC measurements were not correlated in either the summer or the fall (Fig. 2.11). This is most likely due to low levels of coliforms detected during these periods.

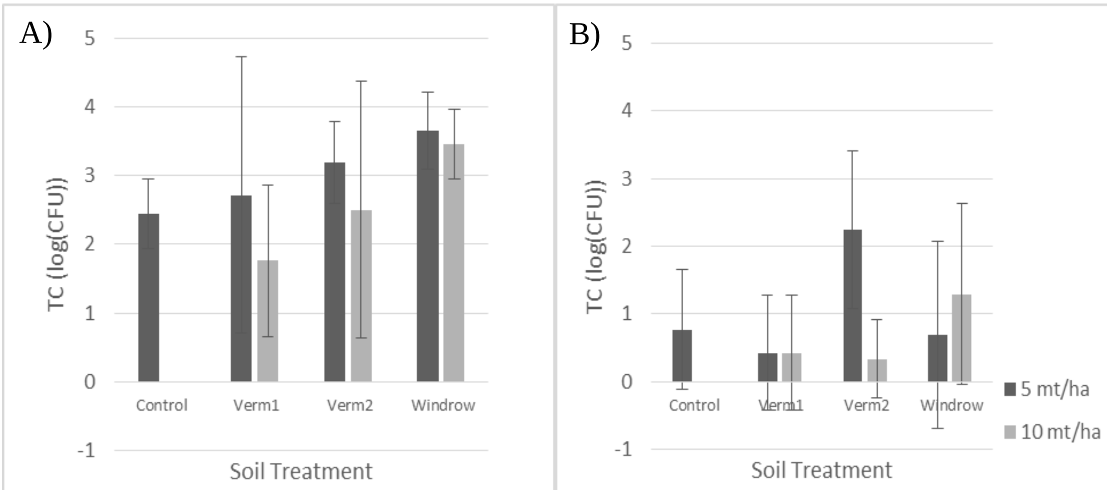


Figure 2.10. Leaf wash total coliforms for A) spring and B) fall. Different colored bars represent different rates of application; dark grey 5 mt/ha, light grey 10 mt/ha. Y-axis represents total coliform counts expressed as the log of colony forming units (CFUs) in undiluted leaf wash samples. Variation between replicates within treatments was extremely large; coliforms were not significantly different between treatments in either season ($P > 0.05$). Error bars represent one SD.

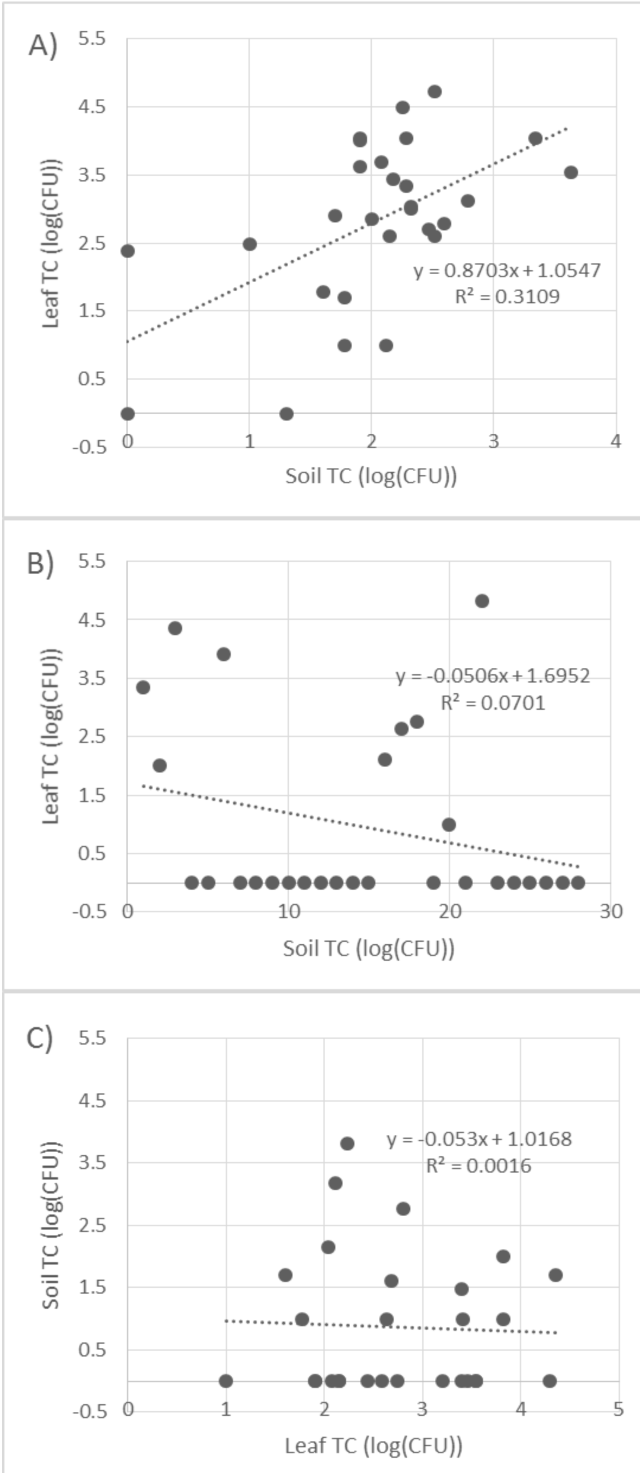


Figure 2.11 Relationship between soil and leaf total coliform (TC) counts for A) spring, B) summer, and C) fall harvests. Y-axis represents total coliform counts expressed as the log of colony forming units (CFUs) in undiluted leaf wash samples. Coliform counts were positively correlated in the spring ($R^2 = 0.3109$, $P = 0.0021$). In the summer and fall harvests there were many samples below the detection level, making relationship between soil and leaf TCs insignificant ($P > 0.05$).

2.4 Discussion and conclusions

2.4.1 Lettuce cultivars

2.4.1.1 Yield and quality

‘Solid King’ produced the largest heads of lettuce and ‘Dov’ the smallest. This difference in size would certainly affect yield in a commercial lettuce operation. However, ‘Dov’ comes from a more distantly related seed line and shows different morphology. ‘Dov’ produced a shorter, rounder head with attractive wavy foliage that appears a slightly different color in the field than some of the other varieties. Customers may perceive these as positive differences when viewing a whole head at market, thus enhancing sales despite the disparity in size. Similarly, ‘Green Forest’ was more likely to bolt but was a shade bluer than the other cultivars, an attribute that may also positively affect sales.

As expected, head weight was significantly less for all the cultivars during the summer. However, USDA standards require that standard lettuce packages containing 24 heads of lettuce weigh 10.0 to 18.1 kg (USDA, 1975). Individual heads should therefore be between 415.8 and 755.8g. Weights of all of the cultivars from all seasons were well above the lower limits of this range. Several individual heads were above the upper limit, most likely due to the presence of wrapper leaves that would be removed by commercial producers before packaging.

