DISSERTATION

ORGANIC NITROGEN FERTILIZERS INFLUENCE NUTRITIONAL VALUE, WATER USE EFFICIENCY, AND NITROGEN DYNAMICS OF DRIP IRRIGATED LETTUCE AND SWEET CORN

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ABSTRACT

ORGANIC NITROGEN FERTILIZERS INFLUENCE NUTRITIONAL VALUE, WATER USE EFFICIENCY, AND NITROGEN DYNAMICS OF DRIP-IRRIGATED LETTUCE AND SWEET CORN

Farmers usually rely on off-farm sources (fish emulsion, feather meal, blood meal) for the additional N needed during the growing season, and they are willing to pay the extra shipping cost. However, there is another fertilizer option being developed that could allow farmers to produce N on-farm, which is cyanobacteria, formerly knowns as the blue green algae. The general objectives of this study were to assess effects of organic N fertilizer application and N rates on nutritional value, water use efficiency, N dynamics of sweet corn and lettuce. A two-year field study was conducted in the summers of 2013 and 2014 at the Colorado State University Horticulture Research Center, Fort Collins, CO. The fertilizers used in this study were blood meal, feather meal, fish emulsion, and cyano-fertilizer. Both fish emulsion and cyano-fertilizer were supplied in four split applications over the growing season through drip irrigation, while the blood meal and feather meal were subsurface banded prior to planting. Lettuce and sweet corn were used as an indicator to evaluate effects of organic nitrogen (N) fertilizers on nutritional value, water use efficiency, and N dynamics. The aims of this study were to evaluate the effect of different types of organic N fertilizer on nutritional value; β-carotene, iron (Fe), zinc (Zn), marketable yield, water use efficiency (WUE), residual soil NO_3^--N, N content, and N use efficiency (NUE) of horticultural crops, particularly lettuce and sweet corn.

All fertilizer treatments in 2013 increased β-carotene concentration in leaf tissue compared to control, while only fish emulsion had a higher β-carotene concentration compared to other treatments in 2014. The high indole-3-acetic acid (IAA) applied in the fish emulsion treatment could have increased β-carotene concentration in lettuce in both years. Amount of IAA applied in the fish emulsion treatment was positively correlated with β-carotene concentration in both years. A significant negative correlation was

found between marketable yield and β-carotene concentration in leaf tissue in 2014. High salicylic acid (SA) applied in the cyano-fertilizer treatment had a higher total leaf area compared to other fertilizers in both years.

In lettuce, the blood meal treatment had a lower leaf Fe and Zn concentrations than other fertilizer treatments at 112 kg N ha^{-1}. The cyano-fertilizer treatment had a higher leaf Fe concentration at 56 kg N ha^{-1}. Leaf N concentration was positively correlated with Leaf Fe and Zn concentrations. Amount of NO_3^--N applied in organic N fertilizers was negatively correlated with leaf Fe concentration. The cyano-fertilizer, fish emulsion, and blood meal treatments increased Fe concentration in sweet corn compared to feather meal. Amount of NO_3^--N, Fe, and Zn applied in organic N fertilizers were positively correlated with kernel Fe concentration, while amount of NH_4^+-N applied was negatively correlated with kernel Fe concentration. There was no N rate or treatment effect on leaf and kernel N concentrations in sweet corn.

The amount of phytohormone, Ca, and Fe applied in organic N fertilizers may have affected field water use efficiency (fWUE), instantaneous water use efficiency (iWUE), kernel number, and leaf gas exchange components of sweet corn. Cyano-fertilizer apparently had a higher WUE, likely due to the high amount of SA applied. A positive relationship was observed between the amount of SA applied with iWUE and fWUE. The amount of Fe applied in organic N fertilizers had a positive correlation with leaf VPD and transpiration rate. The amount of Ca applied in the feather meal treatment may have contributed to increasing leaf temperature and decreasing net photosynthetic rate. The amount of NH_4^+-N and Ca applied in the feather meal treatments were negatively correlated with both iWUE and fWUE.

N rate effect was only observed in lettuce marketable yield and NUE in both years. Blood meal and feather meal fertilizers with higher percentage of N applied as NO_3^--N compared to other fertilizer treatments had a higher residual soil NO_3^--N concentration in 2013. Greater residual soil NO_3^--N was observed in the 0-30 cm depth compared to the 30-60 cm depth in 2014. Organic growers could achieve higher marketable yield and NUE when applying fertilizers at rates between 28 kg N ha^{-1} and 56 kg N ha^{-1} compared with 112 kg N ha^{-1}. In sweet corn, the feather meal and fish emulsion treatments had a higher residual soil NO_3^--N compared with other treatments. The fish emulsion, cyano-fertilizer, and blood meal

had a higher leaf and kernel N contents and NUE compared with feather meal at 56 kg N ha^{-1}. The cyano-fertilizer treatment had a higher marketable ear yield and NUE compared with other treatments at 112 kg N ha^{-1} in 2014.

The amount of C inputs and crop species may have affected soil permanganate oxidizable carbon (POXC) concentration in a single season study. Soil POXC concentration was higher in the cyano-fertilizer treatment compared to the control treatment in sweet corn, while the opposite trend was found in lettuce. Depth effect was observed in soil POXC concentration at 0-30 cm compared to 30-60 cm in lettuce. Soil POXC concentration was higher at 112 kg N ha^{-1} compared to 56 kg N ha^{-1} in sweet corn, but there was no N rate effect in lettuce. Greater soil POXC concentration and marketable ear yield of sweet corn were observed in the cyano-fertilizer treatment compared to others at 112 kg N ha^{-1}. Overall, our results indicate that organic N fertilizer, particularly cyano-fertilizer influenced soil POXC concentration over a short-term growing season of horticultural crops.

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OVERVIEW

Nitrogen (N) is one of the most limiting nutrients in agricultural systems, especially in certified organic vegetable crop production. There are two main classes of N fertilizers, which are solid and liquid; solid fertilizers are often incorporated into the soil before planting, while liquid fertilizers are generally applied season-long through irrigation. Organic agriculture relies heavily on fertilizers from off-farm sources such as fish emulsion, blood meal, feather meal, and compost to meet crop N demand (Gaskell et al., 2006).

Blood meal is a byproduct of slaughterhouse waste and processed into dried powdered forms. Blood meal contains high available forms of NO_3^--N (Rosen and Allan, 2007). Organic zucchini, leeks, and turnips treated with blood meal have shown increases in growth and yield (Termine et al., 1987; Mousa, 2004).

Feather meal has been used as a N fertilizer in sweet corn (Lawson et al., 2012) and field corn (Monem et al., 2014). Feather meal is a byproduct of the poultry industry and consists of 90% keratin, which is an insoluble protein (Gessesse et al., 2003). Keratin is resistant to rapid degradation by soil microorganisms due to its structural rigidity (Mazotto et al., 2011), which classify feather meal as a slow release N fertilizer.

Fish emulsion is derived from processed fish waste and is usually shipped long distances in a concentrated liquid form. Emino (1981) found that fish emulsion treated tomato and pepper plants were larger due to the complex composition of amino acids and humic acids of fish emulsion. Decomposition of organic N in fish emulsion also releases humic acids that lead to the production of auxin substances (Trevisan et al., 2010). In a study conducted by Balraj et al. (2014), xylem vessels of fish emulsion treated eggplants had a wider and more circular structure due to the presence of auxin, particularly indole-3-acetic acid (IAA). Auxin in the form of IAA has also been associated with increased growth and yield in grapes and wheat (Wang and Li., 2007; Agami and Mohamed, 2013).

These organic fertilizers are produced off-farm and are commercially available throughout the United States. Farmers usually rely on off-farm sources for the additional N needed during the growing season, and they are willing to pay the extra shipping cost. However, there is another fertilizer option being developed that could allow farmers to produce N on-farm, by growing cyanobacteria, previously known as blue-green algae, for application through fertigation.

Cyanobacteria have great potential as a N fertilizer due to their ability to fix N_2 from the atmosphere using the nitrogenase enzyme and to fix CO_2 using vegetative cells (Wolk, 1996). Cyanobacteria (cyano-fertilizer) can be produced in managed ponds on-farm, thus reducing the costs and risks associated with relying on off-farm sources. By producing cyano-fertilizer on-farm, organic growers could have better control of fertilizer application timing on their crops.

Different organic N fertilizers possess different biochemical properties. Organic N fertilizers contain phytohormones such as IAA and salicylic acid (SA). Phytohormones, particularly IAA and SA play a significant role in stimulating plant growth and development (Rodgers et al., 1979), enhancing carotenoid biosynthesis, and increasing β-carotene concentration in wheat, mung bean seedlings (Moharekar et al., 2003), and tomato (Srivastava and Handa, 2005), regulating plant-water relations (Kang et al., 2012; Gururani et al., 2015), increasing water use efficiency (WUE) in rice, mustard, and corn (Yusuf et al., 2008; Nazar et al., 2015), and influencing Fe and Zn uptake in plants (Rubio et al., 2009).

Most studies on N fertilization have focused on agronomic crops using conventional fertilizers and only few studies have been conducted on organic horticultural crops. In this dissertation, horticultural crops such as lettuce (*Lactuca sativa*) and sweet corn (*Zea mays*) were chosen to reflect responses towards organic N fertilizer application over the growing seasons of 2013 and 2014.

Nutritional Value

Lettuce is rich in micronutrients, including Fe and Zn (Pillay and Jonnalagadda, 2007; Itanna, 2002). Lettuce has a shallow rooting zone (Knight et al., 2013) and is categorized as a 'Strategy I' crop that releases organic acids from its roots to accelerate the translocation of solubilized Fe to shoots in

response to Fe deficiency in calcareous soil (Zocchi et al., 2007). Sweet corn is one of the most consumed vegetables for its nutritive value, including Fe and Zn. Plants in the family Graminaceae, such as sweet corn, use strategy II to increase soil Fe availability by secreting siderophores in response to Fe deficiency in calcareous soil (Romheld, 1987).

Calcareous soils contain free calcium carbonate and alkaline pH levels 7, which reduce inorganic forms of Fe and Zn for plant uptake. Fe and Zn deficiencies in plant tissues are important micronutrient disorders in calcareous soils (Shenker and Chen, 2005). Micronutrient malnutrition is very common among women and preschool children caused mainly by low dietary intake of micronutrients, especially Fe and Zn (Bouis, 2003). Synergistic relationships have been observed between N fertilizer application and micronutrients in plant tissue. N fertilizer plays an important role in root uptake, shoot transport, and translocation of Fe and Zn in wheat (Cakmak et al., 2010; Kutman et al., 2010). N has been reported to increase Fe and Zn contents in sweet corn (Oktem et al., 2010) and wheat (Cakmak et al., 2010; Aciksoz et al., 2011; Barunawati et al., 2013).

An estimated two billion people suffer from Vitamin A, Fe, and Zn deficiencies in the world, causing a loss of 63 million lives annually (Humphrey et al., 1992; Tulchinsky, 2010). The primary source of β-carotene comes from agricultural products. Leafy green vegetables play an important role in the human diet, particularly, to meet the dietary requirement of vitamin A. Green leafy vegetables, such as lettuce, are one of the most important vegetable types consumed in raw form for its nutritive value, including β-carotene (Khoo et al., 2011). One of the main methods shown to increase β-carotene concentration in plant tissue is by adding fertilizers. N fertilizers have shown positive impacts on β-carotene in leafy vegetables (Mozafar, 1993; Demmig-Adams et al., 1996). Inorganic N forms of N fertilizer influence β-carotene concentration in leaf tissue. Virginia (2001) reported that low NH_4^+-N: NO_3^--N ratio decreases β-carotene concentration in leaf tissue.

Water Use Efficiency

Different sources of N can influence WUE. WUE can be measured on various scales from the leaf to the field (Sinclair and Ludlow, 1984). Field water use efficiency (fWUE) is defined as the yield

produced per unit of water used, and instantaneous water use efficiency (iWUE) is defined as net photosynthetic rate per unit of water loss, which can be measured using a leaf gas exchange approach (Bunce, 2010). Colorado receives less than 50 cm of precipitation per year, and drip irrigation is one of the practices that reduce water use and increase efficiency of water use (Doerge et al., 1989). Adoption of drip irrigation systems has reduced water consumption in horticultural crop production. Drip irrigation coupled with fertigation provides controlled application of water and fertilizer by supplying water and nutrients near the crop roots.

Nitrogen Dynamics

Choosing forms, rates, and methods of organic N fertilizer to ensure efficient use of N and optimize marketable yield and N use efficiency (NUE), while minimizing potential N loss to groundwater is critically important. Post-harvest residual soil NO_3^-N is affected by N rate, timing of application, and precipitation. Improper management of N fertilizers can contaminate groundwater with NO_3^--N (Aneja et al., 2003). Optimizing NUE is important to reduce NO_3^--N losses to groundwater and to supply an adequate amount of plant-available N needed for crops (Thorup-Kristensen, 2001).

Labile carbon (C) is often correlated with the amount of plant-available N in the soil (Ford and Greenland, 1968). Total soil C and N, soil OM, and soil organic C analyses are generally not sensitive to N management practices. Permanganate oxidizable carbon (POXC) is a method that can quantify biologically labile C and is sensitive to N fertilizer management using potassium permanganate ($KMnO_4$), which is an oxidizing agent, which has been found to react with the labile C pool (Blair et al., 1995).