Stem length and percent of total head height for different cultivars were affected differentially by season. This is important because the stem becomes the flowering stalk of the lettuce plant. A longer stem or a stem that makes up a greater percent of the head height indicates that a plant closer to bolting. During the bolting process, morphological and chemical changes take place throughout the plant making it less desirable to the consumer. These changes include increased bitterness, decreased sweetness, and thicker leaves (Drewnowski, 2000, Rappaport, 1956, Dufault, 2006, Simonne, 2002, Bunning, 2010, Van Beek, 1990). Therefore, a longer stem would seem to indicate a less desirable head of lettuce.

All of the cultivars had greater stem length and percent in the summer season, indicating that all were more likely to bolt in the hot summer weather. Within the spring and fall seasons, none of the cultivars had significantly different stem length or percent, so none of them appear to have innately longer stems. During the summer season, 'Green Forest' exhibited greater stem length and percent than other cultivars, indicating that 'Green Forest' was the most sensitive to summer weather and most likely to bolt.

2.4.1.2 Sensory evaluation

Panelists seemed to prefer lettuce grown in the spring slightly to lettuce grown in the summer and fall. This was most notable in scores of acceptability of texture, flavor, and overall quality, all of which were higher in the spring. Spring lettuce was also sweeter and less aromatic than summer and fall; it had more intense texture and less sourness in the spring than in the fall. These intensity attributes likely contribute to the higher acceptability of spring cultivars. However, spring lettuce was only sometimes preferred to summer lettuce, so cultivars may still be marketable as summer varieties.

With regard to cultivar, panelists were unable to detect any difference in any of sensory attributes except intensity of bitterness and acceptability of flavor. This was true for each season. Consumers preferred the flavor of 'Dov' and of the control (grocery store varieties) to ‘Solid King,’ but other cultivars were considered equivalent in flavor. Lettuce cultivars ‘Sunbelt,’ ‘Green Forest,’ and ‘Dov’ show the highest potential for marketability.

Bitterness is most likely the limiting factor for growing lettuce in the warm weather. In this experiment, intensity of bitterness of different cultivars was differentially affected across seasons. ‘Sunbelt’ and ‘Dov’ were not affected by season. The control lettuces were more bitter in the spring than summer and fall. Interestingly, this is opposite of what was expected for cultivars grown in our climate. Commercially available lettuces were not guaranteed to be from the same supplier or produced in the same location for each season. More data would be needed to make an inference about seasonal variation in the quality of commercially available lettuce.

‘Solid King’ and ‘Green Forest’ were judged more bitter in the fall than in the spring, but not in the summer. Similarity in sensory attributes between cultivars and compared to commercially accepted varieties indicates that all field-grown cultivars were fairly acceptable to consumers and of a similar quality to lettuce they are accustomed to buying. With regard to sensory attributes, cultivars may have the potential to be used in the summer season in the hot Mid-Atlantic climate.

2.4.1.3 Overall Conclusions

‘Solid King’ appears to be the best choice for a wholesale growers trying to maximize yield. ‘Dov’ may be suited to small local growers because their audience is usually more concerned with quality, flavor, and appearance. Although all cultivars were preferred in the spring, they still may be marketable at farmers market or CSAs in the summer and fall seasons. Consumers perceived the selected cultivars as being equivalent to grocery store varieties with regard to overall flavor and quality with the exception of ‘Solid King’ slightly less favorable flavor. Given the choice, these consumers would most likely select locally grown cultivars of equivalent quality over cultivars shipped from distant parts of the country. The ability to grow these cultivars—‘Solid King,’ ‘Sun Belt,’ ‘Green Forest,’ and ‘Dov’—in a warmer climate provides local growers with a novel summer crop to complement the ample summertime tomato harvests and offers another opportunity to satisfy consumer demand for locally-sourced produce.

2.4.2 Vermicompost

2.4.2.1 Yield and quality

Soil treatments seemed to have very similar effects on lettuce. There were very few significant differences in yield, quality, and food safety characteristics. However, some trends did emerge from statistical analysis and graphical representation of the data.

Higher rates of application for all organic amendments tended to increase yield. Consistent with the majority of research on vermicompost, these materials were beneficial to the crop. The same rates (5 and 10 t ha^{-1}) of vermicomposts applied to strawberry crop significantly increased growth and yield in another study (Arancon, 2004b). Diverse vermicomposts also applied at these rates were adequate for increasing growth and yields of peppers (*Capsicum annuum*) grown in the field. The particular materials used in this study may need to be applied at higher rates to justify using them to increase crop yield and performance on lettuce.

V1 tended to be the highest performing amendment, followed by W, V2, and the control respectively. Total nutrient content of the original materials and nutrient release rate are the most likely causes of differential lettuce yield and quality. Nitrogen is the most important nutrient for production of many crops. Lettuce in particular has very high nitrogen and water requirements. Total N and nitrate-N levels in the compost materials corresponded with N levels in soil analyses of amended plots and lettuce yields. Specifically, V1 was highest in total N and lettuce plots amended with V1 experienced the highest yields, while V2 was the lowest in total N of the three organic amendments and produced the lowest yield.

However, V1 was also highest in soluble salts, and soils amended with V1 were much higher in Cu and B. These high salt concentrations may be detrimental to plant growth and yield. In other research, containerized greenhouse pepper plants showed decreased growth and yield at vermicompost rates at 60% of potting mix and above. This was attributed to high soluble salt (SS) concentration, poor aeration, and heavy metal toxicity (Arancon, 2004a). It is important to have materials tested for salts and metals before application and to apply materials accordingly. Feedstock may be an important contributor to high salinity. V1 was produced using dairy manure. This is consistent with other studies in which cattle manure and biosolids exhibited higher concentrations of SSs than leaf composts and food waste vermicomposts (Arancon, 2005). While it may be necessary to increase rates of these materials to see a significant increase in yield, caution should be taken not to apply at rates high enough to cause detrimental effects on the crop.

Nutrient release rate may be even more important than initial N content of soil amendments. While N requirements for lettuce are high, N uptake is very low in the first

3-4 weeks in the field (Bottoms, 2012, Jackson, 1994). It then increases linearly until harvest (Bottoms, 2012). N fertilization applied pre-plant is likely to experience high losses of NO_3^-, especially following irrigation events, through nutrient-leaching of the soil (Jackson, 1994). Therefore, best management practices recommend low pre-plant N fertilization and applying most N in one or two side-dressings scheduled around an appropriate crop N fertilization template (Hartz, 2006). For lettuce production, the *Commercial Vegetable Production Recommendations for Maryland 2013* advise nearly half of Nitrogen requirement be applied through sidedressing (UMD, 2013). Iceberg lettuce recommendations are a pre-plant application of 25-50 lbs N A^{-1} followed by 25-30 lbs N A^{-1} applied 3-5 weeks after planting.

Slow-release fertilizers (SRF) and controlled-release fertilizers (CRF) are another tool demonstrated to maximized crop use efficiency while minimizing nutrient leaching. Urea formaldehyde, isobutylidene diurea, and methylene urea are examples of materials that have been used successfully as SRFs and CRFs (Morgan, 2009). Composts, including vermicomposts, are considered SRFs, and may be another suitable solution. Nitrogen mineralization rates of materials used in this research are being determined in ongoing experiments.

2.4.2.2 Food safety

Food safety did not appear to be a concern for any of the soil treatments. Compared to the spring planting, very few coliforms were detected in either the summer and fall plantings. Crops in both of these seasons experienced significant rain events just before the harvest. The summer and fall received 2.49 and 11.71 cm (0.98 and 4.61 in) respectively in the week leading up to harvest. Heavy rainfall may have diluted coliform concentration in both the soil and on leaf tissue to levels below the detectable threshold of the petrifilms. However, this reasoning is questionable. Other unpublished research has shown that after rain events, there tends to be a resurgence in *E. coli* levels (Spanninger *et al.*, 2013).

In the spring planting, however, there were detectable TC levels in all but two soil and two leaf wash samples. Leaf coliform load was positively correlated with soil coliform load. This suggests that soil coliforms may be a good indicator of and possibly a contributor to leaf coliform levels. This is somewhat surprising considering black plastic mulch was used, which might be expected to separate harvestable crop from soil and water splash. Ongoing research is evaluating how soil mulch treatments affect coliform load on produce and which mulches act as effective barriers.

TC counts did not seem to be related to TCs in organic materials prior to amending soils. On average, unamended soil had higher TCs than W or V2. It may be possible that beneficial microorganisms present in vermicompost are outcompeting coliforms when added to the soil. Beneficial microorganisms may include plant beneficial microorganisms such as generally classified plant growth promoting rhizobacteria (PGPR) or more specificially actinomycetes, nitrogen-fixing bacteria, and cellulolytic bacteria.

The results from this experiment suggest lack of food safety risk in the use of vermicompost as a soil amendment on high risk crops. However, the practical implications of this information requires several important considerations. First, TCs includes a very broad range of organisms, including genera such as *Citrobacter, Hafnia,* and *Klebsiella* which are rarely the cause of illness. Because of this, TCs are not an FDA recognized food safety indicator organism. Unfortunately, due to a lack of meaningful *E. coli* data, TCs were the sole assessment of food safety in this research.

In addition, due to the highly variable nature of vermicomposting and vermicompost final products, it is impossible for this work to be representative of all vermicomposting systems—particularly the food safety aspects. All three compost materials used here underwent thermophilic treatment. This contributes significantly to the presence of human and plant pathogens present in the finished material. There is still considerable debate over whether vermicomposting is sufficient in itself to eliminate human pathogens. In what has been called conclusive evidence, Eastman *et al.* (2001) found vermicompost was effective at reduction of four human pathogens (*Salmonella*, fecal coliforms, enteric viruses, and helmintha ova) in biosolids to safe levels. Other research showed that earthworms were capable of eliminating both fecal coliforms and *E. coli* during the process of stabilizing sewage waste (Sinha, 2010). However, earthworms may also be effective vectors of pathogenic organisms. In materials inoculated with *E. coli* O157:H7, pathogen levels were found to be significantly higher after vermicomposting than in control treatments, and both lateral and vertical movement was attributed completely to the earthworms (Williams, 2006). In general, research on control of plant and human pathogens by vermicomposting is extremely inconsistent, with results depending on factors including feedstock material and vermicomposting process as well as sampling methods. Additional research is needed to determine the influence of a multitude of factors in vermicomposting on persistence of pathogens and the quality of specific vermicomposting operations. For now, pre-vermicomposting thermophilic treatment is a prudent practice for good food safety practice.

Chapter 3: Further Discussion

3.1 Reflections and future research

I set forth at the beginning of this work to find a way to increase the sustainability of agriculture in our region. The system that I chose to test included heat-tolerant lettuce for season extension and vermicompost to boost yield and quality of lettuce crop. One year of testing this system has been successfully completed with promising results. At least one more year replicating these trials is needed to verify results. Year two (2014) has been planned and the lettuce for the first seasonal planting was transplanted in mid-April.

Like any research, this project most likely generated more questions than it answered. Some I will be able to address in the subsequent season(s), but some I will not. These questions include improvements to the experimental design and execution, as well as future directions that have arisen from this work. Inclusion of these things during the first year of the study was limited partly by time and resources. More so, it was limited by my personal experience with the scientific literature and process. Following is an examination of some of these questions and suggestions of additional experiments that arise from discussion of the present study.

3.1.1 Lettuce cultivars

Several factors limited the quality and breadth of data collected on lettuce cultivars grown for heat tolerance. Only four lettuce cultivars were evaluated in this study for performance in high temperature growing conditions. This is a meager amount compared to other studies, in particular a variety trial conducted at University of

Delaware which tested twenty romaine cultivars (Ernest, 2012). However, the limited number of cultivars tested in this research were the top performers of ten original cultivars planted in a preliminary trial in 2012. As breeding projects and possible genetic modification of lettuce identify and create new cultivars, additional variety trials will need to be conducted in various regions to determine the potential of these cultivars.

For the cultivars that were tested, logistics were problematic. As soon as the lettuce heads are cut they begin to wilt. We made every effort to minimize wilting by keeping the lettuce heads cool during transportation from the field to the lab using a highly air-conditioned van. However, WyeREC is over an hour away from the processing laboratory at UMD. Some water loss occurred between harvesting and processing, and probably affected fresh weight. If more resources had been available, a better option would have been shipping in a climate controlled (refrigerated) truck. Standard practice for producers in California is to vacuum cool lettuce to 1°C (34°F), as soon as possible after harvest. Some large operations also remove lettuce cores and bag heads individually in the field to prevent such water loss. Neither of these were an option for this research project.

Other variables could have provided a more detailed description of lettuce quality. Molecular analysis using gas chromatography mass spectroscopy (GCMS) technology has been used to identify and quantify bitter constituents as well as vitamins and nutrients in lettuce in other studies. A collaboration with ARS Food Composition and Methods Development Lab in Beltsville was investigated at the beginning of this research. However, there was insufficient time and resources to conduct such analysis. Future studies should use GCMS to quantify compounds in lettuce that confer bitterness and nutrition to consumers.

3.1.2 Vermicompost

Vermicompost has effects on physical, chemical, and biological conditions of the soil. Measuring additional variables covering a combination of these conditions would generate a more comprehensive illustration of the various soil amendments and their effects on lettuce, and should be added to future research. A more complete study should also incorporate multiple field locations to take into account variation in physical, chemical, and biological aspects of different sites.