Many studies have been conducted on N response of crops to organic fertilizers, but none of them have compared cyano-fertilizer with commonly used off-farm organic N fertilizers (blood meal, feather meal, and fish emulsion). In this study, commonly used organic N fertilizers in Colorado were evaluated for their impact in lettuce and sweet corn. There is no literature dealing specifically with the relationship between how inorganic N forms and phytohormone concentrations in fertilizers affect nutritional properties, marketable yield, WUE, leaf gas exchange components, residual soil inorganic N, soil POXC, and NUE of horticultural crops.

This dissertation consists of a two-year field study at the Horticulture Farm Research Center, Colorado State University. Four sections were covered regarding the influence of organic N fertilizers on β-carotene, Fe, and Zn nutrition; WUE; residual soil inorganic-N; and soil POXC of lettuce and sweet corn. In section I, the objective was to evaluate β-carotene content in lettuce under field conditions (Chapter 1). In section II, the objective was to investigate effects of organic N fertilizer sources and rates on accumulation of Fe and Zn in lettuce (Chapter 2) and sweet corn (Chapter 3) under field conditions. The objective of section III was to assess whether inorganic N forms in organic N fertilizer on WUE and leaf gas exchange components of drip irrigated sweet corn under field conditions (Chapter 4). The objective of section IV was to determine any relationships among organic N fertilizers and N application rates on residual soil inorganic N and NUE of lettuce (Chapter 5) and sweet corn (Chapter 6), and soil POXC concentration of lettuce and sweet corn (Chapter 7) under field conditions.

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CHAPTER 1. PHYTOHORMONES IN ORGANIC NITROGEN FERTILIZERS INFLUENCED β-CAROTENE CONCENTRATION AND MARKETABLE YIELD COMPONENTS OF LETTUCE

1.1 Introduction

Most carotenoids are present in the chlorophyll-protein complex, whose main functions are light harvesting and photoprotection (Tobin and Silverthorne, 1985). β-carotene is one of the important plant-derived carotenoids, which is a precursor to vitamin A (Ramos and Rodriguez-Amaya, 1987). An estimated two billion people suffer from Vitamin A deficiency globally (Tulchinsky, 2010). Vitamin A deficiency can result in blindness and premature death (Mayne, 1996).

The primary source of β-carotene comes from agricultural products. Leafy green vegetables play an important role in meeting the vitamin A requirement in the human diet. Green leafy vegetables, such as lettuce, are one of the most widely consumed vegetables in raw form (Khoo et al., 2011). It is well known that fertilizers can improve the nutritional quality of vegetables (Somers and Beeson, 1948; Maynard, 1956; Eppendorfer, 1978; Poulsen et al., 1995). Maximizing organic nitrogen (N) fertilizer use for horticultural produce, could be achieved by selecting organic N fertilizer source to meet crop yield and nutritional requirements.

N is one of the most limiting nutrients in agricultural systems, especially in certified organic vegetable crop production. Organic agriculture relies heavily on fertilizers and soil amendments from off-farm sources such as fish emulsion, blood meal, feather meal, compost, and animal manures to meet crop N demand (Gaskell et al., 2006). There are two main types of N fertilizers, solid and liquid. In organic agriculture, solid fertilizers are often incorporated into the soil before planting, while liquid fertilizers are generally applied season-long through irrigation.

Most organic N fertilizers are rich in slow-releasing organic N. In a study conducted by Hartz and Johnstone (2006), blood meal and feather meal were found to have high levels of organic N. While these

organic fertilizers are commercially available, many of them are produced off-farm and distributed widely throughout the United States. Fish emulsions are usually shipped long distances in a concentrated liquid form. Farmers usually rely on off-farm sources for the additional N needed during the growing season and they are willing to pay the extra cost of shipping heavy liquids around the United States due to the relatively fast-acting effect of fish emulsion. However, there is another fertilizer option being developed that allows farmers to produce N on-farm, which is cyanobacteria, previously known as blue-green algae.

Cyanobacteria have been documented as a source of N fertilizer in rice paddies as early as the 1970s (Roger and Kulasooriya, 1980). Cyanobacteria have the ability to fix N_2 from the atmosphere using the nitrogenase enzyme (Wolk, 1996), and they have great potential as N fertilizer due to their phototrophic ability to fix CO_2 using vegetative cells. Cyanobacteria also contain β-carotene pigments on their membranes (Stransky and Hager, 1970; Hertzberg et al., 1971; Takaichi and Mochimaru, 2007).

It was reported that cyanobacteria secretes a natural form of auxin, indole-3-acetic acid (IAA) (Prasanna et al., 2012). Srivastava and Handa (2005) reported that IAA regulates carotenoid biosynthesis and increased β-carotene concentration in tomato. To date, no literature on cyanobacteria excretion of salicylic acid (SA) or the importance of SA in organic fertilizers exists. Most studies conducted on SA applied to plants evaluated foliar application of SA. Carotenoid concentration in leaf tissues increased with an increased amount of SA applied to wheat or mung bean seedlings (Moharekar et al., 2003) and Arabidopsis (Rao et al., 1997) due to SA induces carotenoid biosynthesis (Moharekar et al., 2003). SA can enhance plant resistance to environmental stress, such as chilling (Janda et al., 1999).

Fortification of horticultural produce, leafy vegetables in particular, using fertilizer could be one of the options for improving the nutritional value of human diets (Neeser et al., 2007) and nutritional value of the produce itself (Stefanelli et al., 2010). N fertilizers have been shown to increase the concentrations of β-carotene in potato and tomato (Mozafar, 1993; Demmig-Adams et al., 1996). Inorganic forms of N, such as nitrate-N (NO_3^--N) applied in the fertilizers have a significant role in influencing β-carotene concentration in plant tissue. Virginia (2001) reported that lettuce accumulates high NO_3^--N in leaf tissues. N fertilizer applied in forms of NO_3^--N resulting in decreased β-carotene

concentration in leafy vegetables, which was due to the oxidation process during NO_3^--N assimilation in leaf tissue (Booth et al., 1992).

Other macronutrients in fertilizer, such as the amount of calcium (Ca) applied affect β-carotene concentration in leaf tissue because Ca is the main constituent in carotenoid biosynthesis (Goodwin, 1980; Mou, 2009; Kopsell et al., 2013). In general, high Ca: Mg ratio in the fertilizer increased β-carotene concentration in leafy vegetables and was a reliable indicator of crop response to nutritional quality (Jarvan and Poldma, 2004). Umeno et al. (2005) found that Ca influenced carotenoid cleavage enzymes in carotenoid biosynthesis of lettuce. However, it is unknown whether organic N fertilizer source with different Ca: Mg ratio could influence β-carotene concentration in lettuce because β-carotene concentrations of lettuce vary across species and genotypes (Mou, 2005).

Therefore, this study was conducted to compare the effects of different types of organic N fertilizer on β-carotene concentration and lettuce marketable yield components (marketable yield and total leaf area). In this two-year field study, organic N fertilizers in northern Colorado were compared with cyano-fertilizer to evaluate the β-carotene concentration and marketable yield components of lettuce. We hypothesized that (i) either high IAA or SA applied in the fertilizers would increase β-carotene concentration and influence marketable yield components, (ii) high amount of Ca applied in organic N fertilizers would increase β-carotene concentration, and (iii) high NO_3^--N concentration in the fertilizer would decrease β-carotene concentration.

1.2 Materials and Methods

1.2.1 Experimental site

Field experiments were carried out on organically-certified land at the Colorado State University Horticulture Research Center (CSU HRC), Fort Collins, CO. Previously, the field area was planted with winter cover crops of rye (*Secale cereal*) and turnips (*Brassica rapa*). The experimental site is in a semi-arid zone with clay loam soils. The soil at the research site was classified as a fine, smectitic, mesic Aridic Argiustoll of the Nunn series (NRCS, 1980).

1.2.2 Soil analysis

Soil samples were collected to a depth of 30 cm from a representative area of the field (15 m x 30 m) and analyzed by the Soil, Water, and Plant Testing Laboratory at Colorado State University. Particle size distribution was determined using the hydrometer method based on Stoke's law (Gee and Bauder, 1979). Chemical analyses included soil pH and electrical conductivity (EC) measured in the supernatant suspension of 1:1 soil to water using a Mettler Toledo pH/EC meter (Thermo Fischer Scientific, Waltham, MA). Cation exchange capacity (CEC) was determined by summation of the exchangeable bases (Ca^{2+}, K^{+}, Mg^{2+}, Na^{+}) plus hydrogen (H^{+}) using ammonium acetate (1N $NH_4C_2H_3O_2$, pH 7.0) as described by Hajek et al. (1972). Organic matter (OM) content was determined by loss on ignition (Blume et al., 1990). Soil inorganic N (NH_4^{+}-N and NO_3^{-}-N) was extracted using 2M KCl, and the filtered extract was analyzed using an Alpkem Flow Solution IV Auto Analyzer (OI Analytical, College Station, TX). Phosphorus (P) was extracted with 0.5M sodium bicarbonate ($NaHCO_3$) solution, which was developed by Olsen et al. (1954). Major cations (K, Ca, and Mg) and micronutrients (Fe and Zn) were extracted with ammonium bicarbonate –diethylenetriaminepentaacetic acid (AB-DTPA) (Jones, 2001) and analyzed using a Perkin Elmer Inductively Coupled Plasma/Optical Emission Spectrometer (ICP-OES) (Perkin Elmer, Waltham, MA) (Table A1).

1.2.3 Experimental design and plot layout

Treatments consisted of four different types of N fertilizer including control (no fertilizer) arranged in a Randomized Complete Block design with four replications. The plot size for each treatment was 76 cm x 457 cm. The whole experimental plot area was 15 m x 30 m.

1.2.4 Planting materials

The certified organic seeds of 'Concept' lettuce (Johnny's Selected Seeds, Waterville, ME) were planted in 72-cell trays containing a well-mixed Sunshine® organic potting soil mix (SunGro Horticulture, Agawam, MA) in the first week of May 2013 and the first week of May 2014. All seeds were started at the Plant Environmental Research Center's (PERC) greenhouse facility on the Colorado State University (CSU) campus. After four weeks, seedlings were transplanted to the field.

1.2.5 Irrigation

Two lines of John Deere® drip tape (Deere & Co., Moline, IL) with a flow rate of 125 L $hour^{-1}$ 100 m^{-1} were stretched across each bed spaced 30 cm apart with a double header in each plot. Water was applied daily at 08:00 to 08:30 using an automated drip irrigation system. The drip system used for this study comprised of laterals (15 mm diameter) with a spacing of 30 cm between inline emitters. Irrigation was applied at 08:00 for 15 min day^{-1} at the initial stage and was changed to 30 min day^{-1} at a later stage. Irrigation was applied twice per day in 2013, once per day in 2014. The amounts of precipitation and irrigation water applied over the growing seasons are presented in Table A2. Weather data was obtained from a nearby CoAgMet weather station (http://www.coagmet.colostate.edu/).

1.2.6 Fertilizer treatments

The fertilizers compared in this study were liquid fertilizers [Alaska® fish emulsion (Planet Natural, Bozeman, MO) and cyano-fertilizer grown on-site] and solid fertilizers [blood meal and feather meal (Down To Earth Inc., Eugene, OR)]. The N-fixing cyanobacteria (*Anabaena* sp.*)* were cultured from local soils and inoculated into nutrient- supplemented raceways according to the method of Barminski (2014).

1.2.7 Methods of fertilizer application

Fertilizers were applied at 56 kg N ha^{-1} in 2013 and 2014. Both the fish emulsion and cyano-fertilizer were in liquid form and were supplied in four split applications via fertigation over the growing season. The irrigation was cut off the night before any fertigation treatments. Cyano-fertilizer was pumped directly from production raceways into the drip irrigation system using a Flotec® sump pump (Flotec Water, Delavan, WI). Fish emulsion fertilizer was mixed into watering troughs and applied directly into the drip irrigation using the same pump after being flushed with water. The analysis of water used for irrigation and fertigation is presented in Table 3. The blood meal and feather meal were in dry powdered form and incorporated into the soil prior to planting. Blood meal and feather meal were applied 6 cm from the rooting zone in a band 6 cm deep using a hoe. The amounts of water applied via fertigation

in the fish emulsion and cyano-fertilizer treatments were also applied in the blood meal and feather meal plots, so that all plots received equal amounts of water.

1.2.8 Fertilizer analysis

All fertilizer samples were sent to the Soil, Water, and Plant Testing Laboratory at CSU and analyzed for N, P, K, Ca, Mg, Fe, and Zn (Table 3a). Fertilizer samples were digested with nitric acid (HNO_3) and hydrogen peroxide (H_2O_2). Major cations (K, Ca, and Mg) and micronutrients (Fe and Zn) were analyzed in the digests using a Perkin Elmer Inductively Coupled Plasma/Optical Emission Spectrometer (ICP-OES) (Perkin Elmer, Waltham, MA). Inorganic-N concentrations and nutrient ratio in organic N fertilizers (Table A3b) and amount of Ca, Mg, Fe, and Zn applied inorganic N fertilizers was calculated (Table A3c).

1.2.9 Phytohormone analysis

Phytohormone analyses were conducted at the Proteomics and Metabolomics Facility, CSU. IAA and SA were found to be quantified without interference (Table 4a). Fertilizer samples were adjusted to pH 7.0 with 1 N NaOH and extracted three times with water-saturated n-butanol followed by vacuum drying (Pan et al., 2010). The extracts obtained were filtered through membrane filters (pore size 0.45 µm). Supernatants were harvested by centrifugation at 5,000 g for 20 minutes at 4^{o}C. Supernatants were homogenized in liquid nitrogen using a cold mortar and pestle at 4^{o}C. The resulting supeRnatant was extracted using 80% methanol containing 10 mg L^{-1} butylated hydroxytoluene at 4^{o}C. Samples were methylated with diazomethane and dissolved in heptane. Gas chromatography-mass spectrometry (GC-MS) analyses were performed according to Edlund et al (1995). Amount of phytohormone applied over the growing season was calculated from the concentration (Table A4b).

1.2.10 Measurements

1.2.10.1 Marketable yield and total leaf area

Harvesting was done 39 days after transplanting. Brown leaves from the harvested lettuce heads were stripped and removed to obtain a marketable yield and weighed. The total leaf area per lettuce head was measured using a LICOR-3100 leaf area meter (LI-COR, Lincoln, NE) after the harvesting process.

1.2.10.2 β-carotene extraction

Marketable lettuce samples (oldest most expanded leaves) were lyophilized using VirTis Genesis 25LL freeze dryer (Biopharma Process Systems, Winchester, UK) at a constant temperature of -40^{o}C. Then, 100 mg of freeze-dried tissue samples were weighed in to 20 mL glass vials. 800 µL Millipore water (EMD Millipore, Billerica, MA) was added to each sample. Vials were placed in a 40^{o}C water bath for 20 minutes. 2.5 mL of HPLC-grade tetrahydrofuran (THF) (Sigma-Aldrich Corporation, St. Louis, MO) were added to the samples. The sample mixtures were vortexed using a vortex mixture (Genie-2 Scientific Calibration, Apex, NC) and centrifuged for 3 minutes at 500 g. The supernatant was collected with a Pasteur pipet, placed into a 50 mL conical tube, capped and placed on ice. The sediment was re-suspended in 2.0 mL THF and homogenized. During homogenation, the tube was immersed in ice to dissipate heat. The samples were centrifuged again for three minutes at 500 rpm. The supernatants then were concentrated to 2 mL by evaporating the samples under a stream of nitrogen gas (UK Flowtechnik Ltd., Nottingham, UK). The sample was diluted in 100 µL THF in a vortex mixer (Genie-2 Scientific Calibration, Apex, NC). A 2 mL aliquot was filtered through a 0.21 µm polytetrafluoroethylene (PTFE) filter (Agilent Technologies, Wilmington, DE) and transferred to 2 mL amber vials (Agilent Technologies, Wilmington, DE) prior to Ultra Performance Liquid Chromatography (UPLC) analysis.

1.2.10.3 β-carotene determination

β-carotene determination was conducted using a modified method by Schierle et al. (2004). A Waters Acquity^{TM} Ultra Performance Liquid Chromatography (UPLC) system (Waters Corporation, Milford, MA) was used for pigment separation. Chromatographic separations were achieved using a C_{30} reverse-phase column (Waters Corporation, Milford, MA). All separations were achieved using a binary mobile phase of 11% methyl tert-butanol (MTBE), 89% MeOH, and 0.01% triethylamine (TEA). The flow rate was 1.0 mL min^{-1}, with a run time of 23 min, followed by 5 min equilibration prior to the next injection. Eluted compounds from a 10 µL injection loop were detected at 450 nm. All analyses were performed in triplicate. The carotenoid standards were obtained from Sigma-Aldrich (Sigma-Aldrich Corporation, St. Louis, MO). The β-carotene concentration was calculated using the following formula:

$$\beta\text{-carotene } (\mu g\ g^{-1}) = \frac{A_{Crt} \times C_{standard}\ (\mu g\ mL^{-1}) \times V\ (mL)}{A_{standard} \times W_{sample}\ (g)}$$

where, A_{Crt} = Carotenoid peak area; $A_{standard}$ = Standard area; $C_{standard}$ = standard concentration; V = Total extract volume; W_{sample} = Sample weight

1.2.11 Statistical analysis

The experimental units were arranged in a Randomized Complete Block Design (RCBD). Data were analyzed using SAS version 9.3 (SAS Institute Inc., Cary, NC). Univariate and Boxplot procedures were used to evaluate the normality of data distribution. Analysis of variance (ANOVA) was performed on the data with the MIXED procedure ($P < 0.1$). Fertilizer treatment was categorized as a fixed effect, and replication was categorized as a random effect. When treatment or interaction effects were significant, means were separated by Tukey's Studentized Range (HSD) test of mean separation. PROC REG with the stepwise selection method was used to select the best combination of independent variables (amount of Ca, Mg, Fe, Zn, NH_4^+-N, NO_3^--N, IAA, and SA applied in the fertilizers) to predict the dependent variables (β-carotene concentration, marketable yield, and total leaf area). The relationships between variables were assessed by linear correlation using the CORR procedure.

1.3. Results and Discussion

1.3.1 Amount of Indole-3-acetic acid (IAA) application on β-carotene concentration

All fertilizer treatments in 2013 increased β-carotene concentration in leaf tissue compared to the control ($P < 0.001$; Figure A1a). The blood meal treatment had a lower β-carotene concentration compared to cyano-fertilizer, fish emulsion, and feather meal treatments possibly due to high NO_3^--N concentration in the blood meal fertilizer compared to others (Table A3b). Booth et al. (1992) found that high NO_3^--N concentration in the fertilizer (0.1% of N applied as NO_3^--N) decreased β-carotene concentration in leafy vegetables, due to oxidation process during NO_3^--N assimilation in leaf tissue (Booth et al., 1992).

In 2014, only fish emulsion had a greater β-carotene concentration compared with other treatments including control ($P = 0.003$; Figure A1b). The β-carotene concentration in leaf tissues of

lettuce was within range of the data collected by Cruz et al. (2012). The β-carotene concentration in the fish emulsion treatment in 2014 and all treatments in 2013 except control was adequate to meet the recommended daily allowance of between 800 to 1000 µg of vitamin A (Musa and Ogbadoyi, 2012; FAO and WHO, 2000), which is assuming consumption of 340 g of lettuce daily (USDA, 2013). Enhancing the β-carotene concentration of lettuce would improve the nutrient intake without requiring an increase in daily dietary intake (Mou, 2005).

The greater IAA concentration (Table A4a) and IAA applied (Table A4b) in the fish emulsion treatment could be related to the increased β-carotene concentration in plant tissue in that treatment (Figure A1). Among independent variables (amount of Ca, Mg, Fe, Zn, NH_4^+-N, NO_3^--N, IAA, and SA applied in the fertilizers) analyzed, our results showed that greater IAA application (IAAapp) was positively correlated with β-carotene concentration in 2013 ($r = 0.49$; $P = 0.0083$) and 2014 ($r = 0.53$, $P = 0.0029$). A stepwise selection model showed that IAAapp could be used to predict β-carotene concentration in both years (Table A5). Karatas et al. (2010) found that IAA can regulate the synthesis of antioxidant enzymes and influence carotenoid concentration in an herbaceous flowering plant, *Tropaeolum*. However, the full mechanism of how IAA regulates carotenoid synthesis in lettuce is still unknown.

1.3.2 β-carotene concentration and marketable yield

Significant differences were observed in the marketable yield of lettuce in 2014 ($P < 0.001$), but no treatment effect was observed in 2013 (Figure A2a). The fish emulsion treatment had a lower marketable yield than the other treatments in 2014 (Figure A2b). The greater marketable yield observed in 2014 compared to 2013 was possibly due to higher precipitation in 2014 (Table A2). Some of the lettuce plants were damaged in a hailstorm in 2014.

In 2014, β-carotene concentration decreased by 180 µg g^{-1} with a marketable yield increase of 30 g per plant compared to 2013 in the fish emulsion treatment. A significant negative correlation ($r = -0.7565$, $P = 0.0044$) was found between β-carotene concentration and marketable yield of lettuce in 2014, but not in 2013. The β-carotene concentration in lettuce decreased with increasing marketable yield,

similar to what was previously reported by Oyama et al. (1999) and Mou (2005). In 2014, the β-carotene concentration in leaf tissue was greater in the fish emulsion treatment, apparently due to its higher concentration in smaller plants (Figures A1b and A2b).

1.3.3 Amount of salicylic acid applied affected leaf surface area

Leaf area is an important marketable yield component of lettuce produce. Environmental stress such as the hailstorm on June 22, 2014 can impose an effect on leaf growth and influence post-harvest quality of lettuce produce. Kang et al. (2007) reported that SA enhanced the chilling tolerance of banana seedlings. SA increased leaf cell wall structure and induced high expression of selected genes during chilling conditions (Kang et al., 2003). Among independent variables (amount of Ca, Mg, Fe, Zn, NH_4^+-N, NO_3^--N, IAA, and SA applied in the fertilizers) analyzed, a stepwise selection model showed that SA application (SAapp) influenced the total leaf area of lettuce in both years (Table A6). A consistent trend in the total leaf area under the cyano-fertilizer treatment in both years (Figure A3) could be due to the higher amount of SA applied in the cyano-fertilizer treatment compared to other fertilizers (Table A4b).

SA affects leaf area, thus more light can be captured by a larger leaf surface area when plants are treated with SA (Young, 1991). In our study, lettuce treated with cyano-fertilizer, which had the highest amount of SA applied (Table A4b) and higher total leaf area compared to other treatments in both years (Figure A3). The total leaf area plays a significant role as the main source of light absorption for plant growth and development. In general, there was a positive relationship between leaf area and biomass accumulation, which often determines yield. However, depending on environmental factors, higher leaf area does not necessarily result in higher biomass due to partitioning at several levels, such as new leaf formation, root mass, and respiration (Weraduwage et al., 2015; Zhang et al., 2015).

1.4 Conclusion

In conclusion, all fertilizer treatments in 2013 increased β-carotene concentration in leaf tissue compared to control, while only fish emulsion had a higher β-carotene concentration compared to other treatments in 2014. The high indole-3-acetic acid (IAA) applied in the fish emulsion treatment could have increased β-carotene concentration in lettuce in both years. Amount of IAA applied in the fish emulsion

treatment (IAAapp) was positively correlated with β-carotene concentration in both years. A significant negative correlation was found between marketable yield and β-carotene concentration in leaf tissue in 2014. High amount of salicylic acid (SA) applied in the cyano-fertilizer treatment may have contributed to a higher total leaf area compared to other fertilizers in both years. This study contributes to the fundamental knowledge of β-carotene concentration response to N fertilizer application in lettuce. The relationships between phytohormones present in organic N fertilizers and β-carotene concentration in lettuce should be explored in future research.

TABLES

Table A1. Initial soil properties of the 0-30 cm soil depth of the experimental site in lettuce.

Soil properties	2013	2014
pH[‡]	7.3	7.5
Electrical conductivity[‡] (dS m^{-1})	0.5	0.6
Cation exchange capacity[§] (meq 100 g^{-1})	30	29
Organic matter [#](%)	2.9	2.7
NH_4^+-N[¶] (ppm)	1.9	2.4
NO_3^--N[¶] (ppm)	5.2	4.0
P[‖] (ppm)	26	31
K[∫] (ppm)	432	473
Ca[∫] (ppm)	4411	4513
Mg[∫] (ppm)	622	634
Fe[∫] (ppm)	6.5	6.3
Zn[∫] (ppm)	1.5	1.6

[‡]pH and electrical conductivity were determined in water (1:1).
[§]CEC was determined using $NH_4C_2H_3O_2$ extraction.
[#]OM was determined by the loss on ignition method.
[¶]Samples were extracted using 2M KCl
[‖]Samples were extracted using 0.5M $NaHCO_3$.
[∫]Samples were extracted using ammonium bicarbonate diethylenetriamine pentaacetate (AB-DTPA).

Table A2. Average monthly precipitation and the amount of water applied during growing season in 2013 and 2014 in lettuce.

	2013	2014
Planting	July 1	June 9
Harvest	August 9	August 4
Precipitation (mm)		
June	--	34
July	39	69
August	9	0.3
Water applied (mm)		
Irrigation	210	221
Fertigation	230	230
Total (mm)	488	554

[‡]Total amount of water applied over the growing season is the combination of precipitation, irrigation, and fertigation. All plots received the same amount of water applied via irrigation. When liquid fertilizers were applied, the control and solid fertilizer treatments were irrigated with the same amount of water as that used to fertigate the liquid fertilizer treatments.

Table A3a. Nutrient analysis of fertilizer samples.