Other research has measured physical variables of vermicompost such as water holding capacity, porosity, drainage, and aeration. While it is well documented that vermicompost improves the physical characteristics of the soil, quantification of these variables for specific materials would be beneficial.

Nutrient availability is one chemical variable that is highly dependent on environmental conditions. This is particularly important for vermicompost, an SRF whose nutrient release is dependent on microbial activity, soil type, temperature, and moisture content. Executing this study at multiple sites with different soil conditions would provide much broader insight into the nature of these materials.

Quantification of N uptake would also be useful information. While green color and chlorophyll content measured in this experiment can be correlated with N content of leaf tissue, they are not a direct measurement. Instead, leaf N could be quantified by leaf mid-rib analysis utilizing dry tissue or stem sap for analysis. Such samples could have also been taken over the course of the growing season to determine the time course of N uptake.

While the present research concentrated on the physical and chemical aspects of vermicompost, many of the effects of vermicompost have also been attributed to their biological effects. It would be interesting to quantify microbial activity via microbial respiration and microbial biomass. Future work should also take advantage of Next-Generation Sequencing technology to characterize the microbial community structure in the vermicompost amendments as compared to windrowed compost and surrounding soil. The diversity and speciation of microorganisms present in these communities may be an influential factor in the performance of lettuce crop.

In a study characterizing the microbial communities of various compost materials, experiments would involve isolating and sequencing genetic material from samples taken of the finished compost material and amended soil over the course of the growing season. DNA would be extracted with an extraction kit and target sequences amplified using PCR. Powerful new technology and software, including 454 Pyrosequencing, Illumina Sequencing, and QIIME are available for characterization of amplified sequences. Describing the similarities and differences in microbial communities between various materials may provide insight into which microbes play an important role in improved plant performance.

Earthworm biology and the effect on vermicompost is another area with the potential for very productive research. As earthworms decompose organic material, they alter the material significantly by changing physical, chemical, and biological properties. Material passing through the earthworm gut experiences changes in microbial community. It would be interesting to monitor changes in these communities by characterizing them over time. Again this would require significant resources and Next-Generation Sequencing technology.

One practical application of monitoring these changes in microbial communities is food safety. The role of earthworms in control or spread of human pathogens is still debated. Persistence of pathogens could be monitored through inoculation of organic materials and sampling over the course of the vermicomposting process.

3.1.3 Food Safety

Food safety risk analysis in 2013 produced highly erratic data, warranting changes in future research. Coliform counts were extremely low, even at very low dilutions, and *E. coli* was only observed in two samples taken early in the season. It is nearly impossible to use such data to run a statistical analysis and produce meaningful results. While low counts imply low food safety risk, they may not necessarily be accurate. Factors such as weather conditions and dilution volumes can influence these data. Another year of data will help to clarify my results.

The food safety portion of data collection was one of the most time-consuming and largest financial investments of this project. In an effort to reduce wasted time and resources, minor changes will be made to the food safety protocol. In 2014, data will only be collected on leaf washes, instead of taking both soil samples and leaf washes. Also, data will be collected on total coliforms and fecal coliforms instead of total coliforms and *E. coli*. Fecal coliforms are a subset of total coliforms, more fecal-specific in origin, while *E. coli* is one species of fecal coliform. The same 3M petrifilms used to quantify coliforms in 2013 will be used to quantify fecal coliforms: total coliforms are incubated at 37^{0}C and fecal coliforms are incubated at 43^{0}C. Testing for these bacteria will hopefully yield statistically useable data.

3.2 Additional work

In addition to work presented in the manuscript (Chapter 2), other experiments and data analysis was conducted. It is detailed below.

3.2.1 Vermicompost mineralization experiments

Experiments were conducted on nutrient availability of the various soil amendments. The goal of these experiments was to determine the nitrogen release rates (i.e. mineralization and nitrification rates) of the materials. While this is a standard practice for composts and synthetic materials, it has not been reported for many vermicomposts. Results would allow us to compare mineralization and nitrification between soil amendments. They would also help nutrient management specialists provide educated recommendations to farmers about the application rate and time of these vermicomposts.

Unfortunately, my experiments are still incomplete; analysis of samples is ongoing. See Figure D.1 for diagram of experiments. The ongoing (2014) experiments are described below:

To determine nutrient availability to plants, N mineralization rate was determined for each material, in both the field and the lab. In the field, two ion exchange resin bags were planted per plot. The bags were made of 7-10cm sections of nylon filled with 10g of Amberlite IRN-150 ion exchange resin and secured with a knot. A string was tied to each bag for easy location and removal from the soil (Figure D.1). Resin bags were planted and harvested on the same dates as the lettuce. Resin from each bag was emptied into a 50ml centrifuge tube. Resin was extracted by shaking horizontally on an orbital shaker with 35ml 2M KCl for 1 hour at 100rpm. Extracts were filtered and analyzed for NH_4^+ and NO_3^- concentrations using a Lachat continuous autoanalyzer system (Lachat Instruments, Loveland, CO).

In the lab, N mineralization was compared among materials used in the field, plus an additional industry standard produced by Worm Power (Avon, NY). Soil preparation and incubation procedure was modified from protocols created for nationally coordinated research on N mineralization (Honeycutt, 2005). Amendments were applied to field soil at rates similar to those used in the field such that initial total N was approximately equal. Jars containing soil mixtures were held at 25^0C for approximately three months to mimic a full growing season. 5g samples were taken from each jar at days 0, 7, 35, 63, and 98 (on the date of application, one week after application, and then monthly). Samples were extracted and analyzed using the same method described for resin extractions above.

Data is currently being collected on the resin and soil extracts. The field experiments (resin bags) will be used to calculate net N mineralized over the growing period during each season (total NH_4^+ and NO_3^- extracted from resin). Lab incubation data will be used to determine nitrification and mineralization rates over the course of a full growing season (approximately three months). I am hopeful that there will be similarities between the field and soil rates. Unfortunately in other research it has been difficult to correlate lab and field mineralization data due to the multitude of variables affecting nutrient cycling in the field.

3.2.2 Further statistical analysis

Additional statistical analysis will be performed once a full data set has been obtained. Data that are still missing include N mineralization data and the second year of field experiments. Treatments effects were not significant for many of the response variables, such as chlorophyll content, color, and yield. However, it seems unlikely that these things would be unrelated to differential N content of the materials and that they would have no relationship with each other.

In addition, there are still a number of relationships between variables that remain unclear. Several interactions were found between explanatory variables. These may be evidence of biological conditions or the result of insufficient replication for data. For example, there was a significant interaction between season and soil treatment for head weight. It is possible that soil treatments were differentially effected by the seasons, but seems unlikely. Also, bitterness and bolting of several of the cultivars was greater for summer and/or fall seasons. These cultivars may actually respond differently to the hotter conditions, or it may be an artifact of insufficient data.