	N[‡]	P[♯]	K[♯]	Fe[♯]	Ca[♯]	Mg[♯]	Zn[♯]
	%	----------mg kg^{-1}----------					
Cyano-fertilizer	0.2	6	0.1	6	10	18	0.1
Fish emulsion	5	1600	20510	131	725	921	18
Feather meal	13	640	1776	62	1466	575	18
Blood meal	13	32	366	118	904	283	14

[‡]Samples were analyzed using CN analyzer.
[♯]Samples were digested with HNO_3 and H_2O_2 and analyzed using ICP-OES.

Table A3b. Inorganic N concentration, inorganic-N ratio, and percentage of N applied as inorganic-N of fertilizers.

	NH_4^+-N[¶]	NO_3^--N[¶]	NH_4^+-N: NO_3^--N	N as NH_4^+-N	N as NO_3^--N
	----------mg kg^{-1}----------			----------%----------	
Cyano-fertilizer	4.7	0.01	470	0.24	0.0005
Fish emulsion	23.7	0.12	198	0.05	0.0002
Feather meal	1232	2.30	536	0.95	0.002
Blood meal	27.7	8.40	3.3	0.02	0.006

[¶]Samples were extracted using 2M KCl and were analyzed using autoanalyzer.

Table A3c. Amount of Ca, Mg, Fe, and Zn applied in the fertilizers over the course of growing season.

	Ca	Mg	Fe	Zn
	----------kg ha^{-1}----------			
Cyano-fertilizer	1.1×10^{-3}	2.0×10^{-3}	6.7×10^{-4}	1.1×10^{-5}
Fish emulsion	8.1×10^{-2}	1.0×10^{-1}	1.5×10^{-2}	2.0×10^{-3}
Feather meal	1.6×10^{-1}	6.4×10^{-2}	6.9×10^{-3}	2.0×10^{-3}
Blood meal	1.0×10^{-1}	3.2×10^{-2}	1.3×10^{-2}	1.6×10^{-3}

Table A4a. Phytohormone concentrations in the fertilizers.

	Indole-3-acetic acid (IAA)[†]	Salicylic acid (SA)[†]
	----------ng g^{-1}----------	
Cyano-fertilizer	0.06	6
Fish emulsion	1436	77
Feather meal	241	240
Blood meal	58	50

[†]Samples were extracted in 80% methanol containing 10 mg L^{-1} butylated hydroxytoluene at 4°C.

Table A4b. Amount of phytohormones applied in the fertilizer over the growing season.

	Indole-3-acetic acid (IAA) [‡]	Salicylic acid (SA) [‡]
	----------kg ha^{-1}----------	----------kg ha^{-1}----------
Cyano-fertilizer	4.4×10^{-5}	4.8×10^{-3}
Fish emulsion	2.4×10^{-3}	1.2×10^{-4}
Feather meal	1.5×10^{-4}	1.5×10^{-4}
Blood meal	3.9×10^{-5}	3.4×10^{-5}

[‡]Amount of phytohormone applied was calculated as the mass of fertilizer applied at 56 kg N ha^{-1} over the growing season multiplied by the phytohomone concentration analyzed in fertilizers. Fertilizer samples were extracted in 80% methanol containing 10 mg L^{-1} butylated hydroxytoluene at 4°C.

Table A5. A stepwise regression model predicting β-carotene concentration in lettuce in 2013 and 2014. Variables analyzed were amount of Ca, Mg, NH_4^+-N, NO_3^--N, IAA, and SA applied in the fertilizers (N = 16). All variables left in the model are significant at the 0.1 level.

	Estimate	P-value	Model
β-carotene concentration (2013)			
r^2 =0.77			β-carotene concentration$_{2013}$ = 920 +
Intercept	920	<.0001	490IAAapp
Indole-3-acetic acid application,	490	0.0079	
β-carotene concentration (2014)			
r^2 = 0.62			β-carotene concentration$_{2014}$ = 915 +
Intercept	915	<.0001	
Indole-3-acetic acid application,	1315	0.0093	

Table A6. A stepwise regression model predicting lettuce leaf area in 2013 and 2014. Variables analyzed were amount of Ca, Mg, NH_4^+-N, NO_3^--N, IAA, and SA applied in the fertilizers (N = 16). All variables left in the model are significant at the 0.1 level.

	Estimate	P-value	Model
Total leaf area (2013)			
r^2 = 0.84			Total leaf area$_{2013}$ = 1590 + 4674SAapp
Intercept	1590	<.0001	
Salicylic application, SAapp	4674	0.0410	
Total leaf area (2014)			
r^2 = 0.75			Total leaf area$_{2014}$ = 1320 + 3680SAapp
Intercept	1320	<.0001	
Salicylic acid application, SAapp	3680	0.0035	

FIGURES

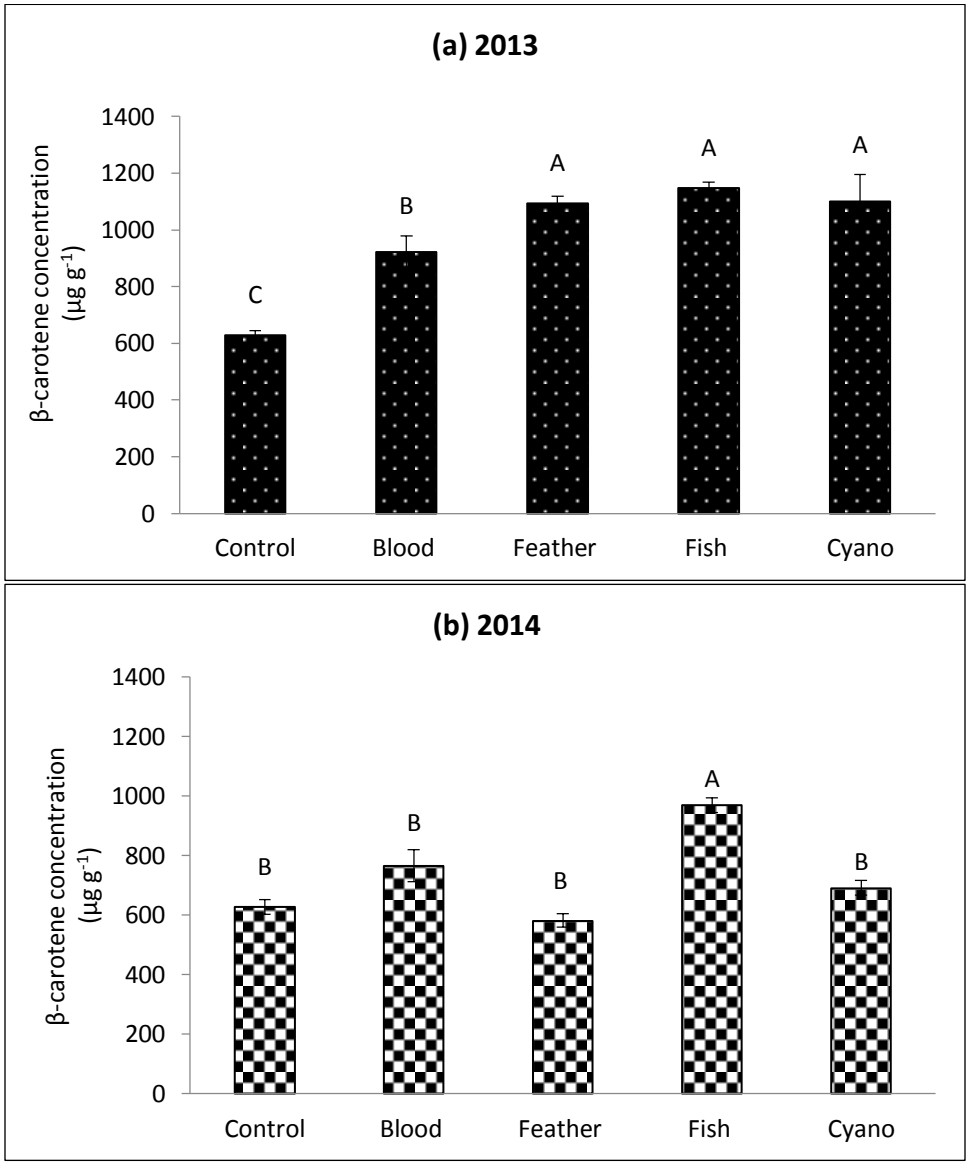

Figure A1. Effects of different organic N fertilizers on β-carotene concentration of drip irrigated lettuce. Fertilizers were applied at 56 kg N ha^{-1}. Means with the same letter are not significantly different at $P < 0.1$ using Tukey's Studentized Range (HSD) test of mean separation. Bars represent standard errors of mean. Blood = Blood meal; Feather = Feather meal; Fish = Fish emulsion; Cyano = Cyano-fertilizer.

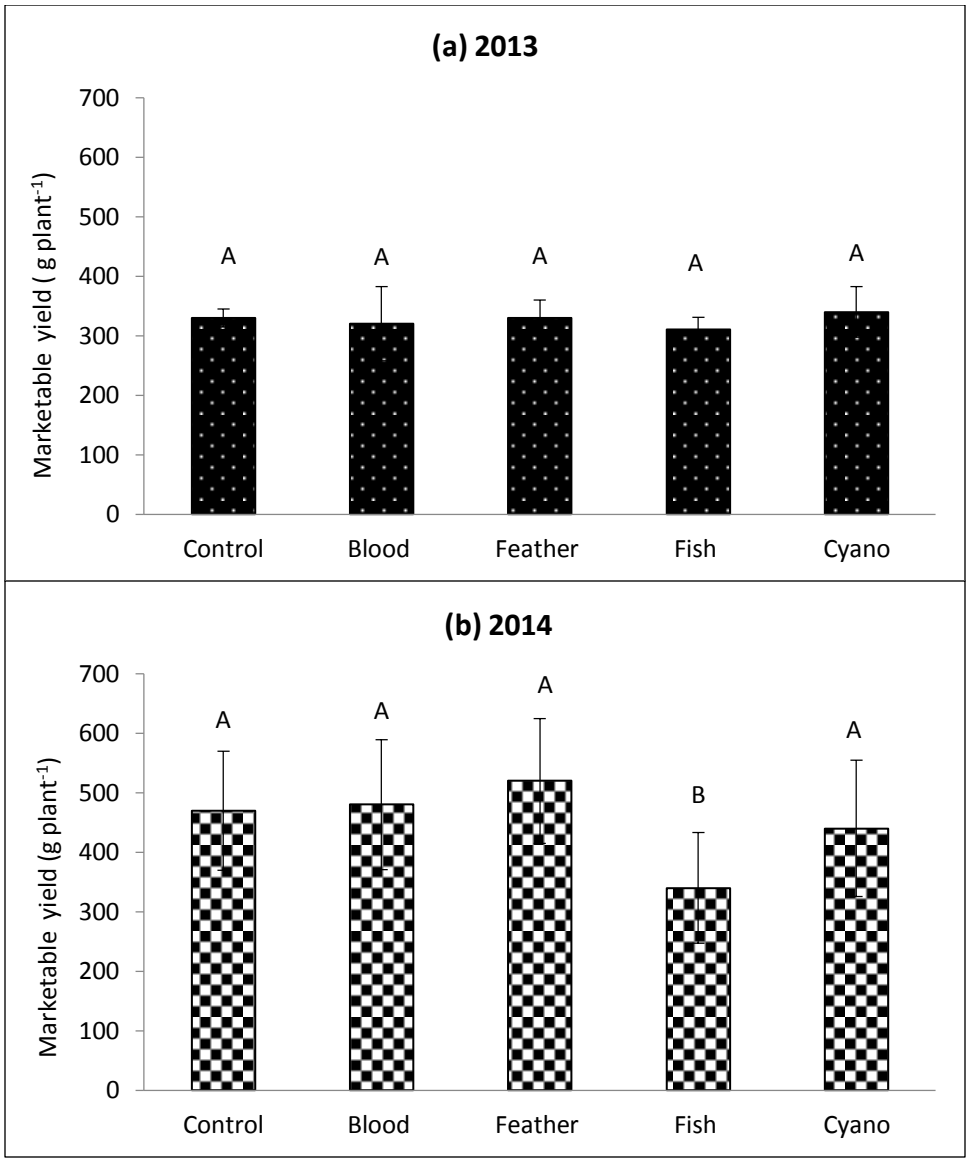

Figure A2. Effects of different organic N fertilizers on fish yield of drip irrigated lettuce. Fertilizers were applied at 56 kg N ha^{-1}. Means with the same letter are not significantly different at $P < 0.1$ using Tukey's Studentized Range (HSD) test of mean separation. Bars represent standard errors of mean. Blood = Blood meal; Feather = Feather meal; Fish = Fish emulsion; Cyano = Cyano-fertilizer.

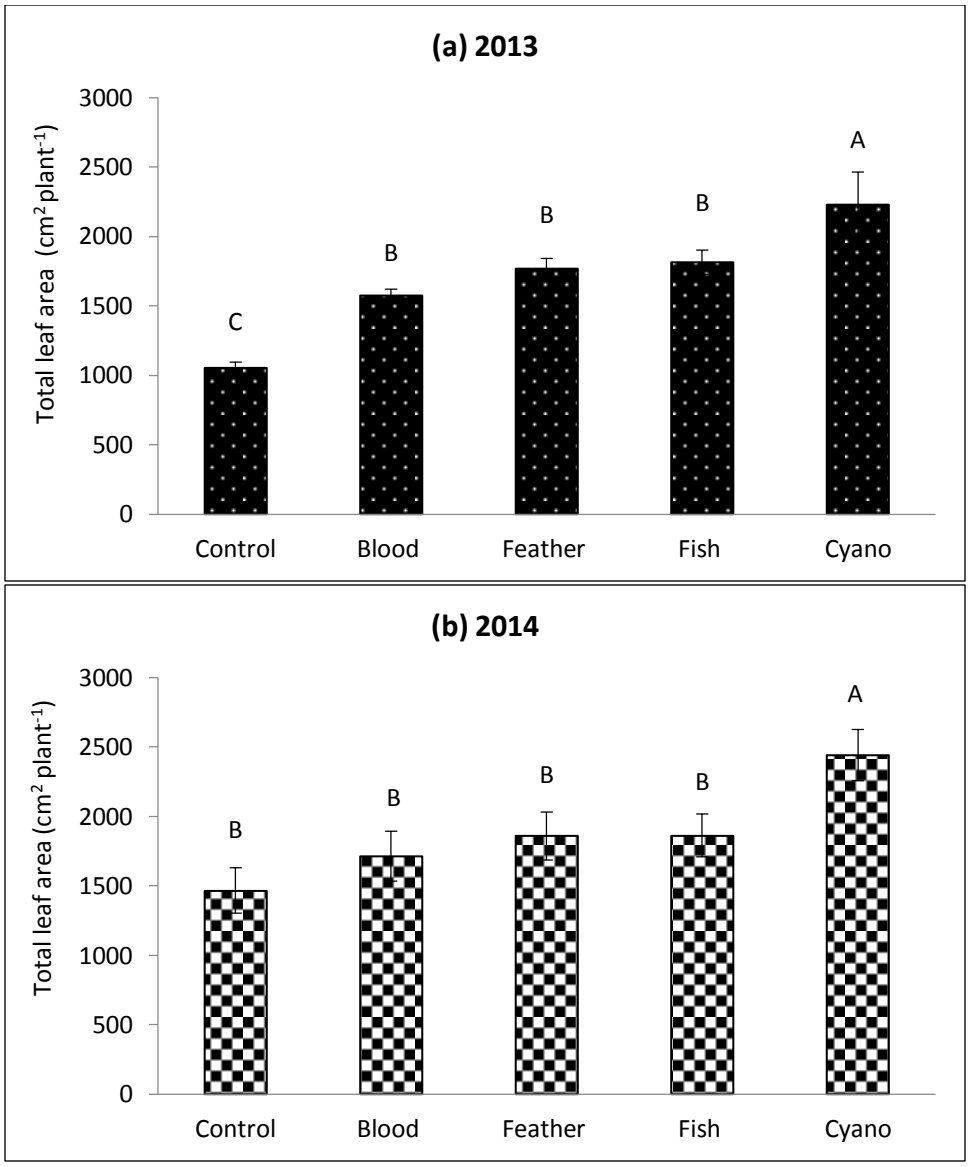

Figure A3. Effects of different organic N fertilizers on the total leaf area of drip irrigated lettuce. Fertilizers were applied at 56 kg N ha^{-1}. Means with the same letter are not significantly different at $P < 0.1$ using Tukey's Studentized Range (HSD) test of mean separation. Bars represent standard errors of mean. Blood = Blood meal; Feather = Feather meal; Fish = Fish emulsion; Cyano = Cyano-fertilizer.

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CHAPTER 2. ORGANIC NITROGEN FERTILIZERS INFLUENCE IRON AND ZINC CONCENTRATIONS IN LETTUCE

2.1 Introduction

Micronutrient malnutrition among women and preschool children is caused mainly by low dietary intake of micronutrients, especially Fe and Zn (Bouis, 2003). Nitrogen (N) fertilizers have shown positive impacts on a plant's ability to accumulate Fe and Zn, and increased N rates can increase Fe and Zn concentrations in leaf tissues (Aciksoz et al., 2011). Some of the main factors affecting Fe and Zn accumulation in edible plant tissue are the availability of macronutrient such as N and the type of N fertilizer applied to crops (Cakmak, 2008).

There are two main classes of N fertilizers, solid and liquid. In organic agriculture, solid fertilizers are often incorporated into the soil before planting, while liquid fertilizers are generally applied season-long through irrigation. Organic agriculture relies heavily on fertilizers and soil amendments from off-farm sources such as fish emulsion, blood meal, feather meal, compost, and animal manures to meet crop N demand (Gaskell et al., 2006).

While all of the aforementioned organic N fertilizers are commercially available, many of them are produced off-farm and distributed widely throughout the United States. Farmers usually rely on off-farm sources such as fish emulsion, feather meal, and blood meal for the additional N needed during the growing season, and they are willing to pay the extra cost of shipping fertilizers around the United States. However, one of the new fertilizer options being developed that allow farmers to produce N on-farm, which is cyanobacteria, which was previously known as blue-green algae.

Cyanobacteria (*Anabaena* sp.) have great potential as N fertilizer derived from solar energy due to their phototrophic ability to fix CO_2 and the ability to fix N_2 from the atmosphere (Wolk, 1996). Cyanobacteria can also be produced in managed ponds on-farm which could reduce the costs and risks

associated with relying on off-farm sources. By using a biological process outside of the soil, the farmer may have improved control of application timing and N rates on crops.

Lettuce (*Lactuca sativa*) is the most commonly consumed salad vegetable in Colorado (Bunning et al., 2010) and is rich in micronutrients, including Fe and Zn (Pillay and Jonnalagadda, 2007; Itanna et al., 2002). Lettuce is categorized as a 'Strategy I' crop in response to Fe deficiency. 'Strategy I' crops rely on ferric-chelate reductase activity, proton excretion, and release of organic acids from roots to accelerate the translocation of solubilized Fe to shoots (Zocchi et al., 2007).

In this study, commonly-used organic fertilizers were compared for their impact on marketable yield, leaf Fe, and Zn concentrations in lettuce. While there are many studies focusing on micronutrient composition of cereal grains (Cakmak, 2008; Cakmak et al., 2010; Kutman et al., 2010, Aciksoz et al., 2011), there is a growing interest in leafy vegetables as a substantial dietary source of Fe and Zn (Broadley et al., 2010). We hypothesized that the amount of inorganic-N (NH_4^+-N and NO_3^--N), Fe, and Zn applied in organic N fertilizers may increase leaf Fe and Zn concentrations in lettuce.

2.2 Materials and Methods

2.2.1 Experimental site

Field experiments were carried out at the Colorado State University (CSU) Horticulture Research Center, Fort Collins, CO. The field area was planted with winter cover crops of rye (*Secale cereal*) and turnips (*Brassica rapa*). The experimental site is in a semi-arid zone with clay loam soils. The soil at the research area was classified as a fine, smectitic, mesic Aridic Argiustoll (NRCS, 1980).

2.2.2 Soil analysis

Soil samples were collected to a depth of 30 cm from the field area (15 m x 30 m) and analyzed by the Soil, Water, and Plant Testing Laboratory at Colorado State University (CSU). Chemical analyses included soil pH and electrical conductivity (EC) measured in supernatant suspension of 1:1 soil to water using a Mettler Toledo pH/EC meter (Thermo Fischer Scientific, Waltham, MA). Cation exchange capacity (CEC) was determined by summation of the exchangeable bases (Ca^{2+}, K^+, Mg^{2+}, Na^+) plus hydrogen (H^+) using ammonium acetate (1N $NH_4C_2H_3O_2$, pH 7.0) as described by Hajek et al. (1972).

Organic matter (OM) content was determined by the loss on ignition method (Blume et al., 1990). Soil inorganic-N (NH_4^+-N and NO_3^--N) was extracted using 2M KCl, and the filtered extract was analyzed using an Alpkem Flow Solution IV Auto Analyzer (OI Analytical, College Station, TX). Phosphorus (P) was extracted with 0.5M sodium bicarbonate ($NaHCO_3$) solution (Olsen et al., 1954) and analyzed using a Perkin Elmer Inductively Coupled Plasma/Optical Emission Spectrometer (ICP-OES) (Perkin Elmer, Waltham, MA). Major cations (K, Ca, and Mg) and micronutrients (Fe and Zn) were extracted with ammonium bicarbonate–diethylene triaminepentaacetic acid (AB-DTPA) (Jones, 2001) and analyzed using a Perkin Elmer Inductively Coupled Plasma/Optical Emission Spectrometer (ICP-OES) (Perkin Elmer, Waltham, MA) (Table B1).

2.2.3 Experimental design and plot layout

Treatments consisted of four different types of organic N fertilizer at two N rates and a control. Thirty-six experimental plots were arranged in a Randomized Complete Block design with four replications. The plot size was 76 cm x 457 cm, and the whole experimental area was 15 m x 30 m.

2.2.4 Planting materials

Lactuca sativa var. 'Concept' seeds (Johnny's Selected Seeds, Waterville, ME) were planted in 72-cell trays containing a well-mixed Sunshine® organic potting soil mix (SunGro Horticulture, Agawam, MA) in the first week of June 2013 and the first week of May 2014. All seeds were started at the Plant Environmental Research Center's (PERC) greenhouse facility on the CSU campus. After four weeks, seedlings were transplanted to the field on July 1, 2013 and June 6, 2014.

2.2.5 Irrigation

Drip irrigation tape (John Deere®, Deere & Co., Moline, IL) with a flow rate of 125 L $hour^{-1}$ 100 m^{-1} was stretched across each plot spaced 30 cm apart with a double header in each plot. The drip system used for this study comprised of laterals (15 mm diameter) with a spacing of 30 cm between inline emitters. Water was applied daily from 8:00 to 8:30 a.m. for 30 min day^{-1} at the initial stage and was changed to 30 minutes day^{-1} at 4^{th} leaf stage using an automated drip irrigation system. The amount of

precipitation and amount of irrigation water applied over the growing season is presented in Table B2. Weather data was obtained from a nearby CoAgMet weather station (http://www.coagmet.colostate.edu/).

2.2.6 Fertilizer treatments

The fertilizers compared in this study were liquid fertilizers [Alaska® fish emulsion (Planet Natural, Bozeman, MO) and cyano-fertilizer], and solid fertilizers [blood meal and feather meal (Down To Earth Inc., Eugene, OR)]. The N-fixing cyanobacteria (*Anabaena* sp.) was cultured from local soils and grown in nutrient-supplemented raceways according to the method by Barminski (2014).

2.2.7 Methods of fertilizer application

Fertilizers were applied at 56 and 112 kg N ha^{-1} in 2013, while rates of 28 and 56 kg N ha^{-1} were applied in 2014. Both the fish emulsion and cyano-fertilizer were in liquid form and were supplied in four split applications via fertigation over the growing season. The irrigation was cut off the night before any fertigation treatments. Cyano-fertilizer was pumped directly from production raceways into the drip irrigation system using a Flotec® sump pump (Flotec Water, Delavan, WI). Fish emulsion fertilizer was mixed in watering troughs and applied directly into the drip irrigation using the same pump after being flushed with city water. The blood meal and feather meal were in dry powdered form and were incorporated into the soil prior to planting. Blood meal and feather meal were applied 6 cm from the plants and 6 cm deep through sub-surface band application using a hoe.

2.2.8 Fertilizer analysis

Fertilizer samples were sent to the Soil, Water, and Plant Testing Laboratory at CSU and analyzed for N, P, K, Ca, Mg, Fe, and Zn (Table B3). Fertilizer samples were digested with nitric acid (HNO_3) and hydrogen peroxide (H_2O_2). Major cations (K, Ca, and Mg) and micronutrients (Fe and Zn) were analyzed using a Perkin Elmer Inductively Coupled Plasma/Optical Emission Spectrometer (ICP-OES) (Perkin Elmer, Waltham, MA). Solid fertilizers were extracted using 2M KCl, and the filtered extracts were analyzed for NH_4^+-N and NO_3^--N. The filtered extracts of solid fertilizers and the filtered liquid fertilizers were analyzed using an Alpkem Flow Solution IV Auto Analyzer (OI Analytical,

College Station, TX). Inorganic-N concentration and ratio (Table B4a) and amount of Fe and Zn applied is shown in Table B4b.

2.2.9 Measurements

2.2.9.1 Marketable yield and leaf dry weight

Lettuce was harvested 39 days in 2013 and 57 days in 2014 after transplanting. The lettuce crops were damaged by a hailstorm on June 22, 2014 resulting in a setback and subsequent longer growing period. Once harvested, brown leaves were stripped and removed to obtain a fresh green marketable yield and the marketable lettuce heads were weighed. All harvested samples were washed with deionized water before oven-drying for elemental analysis. The harvested samples from each subplot were oven-dried at the CSU's Agriculture Research Development & Education Center (ARDEC) at 72^{o}C for 72 hours. The dried samples were weighed and then ground to pass a 1.0-mm screen (20 mesh) using a plant tissue grinder (Thomas Wiley®, Swedesboro, NJ). Sub-samples were then taken from each sample for further analysis.