On the other hand, a number of trends were observed in the data that were not found to be significant in data analysis. For instance, soil treatments and rates generally did not have significantly different effects on lettuce yield and quality. However, graphical representation showed that vermicompost amendments and increased rate of all amendments resulted in increased yield. At least one additional year of data will be required to clarify these relationships. Results from replicated years will be used to make more informed recommendations about this system.

At the end of a second year's work, additional statistical analysis may also be useful. Principle Component Analysis would enable examination of possible correlations between all measured variables.

3.3 Educational Impact

3.3.1 Dissemination of results

The results of this project have been disseminated at grower meetings including Central Maryland Vegetable Growers Meeting (January 2014) and the WMREC Horticultural Crops Twilight Meeting (August 2013). Results of lettuce cultivar research was published in the University of Maryland Extension publication, *Vegetable and Fruit Headline News* (Vol 4 Iss10. October 24, 2013). Growers have been receptive to the project and excited to try components of the proposed system in their operations.

It is my intention to share research with the scientific community. Research questions and findings were also shared with the scientific community through poster presentations at GRID and BioScience Day at the University of Maryland. The project will be replicated in the 2014 growing season to verify results and facilitate publishing in a peer-reviewed scientific journal. The literature review (Chapter 1) will be submitted to the peer-reviewed journal *Agroecology and Sustainable Food Systems*. The manuscript (Chapter 2) will be submitted to the ASHS publication *HortTechnology*.

3.3.2 Undergraduate education

In addition, this project provided an invaluable experience in scientific research for our undergraduate assistant, Elizabeth Prinkey. Elizabeth was a critical resource for accomplishing the heavy workload required by this project. She was also engaged enough in the entire project to provide insights and corrections to the experimental process. Her positive attitude and attention to detail especially made her a tremendous asset to have on my team.

Elizabeth gleaned a tremendous amount from her experience as a research assistant. She expressed interest in graduate school in a scientific discipline at the beginning of work, so I made many efforts to explain to her the scientific process and community as I navigated it myself. She learned critical tools and skills including searching and reading the literature, experimental design, data collection, analysis with SAS, and presentation of data through extension meetings and poster presentations.

3.3.3 Personal Development

The impact of this project was much broader than the specific results inferred from the data collected during research. As the primary investigator of this project, I was tasked with all aspects of the research process. This involved formulating a research idea, examining the scientific literature to determine the current state of knowledge in the field, constructing a testable hypothesis, creating and executing a research plan, and then interpreting and disseminating results.

I have become proficient in examining the scientific literature and dissecting academic papers in order to glean their true scientific merit. I applied for funding through the Northeast SARE Graduate Student Grant and the MDA Specialty Crops Block Grant. Unfortunately the SARE proposal was rejected; reviewers did not understand that one year of data had already been collected and deemed the project to broad and overzealous. Nonetheless, through this writing I have gained valuable experience navigating the funding processes and institutions. I have honed my writing skills by preparing this thesis, and many presentations and reports about this project and other projects in which I have been involved.

This project and the classes I have taken as part of my graduate education have broadened my knowledge in the field of plant science, horticulture, and agriculture. I have learned foundational and advanced knowledge of plant physiology, pathology, identification, insects, soil science, and statistics. I have gained the skills and knowledge to launch a career in the academic and applied sciences through which I will give back to the scientific and agricultural communities by contributing meaningful research and education.

Appendix A – Planting Logistics

Table A.1. Summary of weather conditions

Planting	Avg precipitation (in/day)	Avg high (ºF)	Avg low (ºF)	High (ºF)	Days above 26.7ºC (83.0ºF)
1 – Spring	0.089	70.99	49.55	90.1	7
2 – Summer	0.059	84.79	64.83	95.7	22
3 – Fall	0.133	76.41	53.81	93.2	13

Table A.2. Timetable of plantings

Planting	Seeding	Transplant	Harvest
1 – Spring	March 13	April 14	June 3
2 – Summer	May 17	June 18	August 5
3 – Fall	July 15	August 28	October 16

Appendix B – Compost Materials

Soil amendments were selected based on their ability to represent the vermicompost being produced and distributed locally to the Mid-Atlantic. They were chosen based on the extent of distribution and the expertise of the people producing the compost. All materials were obtained from local operations. See Table 2.5.

Two vermicomposts were chosen for the integrity of the operations producing them, their extensive use in the area, and their ability to complement each other. Vermicompost 1 (V1) was produced by Full Circle and Vermicompost 2 (V2) by ECO City Farms, in Edmonston. Both operations produce vermicompost on a large scale and distribute to an expansive part of our region. However, they are produced from differing feedstock materials. Full Circle's vermicompost is produced from dairy manure solids, while ECO's vermicompost is produced from urban food waste collected from Washington DC by Compost Cab. Both of these materials undergo thermophilic composting prior to vermicomposting to kill human pathogens that may be present.

The windrowed compost was selected to complement the vermicompost materials. It was provided by the USDA compost facility and produce from dairy manure solids. All materials were considered finished compost materials. Samples were sent to Penn State Laboratory for complete compost analyses prior to application (Appendix B).

Appendix C – Compost and Soil Analyses

Table C.1 Compost Analysis – Performed by Penn State Laboratory

Material	pH	Soluble Salts (1:5 w:w mmhos/cm)	Moisture (%)	Organic Matter (%)	Total N (%)	C (%)	C:N Ratio (%)	Nitrate-N (mg/kg)
W	7.5	5.7	0.37	0.74	0.0119	0.124	10.4	475.7
V1	7.1	21.4	0.585	0.296	0.0156	0.157	10	2411.5
V2	6.7	3.68	0.553	0.187	0.0089	0.1	11.3	401.8

Table C.2 Soil Analyses – Performed by A&L Eastern Laboratories, Richmond, VA
Table C. 2a Spring 2013

Treatment	Organic matter	P	K	Mg	Ca	pH	C.E.C	NO_3N	S	Zn	Mn	Fe	Cu
N	1.7	49	83	119	731	5.8	6	46	13	1.5	34	113	1
Wa	2.1	83	180	147	916	5.9	7.6	78	17	1.8	51	120	1
Wb	2.4	123	396	179	1041	6	7.6	94	19	3.9	49	123	1
V1a	2.7	121	292	169	961	5.9	8.4	114	19	2.9	52	123	3.4
V1b	3.5	153	601	221	1199	6.3	10.5	171	28	4.6	48	116	6.6
V2a	2.8	101	148	140	926	5.9	7.5	103	17	3	45	116	1.1
V2b	2.4	101	153	146	935	5.8	7.8	96	14	3.4	49	121	1

Table C.2b Summer 2013

Treatment	Organic matter	P	K	Mg	Ca	pH	C.E.C	NO_3N	S	Zn	Mn	Fe	Cu
N	1.4	103	130	122	813	5.6	7.1	.	.	.	.	.	.
Wa	1.9	109	337	150	913	5.5	2.3	.	.	.	.	.	.
Wb	2.5	148	731	208	1137	5.8	11.5	.	.	.	.	.	.
V1a	2.7	121	419	168	997	5.6	9.7	.	.	.	.	.	.
V1b	3.2	138	771	225	1131	6.1	11	.	.	.	.	.	.
V2a	2.2	123	210	152	922	5.6	8.4	.	.	.	.	.	.
V2b	2.4	160	324	184	1117	5.5	10.7	.	.	.	.	.	.