2.2.9.2 Leaf N, Fe, and Zn determination

Total N of plant tissues was measured using a LECO CN analyzer (Leco Corp., St. Joseph, MI). Then, 0.5 g subsamples of ground tissue were combined with 10 mL 70% nitric acid (HNO_3) and sealed in a closed vessel digestion system for three hours. Digestions were diluted with 2% HNO_3/0.5% hydrochloric acid (HCl) (v/v). Fe and Zn measurements were made using a Perkin-Elmer Inductively Coupled Plasma/Optical Emission Spectrometer (ICP-OES) (Perkin-Elmer, Waltham, MA).

2.2.10 Statistical analysis

Data were analyzed using SAS version 9.3 (SAS Institute Inc., Cary, NC). Univariate and Boxplot procedures were used to evaluate the normality of data distribution. Analysis of variance (ANOVA) was performed on the data with the MIXED procedure ($P < 0.1$). Fertilizer treatment and N rate were categorized as fixed effects, and replication was categorized as a random effect. When treatment or interaction effects were significant, means were separated by Tukey's Studentized Range (HSD) test.

The relationships between parameters were assessed by linear correlation using the CORR procedure. The relationships between parameters were assessed by linear correlation using the CORR procedure.

2.3 Results

2.3.1 Marketable yield

Nitrogen application rate affected marketable yield in both years, while an interaction effect between N rate and treatment on marketable yield was only observed in 2014 (Table B5). Marketable yield was 8.4% greater at 112 kg N ha^{-1} compared to 56 kg N ha^{-1} and control (0 kg N ha^{-1}) in 2013 (Table B6). In 2014, marketable yield was greater at 28 kg N ha^{-1} compared to 56 kg N ha^{-1} and control (0 kg N ha^{-1}). The cyano-fertilizer treatment had a greater marketable yield compared with other treatments and control at 28 kg N ha^{-1} in 2014 (Figure B1). The fish emulsion treatment had the lowest marketable yield at 56 kg N ha^{-1}.

2.3.2 Leaf Fe and Zn concentrations

An interaction between N rate and treatment was observed in leaf Fe ($P < 0.05$) and Zn ($P < 0.10$) concentrations in 2013. Blood meal and cyano-fertilizer treatments had a higher leaf Fe concentration at 56 kg N ha^{-1} than 112 kg N ha^{-1}, while feather meal and fish emulsion had a higher leaf Fe concentration at 112 kg N ha^{-1} (Figure B2). The cyano-fertilizer treatment had a greater leaf Fe concentration compared to other treatments and control at 56 kg N ha^{-1}. The lowest leaf Fe concentrations were observed in the blood meal treatment at 112 kg N ha^{-1} and the feather meal treatment at 56 kg N ha^{-1}.

In 2013, the feather meal, fish emulsion, and cyano-fertilizer treatments had a greater leaf Zn concentration than the blood meal treatment at 112 kg N ha^{-1} (Figure B3). Leaf Zn concentration ($r = -0.64$; $P = 0.02$) was negatively correlated with the amount of Zn applied in organic N fertilizers at 112 kg N ha^{-1}. The blood meal treatment had a higher amount of Zn applied compared to other fertilizer treatments (Table 5b).

A positive relationship was observed between Fe and Zn concentrations in leaf tissues ($r=0.52$; $P=0.0466$). Fe and Zn concentrations in leaf tissues were positively correlated with leaf N concentration ($r=0.86$; $P=0.0003$) and ($r=0.69$; $P=0.0041$), respectively (Table B7). Leaf Fe and Zn concentrations were

not affected by fertilizer treatments in 2014, and no treatment effect was observed in leaf N concentration in either year.

2.4 Discussion

2.4.1 Amount of NO_3^--N applied correlated negatively with leaf Fe concentration in lettuce

Among variables including amount of NO_3^--N, NH_4^+-N (Table B4a), Fe, and Zn applied (Table B4b) in organic N fertilizers, leaf Fe concentration was negatively correlated with NO_3^--N application in organic N fertilizers (Table B7). The blood meal treatment showed a greater percentage of N applied as NO_3^--N compared to other fertilizer treatments (Table B4a) and thus may have resulted in the lowest leaf Fe and Zn concentrations at 112 kg N ha^{-1} (Figures B2 and B3). Safaa and El-Fattah (2007) reported that Fe and Zn concentrations in lettuce were affected by N forms in the fertilizer. In our study, feather meal had a greater percentage of N applied as NH_4^+-N compared to other fertilizers (Table B4a), which may explain greater Fe and Zn concentrations at 112 kg N ha^{-1}. High NH_4^+-N concentration in fertilizer has been shown to increase Fe and Zn concentrations in wheat leaves as compared to wheat treated with NO_3^--N (Gashaw and Mugwira, 1981). NH_4^+-N fertilizer was more effective than NO_3^--N fertilizer in stimulating the acquisition of Fe in winter wheat (Barunawati et al., 2013). NH_4^+-N fertilizer treatment has been shown to increase Fe and Zn concentrations compared to NO_3^--N fertilizer treatment in lettuce and wheat grown in calcareous soils (Safaa and El-Fattah, 2007; Robin et al., 2008; Elgharably et al., 2010; Masarirambi et al., 2010). However, NH_4^+-N application in the feather meal treatment did not show a significant positive correlation with leaf Fe concentration, which may be due to slow release properties of feather meal (Hadas and Kautsky, 1994).

2.4.2 Leaf Zn concentration

In our study, there was no significant fertilizer effect observed in Zn concentration of lettuce at 56 kg N ha^{-1}, which may be due to a lack of lettuce response to soil Zn concentrations. Leaf Zn concentration of more than 50 mg kg^{-1} is considered high for lettuce (Maynard and Hochmuth, 1997). In our study, all treatments had leaf Zn concentration above 50 mg kg^{-1}, except in the blood meal treatment at the high application rate and control (Figure B3). The blood meal fertilizer had a greater percentage of N applied

as NO_3^--N compared to other fertilizers (Table B4a). NO_3^--N uptake by crops releases hydroxide and bicarbonate ions (Bienfait, 1989), which induced alkalinization of the rhizosphere, and thus decreases the amount of plant-available Fe and Zn.

In our study, however, there was no treatment effect on bulk soil pH at harvest. The soil acidification effect from NH_4^+-N fertilizer application was not observed in our study as there was no treatment effect in bulk soil pH post-harvest (data not shown). In calcareous soils, calcium carbonate buffers soil solution pH (Lindsay and Schwab, 1982). Reduction in soil pH is normally impaired by the buffering capacity of calcareous soil with high bicarbonate ion concentration. Bicarbonate ion can neutralize the effect of the released protons (Schenker and Chen, 2005).

2.4.3 N increased leaf Fe concentration

Hanafy Ahmed et al. (2000) reported that Fe and Zn concentrations increased with ammonium sulfate application on lettuce. In our study, leaf Fe ($r = 0.86$; $P = 0.0003$) and Zn ($r = 0.69$; $P = 0.0041$) concentrations were correlated with leaf N concentration (Table B7). In a study by Kutman et al. (2010), N application increased leaf Fe concentrations in winter wheat. In our study, greater leaf Fe concentration in the feather meal treatment at 112 kg N ha^{-1} compared to 56 kg N ha^{-1} may be due to an increase in Fe chelator, a phytosiderophore that could increase Fe concentration in plant tissues (Mori and Nishizawa, 1987; Aciksoz et al., 2011). However, most studies were conducted on Graminaceae, but none of these reports were found in lettuce.

Generally, leaf Fe concentration in lettuce ranges from 50 to 600 mg kg^{-1} dry weight (Chen and Barak, 1982). In our study, the leaf Fe concentration range was within that range. Mature lettuce leaves grown on organic soils ranged from 50 to 150 mg kg^{-1} and 25 to 50 mg kg^{-1} of Fe and Zn, respectively (Hochmuth et al., 2003). In our study, Fe and Zn concentrations of lettuce treated with fish emulsion and cyano-fertilizer exceeded the range reported by Hochmuth et al. (2003) and Pillay and Jonnalagadda (2007). Exceeding the range is not a major problem since there are no detrimental effects to human (Hochmuth et al., 2003).

The greater marketable yield observed in 2014 than in 2013 was probably due to higher precipitation in 2014 (Table B2). Some of the lettuce crops were damaged in a hailstorm, and environmental stress such as the hailstorm on June 22, 2014 can affect leaf growth and influence post-harvest quality of lettuce produce.

2.5 Conclusion

At 112 kg N ha^{-1}, the blood meal treatment had lower leaf Fe and Zn concentrations than other fertilizer treatments, and was similar to control. The cyano-fertilizer treatment had higher leaf Fe concentration at 56 kg N ha^{-1}. Leaf N concentration was positively correlated with Leaf Fe and Zn concentrations. Amount of NO_3^--N applied in organic N fertilizers was negatively correlated with leaf Fe concentration. In addition to fish emulsion and feather meal, organic growers can consider using cyano-fertilizer as a source of organic N fertilizer to maintain an adequate Fe concentration in lettuce grown in calcareous soils.

TABLES

Table B1. Initial soil properties of the 0-30 cm soil depth of the experimental site in lettuce.

Soil properties	2013	2014
pH[‡]	7.3	7.5
Electrical conductivity[‡] (dS m^{-1})	0.5	0.6
Cation exchange capacity[§] (meq 100 g^{-1})	30	29
Organic matter [#](%)	2.9	2.7
NH_4^+-N[¶] (ppm)	1.9	2.4
NO_3^--N[¶] (ppm)	5.2	4.0
P[‖] (ppm)	26	31
K[ʃ] (ppm)	432	473
Ca[ʃ] (ppm)	4411	4513
Mg[ʃ] (ppm)	622	634
Fe[ʃ] (ppm)	6.5	6.3
Zn[ʃ] (ppm)	1.5	1.6

[‡]pH and electrical conductivity were determined in water (1:1).
[§]CEC was determined using $NH_4C_2H_3O_2$ extraction.
[#]OM was determined by the loss on ignition method.
[¶]Samples were extracted using 2M KCl
[‖]Samples were extracted using 0.5M $NaHCO_3$.
[ʃ]Samples were extracted using ammonium bicarbonate diethylenetriamine pentaacetate (AB-DTPA).

Table B2. Average monthly precipitation and the amount of water applied during growing season in 2013 and 2014 in lettuce.

	2013	2014
Planting	July 1	June 9
Harvest	August 9	August 4
Precipitation (mm)		
June	--	34
July	39	69
August	9	0.3
Water applied (mm)		
Irrigation	210	221
Fertigation	230	230
Total (mm)	488	554

[‡]Total amount of water applied over the growing season is the combination of precipitation, irrigation, and fertigation. All plots received the same amount of water applied via irrigation. When liquid fertilizers were applied, the control and solid fertilizer treatments were irrigated with the same amount of water as that used to fertigate the liquid fertilizer treatments.

Table B3. Nutrient analysis of fertilizer samples.

	N[‡]	P[♯]	K[♯]	Fe[♯]	Ca[♯]	Mg[♯]	Zn[♯]
	%	----------mg kg^{-1}----------					
Cyano-fertilizer	0.2	6	0.1	6	10	18	0.1
Fish emulsion	5	1600	20510	131	725	921	18
Feather meal	13	640	1776	62	1466	575	18
Blood meal	13	32	366	118	904	283	14

[‡]Samples were analyzed using CN analyzer.
[♯]Samples were digested with HNO_3 and H_2O_2 and analyzed using ICP-OES.

Table B4a. Inorganic N concentration, inorganic-N ratio, and percentage of N applied as inorganic-N of fertilizers.

	NH_4^+-N¶	NO_3^--N¶	N as NH_4^+-N	N as NO_3^--N
	----------mg kg^{-1}----------		----------%----------	
Cyano-fertilizer	4.7	0.01	0.24	0.0005
Fish emulsion	23.7	0.12	0.05	0.0002
Feather meal	1232	2.30	0.95	0.002
Blood meal	27.7	8.40	0.02	0.006

¶Samples were extracted using 2M KCl and were analyzed using autoanalyzer.

Table B4b. Amount of Fe and Zn applied at 28, 56 and 112 kg ha^{-1} over the course of growing season.

	Iron (Fe)			Zinc (Zn)		
	28	56	112	28	56	112
	----------kg ha^{-1}----------			----------kg ha^{-1}----------		
Cyano-	1.7×10^{-4}	3.4×10^{-4}	6.8×10^{-4}	2.8×10^{-6}	5.6×10^{-6}	1.1×10^{-5}
Fish emulsion	3.7×10^{-3}	7.3×10^{-3}	1.5×10^{-2}	1.6×10^{-7}	3.2×10^{-7}	6.4×10^{-7}
Feather meal	1.8×10^{-3}	3.5×10^{-3}	7.0×10^{-3}	1.6×10^{-7}	3.2×10^{-7}	6.4×10^{-7}
Blood meal	3.3×10^{-3}	6.6×10^{-3}	1.3×10^{-2}	3.9×10^{-4}	7.8×10^{-4}	1.6×10^{-3}

Table B5. Analysis of variance (ANOVA) of marketable yield, leaf Fe and Zn concentrations in 2013 and 2014.