Table C.2c Fall 2013

Treatment	Organic matter	P	K	Mg	Ca	pH	C.E.C	NO_3N	S	Zn	Mn	Fe	Cu
N	2.2	43	83	158	797	5.3	8	.	.	.	.	.	.
Wa	2.5	55	165	164	830	5.5	8	.	.	.	.	.	.
Wb	2.6	71	304	191	981	5.8	9	.	.	.	.	.	.
V1a	3.6	103	571	272	1342	5.9	12.6	.	.	.	.	.	.
V1b	5	181	1305	397	1808	6.3	17.5	.	.	.	.	.	.
V2a	2.7	61	157	189	1040	5.3	10.4	.	.	.	.	.	.
V2b	3.4	111	218	235	1375	5.5	12.7	.	.	.	.	.	.

Appendix D – Vermicompost effects by season

Table C.1. Effect of vermicompost soil amendments and rates on yield and quality characteristics of lettuce in spring 2013.

	Weight (g)	Head Height (in)	Diameter (in)	Diameter (mm)	Stem Length (cm)	Percent (%)	Color L*	Color a*	Color b*	Root Dry weight (g)	Coliforms Log(ltc)[z]	Coliforms Log(stc)[y]
Trt												
V1	745.67[a]	12.04[a]	8.54[a]	25.07[a]	nr	nr	50.19[a]	-7.56[a]	12.44[a]	9.44[ab]	2.73[a]	2.43[a]
V2	624.42[b]	11.31[b]	8.39[a]	23.35[b]	nr	nr	49.66[a]	-7.42[a]	12.31[a]	8.53[b]	3.34[a]	1.99[a]
W	729[a]	11.74[ab]	8.44[a]	25.19[a]	nr	nr	50.75[a]	-7.34[a]	12.32[a]	10.01[a]	4.05[a]	2.47[a]
Rate												
A	668.47[a]	11.53[a]	8.43[a]	23.87[a]	nr	nr	50.25[a]	-7.34[a]	12.09[a]	9.24[a]	3.68[a]	2.56[a]
B	730.92[b]	11.87[a]	8.48[a]	25.21[b]	nr	nr	50.15[a]	-7.54[a]	12.63[a]	9.41[a]	3.07[a]	2.03[a]
ANOVA												
Trt	0.0046**	0.0158*	0.8139[NS]	0.0328*	nr	nr	0.6400[NS]	0.8394[NS]	0.9906[NS]	0.0432*	0.1288[NS]	0.3252[NS]
Rate	0.0378*	0.0783[NS]	0.7694[NS]	0.0331*	nr	nr	0.9154[NS]	0.5207[NS]	0.5224[NS]	0.7079[NS]	0.2364[NS]	0.0730[NS]
Trt*Rate	0.4690[NS]	0.4768[NS]	0.4018[NS]	0.9252[NS]	nr	nr	0.2593[NS]	0.2132[NS]	0.1493[NS]	0.4719[NS]	0.8238[NS]	0.7386[NS]

[a] = means followed by different letters within a column indicate significant difference between treatments
[z] = Log (leaf total coliforms), log of total coliform counts from leaf wash dilutions
[y] = Log (soil total coliforms), log of total coliform counts from soil sample dilutions
*=significant (α=0.05), **=significant (α=0.01), NS=not significant ($p \geq 0.05$)
nr=not recorded

Table C.2. Effect of vermicompost soil amendments and rates on yield and quality characteristics of lettuce in summer 2013.

	Head			Stem			Color			Root	Coliforms		Chlorophyll	
	Weight (g)	Height (in)	Diameter (in)	Diameter (mm)	Length (cm)	Percent (%)	L*	a*	b*	Dry weight (g)	Log(ltc)^{z}	Log(stc)^{y}	Chla^{x} (µg/mg)	Chlb^{w} (µg/mg)
Trt														
V1	634.37^{a}	11.08^{a}	8.91^{a}	22.63^{a}	2.20^{a}	0.20^{a}	52.90^{a}	-6.42^{a}	10.40^{a}	30.89^{a}	0.49^{a}	0.84^{a}	63.97^{a}	805.76^{a}
V2	619.40^{a}	10.82^{ab}	8.50^{a}	21.47^{a}	1.94^{a}	0.18^{a}	53.13^{a}	-6.74^{a}	11.15^{a}	27.31^{a}	1.06^{a}	0.97^{a}	58.46^{a}	736.18^{a}
W	513.30^{a}	10.12^{b}	8.04^{a}	22.41^{a}	1.60^{a}	0.15^{a}	52.94^{a}	-6.77^{a}	10.78^{a}	27.77^{a}	0.80^{a}	0.27^{a}	48.03^{a}	604.95^{a}
Rate														
A	592.00^{a}	10.81^{a}	8.42^{a}	22.89^{a}	1.99^{a}	0.18^{a}	53.00^{a}	-6.82^{a}	10.99^{a}	26.63^{a}	1.04^{a}	0.66^{a}	58.33^{a}	734.62^{a}
B	586.05^{a}	10.54^{a}	8.55^{a}	21.45^{a}	1.84^{a}	0.17^{a}	52.98^{a}	-6.46^{b}	10.56^{a}	30.69^{a}	0.53^{a}	0.73^{a}	55.31^{a}	696.64^{a}
ANOVA														
Trt	0.1132^{NS}	0.0494*	0.1145^{NS}	0.6431^{NS}	0.0722^{NS}	0.1704^{NS}	0.9018^{NS}	0.0758^{NS}	0.2507^{NS}	0.5664^{NS}	0.8028^{NS}	0.5387^{NS}	0.1451^{NS}	0.1447^{NS}
Rate	0.9035^{NS}	0.3735^{NS}	0.6917^{NS}	0.2174^{NS}	0.4240^{NS}	0.5921^{NS}	0.9669^{NS}	0.0115*	0.2544^{NS}	0.1702^{NS}	0.4793^{NS}	0.8937^{NS}	0.6305^{NS}	0.6312^{NS}
Trt*Rate	0.0733^{NS}	0.0597^{NS}	0.7365^{NS}	0.7955^{NS}	0.5900^{NS}	0.8093^{NS}	0.8846^{NS}	0.3452^{NS}	0.8813^{NS}	0.4471^{NS}	0.2514^{NS}	0.9633^{NS}	0.7155^{NS}	0.716^{NS}

^{a} = means followed by different letters within a column indicate significant difference between treatments

^{z} = Log (leaf total coliforms), log of total coliform counts from leaf wash dilutions

^{y} = Log (soil total coliforms), log of total coliform counts from soil sample dilutions

^{x} = chlorophyll *a* concentration (µg pigment per mg dry weight leaf tissue)

^{w} = chlorophyll *b* concentration (µg pigment per mg dry weight leaf tissue)

*=significant (α=0.05), **=significant (α=0.01), ^{NS}=not significant ($p \geq 0.05$)

nr=not recorded

Table C.3. Effect of vermicompost soil amendments and rates on yield and quality characteristics of lettuce in fall 2013.