	2013			2014		
	Yield	Leaf Fe	Leaf Zn	Yield	Leaf Fe	Leaf Zn
	---------- P > F ----------			---------- P > F ----------		
N rate	0.0373*	0.7279	0.5538	0.0337*	0.8515	0.6181
Treatment	0.1555	0.3335	0.4373	0.0657*	0.1216	0.1480
Nrate*Treatment	0.5995	0.0370*	0.0894*	0.0186*	0.3561	0.3080

*P-values are significantly different at $P < 0.1$.

Table B6. Marketable yield of lettuce in 2013 and 2014. Means with the same letter are not significantly different at $P < 0.1$ using Tukey's Studentized Range (HSD) test of mean separation.

	2013[‡]	2014[¶]
N rate (kg ha^{-1})	---------- kg ha^{-1}----------	
0	958 b	779 c
28	--	1483 a
56	939 b	1269 b
112	1018 a	--

[‡]Fertilizers were applied at 56 and 112 kg N ha^{-1} in 2013.
[¶] Fertilizers were applied at 28 and 56 kg N ha^{-1} in 2014.

Table B7. Pearson correlation coefficients of leaf Fe and Zn concentrations with NO3app, NH4app, Feapp, and Znapp in 2013. NO3app = NO_3^--N application, Feapp = Iron (Fe) application, and Znapp = Zinc (Zn) application. Leaf Zn = Leaf Zn concentration, Leaf Fe = Leaf Fe concentration.

	NO3app	NH4app	Feapp	Znapp	Leaf Zn	Leaf Fe
Leaf N	r = 0.12	r = -0.12	r = 0.14	r = 0.25	r = 0.69	r = 0.86
conc.	P = 0.86	P = 0.70	P = 0.65	P = 0.43	P = 0.0041*	P =0.0003*
Leaf Fe	r = - 0.58	r = 0.44	r = -0.41	r = 0.02	r = 0.52	r =1.00
conc.	P =	P = 0.27	P = 0.19	P = 0.94	P = 0.05*	
Leaf Zn	r = 0.15	r = 0.15	r = -0.1	r = 0.18	r =1.00	r = 0.52
conc.	P = 0.64	P = 0.65	P = 0.87	P = 0.59		P = 0.05*

*P-values are significantly different at P < 0.1.

FIGURES

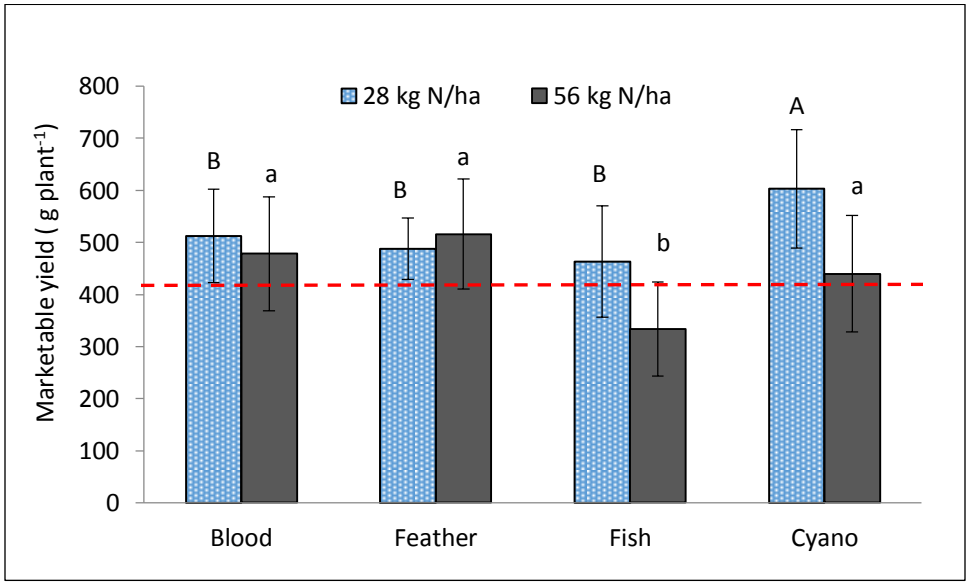

Figure B1. The marketable yield at harvest. Fertilizers were applied at 28 and 56 kg N ha^{-1} in 2014. Bars represent standard deviation of mean. Means with the same letter are not significantly different at $P < 0.1$ using Tukey's Studentized Range (HSD) test of mean separation. Means with capital letters represent the means within 28 kg N ha^{-1}, while means with small letters represent the means within 56 kg N ha^{-1}. The dashed line represents control. All treatments were not significantly different from control except for the cyano-fertilizer at 28 kg N ha^{-1} and the fish emulsion treatment at 56 kg N ha^{-1}. Blood = Blood meal; Feather = Feather meal; Fish = Fish emulsion; Cyano = Cyano-fertilizer.

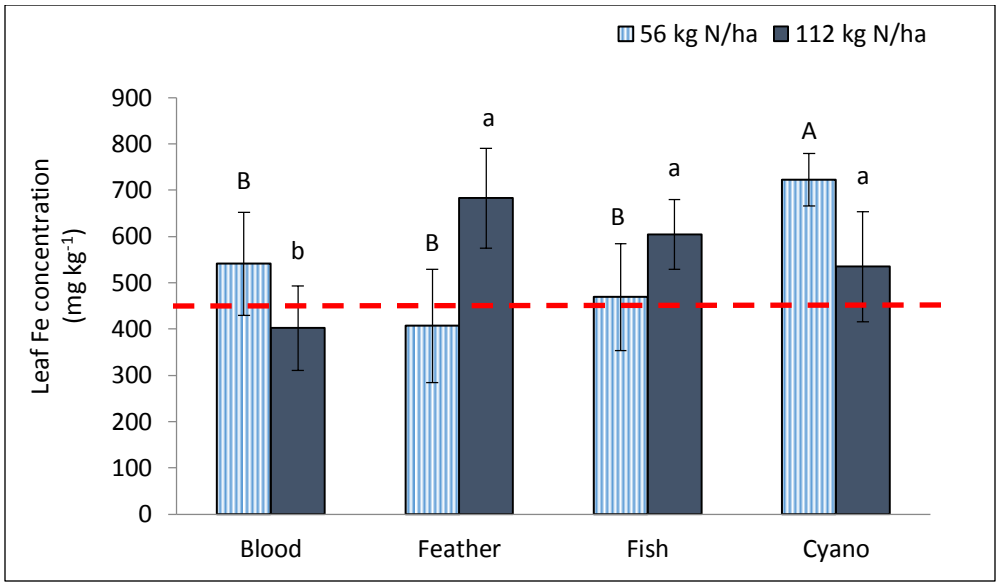

Figure B2. Leaf Fe concentration at harvest. Fertilizers were applied at 56 and 112 kg N ha^{-1} in 2013. Bars represent standard deviation of mean. Means with the same letter are not significantly different at $P < 0.1$ using Tukey's Studentized Range (HSD) test of mean separation. Means with capital letters represent the means within 56 kg N ha^{-1}, while means with small letters represent the means within 112 kg N ha^{-1}. The dashed line represents control. All treatments were significantly different from control except the blood meal treatment at 112 kg N ha^{-1}, blood meal and fish emulsion treatments at 56 kg N ha^{-1} at $P < 0.1$. Blood = Blood meal; Feather = Feather meal; Fish = Fish emulsion; Cyano = Cyano-fertilizer.

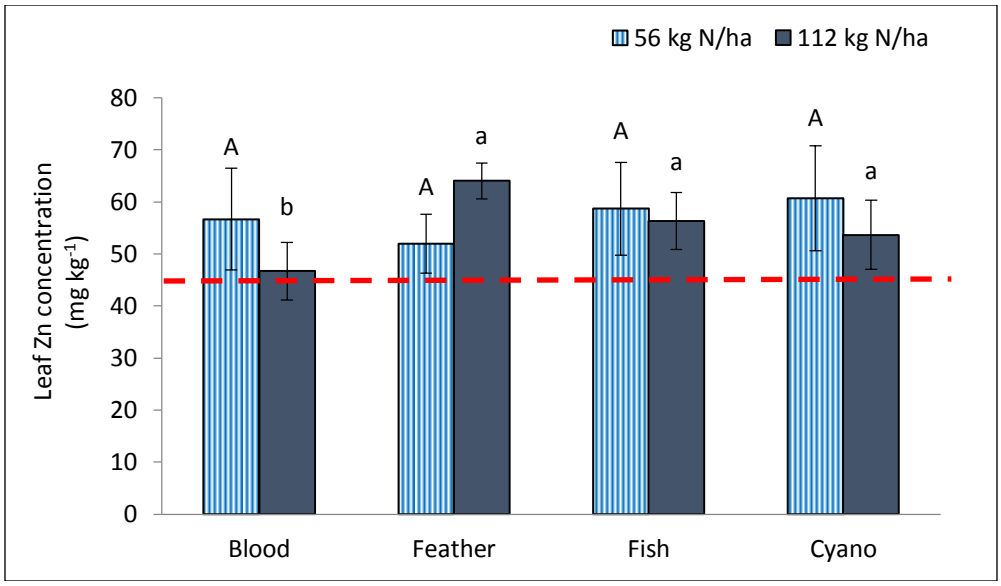

Figure B3. Leaf Zn concentration at harvest. Fertilizers were applied at 56 and 112 kg N ha^{-1} in 2013. Bars represent standard deviation of mean. Means with the same letter are not significantly different at $P < 0.1$ using Tukey's Studentized Range (HSD) test of mean separation. Means with capital letters represent the means within 56 kg N ha^{-1}, while means with small letters represent the means within 112 kg N ha^{-1}. The dashed line represents control. All treatments were significantly different from control except for the blood meal treatment at 112 kg N ha^{-1} at $P < 0.1$. Blood = Blood meal; Feather = Feather meal; Fish = Fish emulsion; Cyano = Cyano-fertilizer.

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CHAPTER 3. ORGANIC NITROGEN FERTILIZERS INFLUENCE IRON CONCENTRATION IN SWEET CORN

3.1 Introduction

An estimated two billion people suffer from micronutrient malnutrition globally, causing a loss of 63 million lives annually (Tulchinsky, 2010). Micronutrient malnutrition, also called hidden hunger, is very common among women and preschool children mainly due to low dietary intake of micronutrients, especially Fe and Zn (Bouis, 2003).

Calcareous soils contain free calcium carbonate and have alkaline pH, which reduce plant availability of Fe and Zn. The pH of plant sap can increase to a level that causes Fe and Zn to precipitate due to hydroxide ions in the root, thus reducing Fe and Zn translocation in plant edible portion (Malakouti, 2008). Fe deficiency is more common in calcareous soils due to Fe precipitation as insoluble Fe^{3+} oxides. Under Fe deficient conditions, plants in the family Graminaceae; including sweet corn (*Zea mays*) use Strategy II to increase soil Fe availability by secreting siderophores to chelate Fe for Fe acquisition (Romheld, 1987).

Some of the factors affecting Fe and Zn concentrations in crops are the presence of siderophores as Fe chelating agents, fertilizer types, inorganic-N forms, nutrient ratios, phytohormone concentrations, and macronutrient levels. Nitrogen (N) plays an important role in root uptake, shoot transport, and translocation of Fe and Zn in wheat (Cakmak et al., 2010). N promotes accumulation of Fe and Zn in grains by activating transporter proteins involved in root uptake and root-shoot translocation of Fe and Zn, thus exerting positive effects on concentration of Fe and Zn in winter wheat (Cakmak et al., 2010; Kutman et al., 2010).

Fertilization is an alternative solution that could provide available Fe and Zn to be taken up by plants. In field studies in the US Corn Belt, Derby et al. (2005) and Mulvaney et al. (2006) reported that sources of N fertilizer affect Fe and Zn concenrations, but the information provided focused on synthetic

fertilizer. The information on organic N fertilizer affecting Fe and Zn concentrations is inadequate for horticultural crops. Organic agriculture relies heavily on fertilizers and soil amendments from off-farm sources such as fish emulsion, blood meal, feather meal, compost, and animal manures to meet crop N demand (Gaskell et al., 2006). These fertilizers vary in nutrient composition, forms of available N (NH_4^+-N and NO_3^--N), and have high transportation costs.

There are two main classes of N fertilizers, solid and liquid. In organic agriculture, solid fertilizers are often incorporated into the soil before planting, while liquid fertilizers are generally applied season-long through irrigation. Blood meal is considered as a quick release organic N fertilizer (Hartz and Johnstone, 2006). In a study conducted by, feather meal was found to have 94-99% N in organic forms, and is usually considered as slow release organic N fertilizer (Hadas and Kautsky, 1994). While these organic fertilizers are commercially available, many of them are produced off-farm and distributed widely throughout the US. Although farmers often use local manure sources, they usually rely on off-farm sources for additional N needed during the growing season. However, there is another fertilizer option being developed that allows farmers to produce N on-farm, which uses cyanobacteria, previously known as blue-green algae.