		Head			Stem			Color		Root	Coliforms	
	Weight (g)	Height (in)	Diameter (in)	Diameter (mm)	Length (cm)	Percent (%)	L*	a*	b*	Dry weight (g)	Log(ltc)^{z}	Log(stc)^{y}
Trt												
V1	1070.25^{a}	12.38^{a}	9.09^{a}	30.22^{a}	4.07^{a}	0.33^{a}	52.86^{a}	-8.40^{a}	12.30^{a}	7.12^{a}	0.42^{a}	2.78^{a}
V2	963.44^{ab}	12.13^{a}	8.78^{a}	28.97^{a}	3.91^{a}	0.32^{a}	53.66^{a}	-11.51^{a}	12.73^{a}	5.98^{a}	1.29^{a}	2.85^{a}
W	867.44^{b}	11.91^{a}	8.59^{a}	28.01^{a}	3.37^{a}	0.28^{a}	53.00^{a}	-2.84^{a}	11.71^{a}	4.91^{a}	0.99^{a}	2.06^{a}
Rate												
a	956.42^{a}	12.17^{a}	8.83^{a}	29.02^{a}	4.05^{a}	0.33^{a}	53.12^{a}	-7.63^{a}	12.13^{a}	6.19^{a}	1.12^{a}	2.52^{a}
b	977.67^{a}	12.10^{a}	8.81^{a}	29.12^{a}	3.53^{a}	0.29^{a}	53.22^{a}	-7.54^{a}	12.37^{a}	5.82^{a}	0.68^{a}	2.61^{a}
ANOVA												
Trt	0.0398*	0.1612^{NS}	0.1939^{NS}	0.0514^{NS}	0.3872^{NS}	0.4788^{NS}	0.3930^{NS}	0.1466^{NS}	0.2040^{NS}	0.1132^{NS}	0.3217^{NS}	0.1865^{NS}
Rate	0.7239^{NS}	0.7460^{NS}	0.9246^{NS}	0.8800^{NS}	0.2354^{NS}	0.2136^{NS}	0.8301^{NS}	0.9811^{NS}	0.5934^{NS}	0.6491^{NS}	0.3557^{NS}	0.8093^{NS}
Trt*Rate	0.8573^{NS}	0.6330^{NS}	0.5649^{NS}	0.1504^{NS}	0.8612^{NS}	0.8348^{NS}	0.9116^{NS}	0.5129^{NS}	0.2128^{NS}	0.4590^{NS}	0.1039^{NS}	0.8650^{NS}

^{a} = means followed by different letters within a column indicate significant difference between treatments

^{z} = Log (leaf total coliforms), log of total coliform counts from leaf wash dilutions

^{y} = Log (soil total coliforms), log of total coliform counts from soil sample dilutions

^{x} = chlorophyll *a* concentration (µg pigment per mg dry weight leaf tissue)

^{w} = chlorophyll *b* concentration (µg pigment per mg dry weight leaf tissue)

*=significant (α=0.05), **=significant (α=0.01), ^{NS}=not significant ($p \geq 0.05$)

Appendix E – Nitrogen mineralization experiments

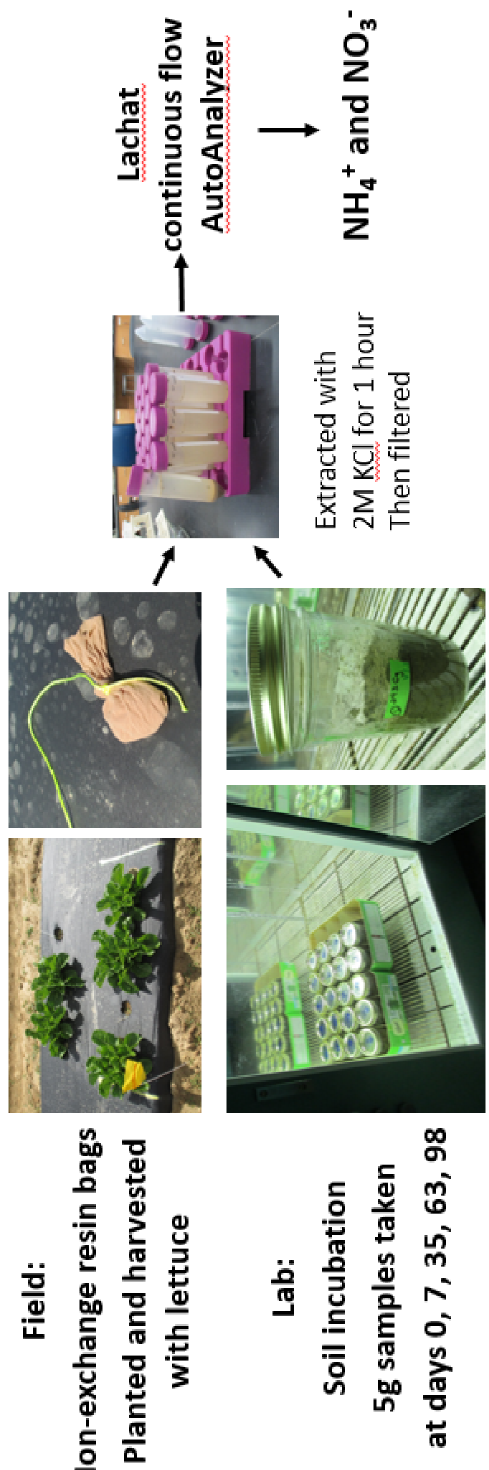


Figure D.1. Nitrogen mineralization flow chart. Field: resin bags containing 10g Amberlite ion-exchange resin were planted at the same time as lettuce transplants and harvested at the time of lettuce harvest. Resin was extracted with 2M KCl. Lab: field soil amended with soil treatments was held at 21.1^{0}C (70^{0}F); 5g samples were taken at day 0, 7, 35, 63, and 98, and extracted with 2M KCl. Extractions from field and lab experiments are currently under analysis by Lachat continuous flow analyzer for NH_4^+ and NO_3^- concentration

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ANNA E. WALLIS

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Education

University of Maryland, College Park, MD

Masters Candidate of Plant Science	B.S. in Biology, B.A. in Music
August 2012 – Present	Graduated: May 2011
GPA: 4.0/4.0	GPA: 3.89/4.0

Honors and Awards

Dean's List, *Fall 2006-Spring 2011*
Primannum Honors Society and NSCS Honors Societies, *December 2006 – present*
College Park Scholars Program, *September 2006 –May 2008*
PSLA Outstanding Graduate Teaching Assistant *2012-2013 Academic Year*
Wallace Bailey, Sr. Research Grant Award, *October 2012*
Poster Winner, UMD Bioscience Day, *2013*

Relevant Coursework

Insect pests of the Mid-Atlantic with IPM, fruit and vegetable technology, food safety and GAPs, biostatistics with SAS, plant physiology, plant pathology, international agriculture, research methods, microbiology, organic and inorganic chemistry with lab, biochemistry

Experience

Plant Science Department, University of Maryland, College Park
Dr. Christopher Walsh, Professor of Horticulture
Graduate Student *August 2012– present*

- Designed and conducted lab and field research project evaluating sustainable lettuce production assessing the effects of summer varieties and vermicompost soil amendment on quality and food safety
- Collaborated on specialty crop research projects including apple rootstocks, raspberry post-harvest storage
- Interpreted results and communicated research findings at extension and grower meetings
- Managed undergraduate student research assistant; advised her on an independent project

Teaching Assistant *August 2010 – present*

- Taught horticultural topics such as plant anatomy, physiology, propagation, grafting, woody plant identification
- Independently instructed classes of 25 students in classroom, laboratory, greenhouse, and outdoor settings
- Developed, evaluated, and revised course material including laboratory manual, exams, assignments
- As lead TA, managed a team of 4 teaching assistants and coordinated all lab materials and events, taught weekly instruction lab, served as the main contact point for students

ECO City Farms, Edmonston, MD
Christian Melendez, Farm Manager and Margaret Morgan-Hubbard, Founder and CEO
Volunteer Intern, *March– September 2013*

- Performed daily responsibilities on a 2-acre urban farm growing vegetables for CSA and farmers market using innovative organic practices in nursery, high tunnel, open fields with other volunteers and interns

- Worked at Riverdale Park farmer's market: harvested and packed produce, organized stand, and sold produce
- Maintained vermicompost bins and castings
- Volunteered at events to communicate and promote ECO's mission to foster community offshoots and provide equitable, healthy food

The Green Farmacy Garden, Fulton, MD
Dr. James A. Duke, world-renown ethnobotanist and author of *The Green Pharmacy*
Gardener and Botanical Researcher, *March – September 2012*

- Managed microclimates of 80-bed 4-terrace organic medicinal plant garden with two other women
- Led private and public tours of the garden; hosted guests and volunteers
- Studied the botany, medicinal properties, and preparations of plants
- Created signs for over 200 plants, making the garden self-guided

Entomology Department, University of Maryland, College Park
Dr. Jeff Pettis, ARS Entomologist and Research Leader
Bee Researcher, *October 2011 – February 2012*

- Logged, managed and analyzed bee samples in a country-wide survey of varroa mites and nosema spores
- Corresponded with labs in California on a daily basis to coordinate and manage samples and data
- Blogged about daily lab work and entomology research for a non-scientific audience

Entomology Department, University of Maryland, College Park
Dr. Galen Dively, Professor Emeritus and IPM Consultant
Entomology Field Technician, *June 2011 – September 2011*

- Planted, maintained, harvested ~10 acres of agricultural and specialty crops in a team of 5-10 people
- Assessed insect damage to crops in several agricultural studies involving organic pesticides and susceptibility to common pests, i.e. BMSB, by measuring insect populations, rating fruits, and measuring crop yield
- Instructed groups of 5-10 people on daily tasks and communicated knowledge of bees and crops
- Kept bees in 26 hives in a team of 3; performed full hive inspections and analyzed hive samples in lab

Research Experience for Undergraduates (REU) Internship, Mount St Helens, Washington
Christian Che-Castaldo, PhD Candidate under Dr. Bill Fagan (BEES Program)
REU Intern Research Assistant, *May – August 2010*

- Designed, planned, and implemented field experiment on species interaction between willow shrubs *(Salix sitchensis)* and the stem-boring weevil (*Cryptorynchus lapathi)*.
- Collected data independently and as part of a team in several studies concerning *Salix sitchensis*
- Studied ecological research, primary succession ecosystems, and volcano ecology
- Spent up to seven consecutive 10-hour days working in the field: camping, hiking, collecting data, and carrying 35lb+ backpacks and equipment in remote areas and in all weather conditions

Ecology Lab, University of Maryland, College Park
Kevin Barry, PhD Candidate under Dr. Michele Dudash, Associate Professor (BEES Program)
Research assistant, *December 2009 – May 2010*

- Processed plants in study comparing competition of native vs. invasive species
- Collected and organized data using Microsoft Excel
- Learned to identify plant families and local mid-Atlantic species and how to use plant key and guide books

America Reads America Counts, College Park, MD
Math Counts mentor at Springhill Lake Elementary School
Mentor volunteer, *June 2007 – August 2009*

- Tutored small groups of 4th grade students twice a week after school on math skills and homework
- Planned activities in a group of other mentors for small (3-5 students) and large (30 students) groups
- Led large groups in interactive math games

Emory Knoll Farms, Harford County, Maryland
Ed Snodgrass, internationally recognized supplier of plants and plant expertise for extensive green roof systems
Summer farm and nursery intern, *June 2007 – August 2009*

- Worked in a team growing, maintaining, propagating, shipping, and studying green roof plants and systems in a sustainable farm and nursery setting
- Implemented techniques used for plant care in a mass-production farm and greenhouse setting
- Learned about a variety of green technologies and visited universities to learn about green roof systems

Invited Talks and Presentations

- 89th Annual Cumberland-Shenandoah Fruit Worker's Conference. Field-testing advanced selections from the Geneva apple rootstock breeding program 12/5/13 – 15 attendees
- Central Maryland Vegetable Growers Meeting. Heat Tolerant Romaine Lettuce Cultivars for Season Extension in the Mid-Atlantic 1/24/14 – 100 attendees
- WMREC Horticultural Crops Twilight Meeting. Sustainable Lettuce Production: Summer Varieties and Vermicompost 8/28/13 – 40 attendees

Publications

- Wallis, Anna. Sustainable Lettuce Production: Summer Varieties and Vermicompost. *Vegetable and Fruit Headline News.* Vol4 Iss10. October 24, 2013. UMD Extension.