Cyanobacteria has unique dual properties because it can both fix N from the atmosphere and photosynthesize to produce N fertilizer without fossil fuels. Cyanobacteria secretes Fe-chelating compounds, which enable them to solubilize soil Fe for subsequent uptake by plant roots (Lopez-Millan et al., 2000). Cyanobacteria, such as *Anabaena* sp., have been shown to produce phytohormones that stimulate plant growth and development (Rodgers et al., 1979). Phytohormones are not only required for plant growth and development, but they also play a significant role in regulating Fe and Zn concentrations in plants (Rubio et al., 2009).

Oktem et al. (2010) reported positive effects of N fertilization on Fe and Zn concentrations in sweet corn. However, a number of other researchers have found that N fertilization had no effect on grain Fe and Zn concentrations in corn (Feil et al., 2005; Losak et al., 2011; Yu et al., 2011). Therefore, we would like to investigate whether different sources of organic N fertilizer affect Fe and Zn concentrations

in sweet corn under field conditions. We hypothesized that amount of NH_4^+-N applied in the organic N fertilizers would increase Fe and Zn concentrations in sweet corn.

In this study, commonly used organic fertilizers were evaluated in terms of sweet corn ear yield and N, Fe, and Zn concentrations and contents. Numerous studies have shown that Fe and Zn fertilizer increases Fe and Zn availability in soil, but most of the indicator crops were agronomic crops, and very few studies have been conducted in horticultural crops. While there are many studies focusing on micronutrient composition in cereal grains, there is a growing interest in horticultural crops that can provide a substantial dietary source of Fe and Zn. We hypothesized that amount of inorganic-N (NH_4^+-N and NO_3^--N), Fe and Zn applied in organic N fertilizers may increase leaf Fe and Zn concentrations in sweet corn.

3.2 Materials and Methods

3.2.1 Experimental site

Field experiments were carried out at the Colorado State University (CSU) Horticulture Research Center, Fort Collins, CO. Previously, the field area was planted with buckwheat followed by winter cover crops, rye (*Secale cereal*) and turnips (*Brassica rapa*). The experimental site is in a semi-arid zone with clay loam soils. The soil was classified as a fine, smectitic, mesic Aridic Argiustoll of the Nunn series (NRCS, 1980).

3.2.2 Soil analysis

Prior to each field season, soil samples were collected to a depth of 30 cm from the field (15 m x 30 m) and analyzed by the Soil, Water, and Plant Testing Laboratory at Colorado State University. Chemical analyses included soil pH and electrical conductivity (EC) measured in supernatant suspension of 1:1 soil to water using a Mettler Toledo pH/EC meter (Thermo Fischer Scientific, Waltham, MA). Cation exchange capacity (CEC) was determined by summation of the exchangeable bases (Ca^{2+}, K^+, Mg^{2+}, Na^+) plus hydrogen (H^+) using ammonium acetate (1N $NH_4C_2H_3O_2$, pH 7.0) as described by Hajek et al. (1972). Organic matter (OM) content was determined by the loss on ignition method (Blume et al., 1990). Soil inorganic N (NH_4^+-N and NO_3^--N) was extracted using 2M KCl, and the filtered extract was

analyzed using an Alpkem Flow Solution IV Auto Analyzer (OI Analytical, College Station, TX). Phosphorus (P) was extracted with 0.5M sodium bicarbonate ($NaHCO_3$) solution (Olsen et al., 1954) and analyzed using a Perkin Elmer Inductively Coupled Plasma/Optical Emission Spectrometer (ICP-OES) (Perkin Elmer, Waltham, MA). Major cations (K, Ca, and Mg) and micronutrients (Fe and Zn) were extracted with ammonium bicarbonate–diethylene triaminepentaacetic acid (AB-DTPA) (Jones, 2001) and analyzed using a Perkin Elmer Inductively Coupled Plasma/Optical Emission Spectrometer (ICP-OES) (Perkin Elmer, Waltham, MA). The results of soil physical, chemical, and nutrient analyses are presented in Table C1.

3.2.3 Experimental design and plot layout

Treatments consisting of four different types of N fertilizer and control were arranged in a Randomized Complete Block Design with four replications. The plot size was 76 cm x 457 cm.

3.2.4 Planting materials

Luscious se^{+} sweet corn seeds were purchased from Johnny's Seeds (Johnny's Selected Seed, Waterville, ME). Seeds were planted at a depth of approximately 2.5 cm. Sweet corn seeds (*Zea mays* var. Luscious F1 se+) were hand planted on July 1, 2013 and July 2, 2014 with an in-row spacing of 12 cm in rows spaced 75 cm apart.

3.2.5 Irrigation

Two lines of John Deere® drip tape (Deere & Co., Moline, IL) with a flow rate of 125 L hour^{-1} 100 m^{-1} were stretched across each row spaced 30 cm apart with a double header in each plot. Water was applied daily from 08:00 to 08:30 using an automated drip irrigation system. The drip system used for this study comprised of laterals (15 mm diameter) with emitters 30 cm apart. Irrigation was applied at 08:00 for 15 min day^{-1} at the initial stage and was changed to 30 min day^{-1} after plants reached the V6-stage. The amount of precipitation and irrigation over the growing season is presented in Table C2. The amount of precipitation was obtained from a nearby CoAgMet weather station (http://www.coagmet.colostate.edu/).

3.2.6 Fertilizer treatments

The fertilizers compared in this study were liquid fertilizers [Alaska® fish emulsion (Planet Natural, Bozeman, MO) and cyano-fertilizer], and solid fertilizers [blood meal and feather meal (Down To Earth Inc., Eugene, OR)]. For this study the N-fixing cyanobacteria (*Anabaena* sp.) was cultured from local soils and inoculated into nutrient-supplemented raceways according to the method by Barminski (2014).

3.2.7 Fertilizer application method

Both the fish emulsion and cyano-fertilizer were in liquid form and were supplied in four split applications over the growing season, while the blood meal and feather meal were in dry powdered form and sub-surface banded prior to planting. Blood meal and feather meal were applied 6 cm from the plants by 6 cm deep through band application using a hoe. All fertilizers were applied at 56 and 112 kg N ha^{-1}.

3.2.8 Fertilizer analysis

Fertilizer samples were sent to the Soil, Water, and Plant Testing Laboratory at CSU and analyzed for N, P, K, Fe, Ca, Mg, and Zn (Table C3). Fertilizer samples were digested with nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) (Jones, 2001). Major cations (K, Ca, and Mg) and micronutrients (Fe and Zn) were analyzed using a Perkin Elmer Inductively Coupled Plasma/Optical Emission Spectrometer (ICP-OES) (Perkin Elmer, Waltham, MA). Solid fertilizers were extracted using 2M KCl, and the filtered extracts were analyzed for NH_4^+-N and NO_3^--N. The filtered extracts of solid and the filtered liquid fertilizers were analyzed using an Alpkem Flow Solution IV Auto Analyzer (OI Analytical, College Station, TX). The nutrient ratio, amount of inorganic-N (Table C4) and Fe and Zn applied (Table 5) were calculated based on nutrient analysis.

3.2.9 Measurements

3.2.9.1 Ear yield and kernel dry weight

Harvesting was done 79 days after planting in both years. Sweet corn ears with marketable quality (kernel filled to the tip) were harvested from each plot and weighed. Husks were removed from the marketable ear and samples from each subplot were then oven-dried at CSU's Agriculture Research

Development & Education Center (ARDEC) at 72°C for 72 hours. The dried kernel samples removed from each ear were then weighed for dry matter and then ground to pass a 1.0-mm screen (20 mesh) using a plant tissue grinder (Thomas Wiley®, Swedesboro, NJ). Sub-samples were then taken from each sample for further analysis.

3.2.9.2 Leaf and kernel N, Fe, and Zn determination

All harvested leaf samples were washed with deionized water before oven-drying and grinding for elemental analysis, as described above. Total N of leaf and kernel tissues were measured using a LECO CN analyzer (Leco Corp., St. Joseph, MI). Fe and Zn concentrations were determined by a Perkin-Elmer Inductively Coupled Plasma/Optical Emission Spectrometer (ICP-OES) (Perkin-Elmer, Waltham, MA) after wet digestion of plant sample powder with HNO_3 (Jones, 2001). Leaf and kernel N, Fe, and Zn concentrations were multiplied by leaf and kernel dry weights, respectively, to determine the total N, Fe, and Zn contents.

3.2.10 Statistical analysis

Data were analyzed using SAS version 9.3 (SAS Institute Inc., Cary, NC). Univariate and Boxplot procedures were used to evaluate the normality of data distribution. Analysis of variance (ANOVA) was performed on the data with the MIXED procedure ($P < 0.1$). Fertilizer treatment and N rate were categorized as fixed effects, and replication was categorized as a random effect. Means were separated by Tukey's Studentized Range (HSD) test for mean separation. Mean separation testing on interaction effects was simplified within N rate using the SLICE option. The relationships between parameters were assessed by linear correlation using the CORR procedure.

3.3 Results

N rate, treatment, and the interaction between N rate and treatment had significant effects on marketable ear yield in both years (Table C6). Treatment effects were observed in leaf and kernel dry weights, kernel Fe concentration, leaf and kernel Fe and N contents, and kernel Zn content in 2013. No significant effect was observed in leaf N, Fe, or Zn concentrations in either year (Table C6).

3.3.1 Marketable ear yield

There was a significant interaction between N rate and treatment in 2013 (P = 0.0209) and 2014 (P = 0.0025) in ear yield (Table C6). The fish emulsion and cyano-fertilizer treatments had a higher marketable ear yield in 2013 compared with other treatments at the high N rate. The fish emulsion treatment had a higher ear yield compared to other treatments including control at the low N rate in 2013 (Figure C1). The marketable ear yield in the cyano-fertilizer treatment was higher at the high N rate compared with the low N rate in both years. The marketable ear yield was higher in the cyano-fertilizer treatment compared with other treatments at the high N rate, while no fertilizer treatment effect was observed at the low N rate in 2014 (Figure C1).

3.3.2 Dry weights and Fe and N contents

The blood meal, fish emulsion, and cyano-fertilizer treatments had a higher leaf and kernel dry weights compared to feather meal and control (Table C7). In 2013, leaf N and Fe contents were influenced by leaf dry weights, as there was no significant effect observed in leaf N (Table C8) and Fe (Table C9) concentrations.

Leaf N and Fe contents had a significant treatment effect in 2013 due to the treatment effect in leaf dry weight (P = 0.0056). The fish emulsion, cyano-fertilizer, and blood meal treatments had higher leaf N and leaf Fe contents compared to feather meal and control (Tables C8 and C9).

Kernel N, Fe, and Zn contents were affected by fertilizer treatment in 2013 (P = 0.0185) due to the treatment effect on kernel dry weight (P = 0.0243; Table C6). The feather meal treatment had the lowest kernel N (Table C10), Fe (Table C11), and Zn (Table C12) contents compared to other fertilizer treatments in 2013.

3.3.3 Kernel Fe concentration

Treatment and N rate effects were observed in kernel Fe concentration in 2013. Higher kernel Fe concentration was observed at 112 kg N ha^{-1} compared to 56 kg N ha^{-1} (P = 0.0821; Table C6). The blood meal treatment had a higher kernel Fe concentration compared with other treatments in 2013 (Table C11).

Kernel Fe concentration treated with cyano-fertilizer was on par with fish emulsion and higher than feather meal and control.

3.3.4 Relationships among fertilizer treatments with N, Fe, and Zn concentrations

Leaf N concentration was positively correlated with leaf Fe concentration in 2013. Kernel N and Zn concentrations were positively correlated in both years (Table C13). Kernel Fe concentration was positively correlated with marketable ear yield. Amounts of NO_3^--N, Fe, and Zn applied in organic N fertilizers were positively correlated with kernel Fe concentration, while amount of NH_4^+-N applied was negatively correlated with kernel Fe concentration. Amounts of inorganic-N (NH_4^+-N and NO_3^--N) and micronutrients (Fe and Zn) applied in organic N fertilizers were negatively correlated with ear yield (Table C14).

3.4 Discussion

3.4.1 Effect of N on Fe concentration in sweet corn

Timsina (2013) reported that N fertilizer influences Fe and Zn concentration in wheat grain. Sweet corn treated with N fertilizer showed an increase in kernel Fe content due to N retranslocation from leaves to kernel (Shi et al., 2012). Positive correlation of Fe and Zn concentrations with N fertilizer applied were possibly due to increased uptake from soil or increased translocation of Fe and Zn from vegetative parts to the grain (Cakmak et al., 2010). Kutman et al. (2010) and Barunawati et al. (2013) found that N affects transporter proteins in the translocation of Fe from leaves to wheat grain.

Under N fertilizer treatment, Fe concentration in kernels can be facilitated by Fe-chelating compounds called siderophores in Strategy II plants (Romheld and Marschner, 1986; Cakmak et al., 2010). Sweet corn relies on enhanced release of siderophores (Romheld, 1987; Briat et al., 2007). However, siderophores were not measured in our study. To our knowledge, there has been no literature found either on N rate or N source influencing siderophore production.

No treatment effect was observed on kernel Fe concentration in 2014 compared to 2013, which may be due to the dilution effect of increased marketable yield in 2014 compared to 2013, similar to results reported by Liu et al. (2006), Sperotto et al. (2013), and Gomez-Becerra et al. (2010). A two-fold

increase in marketable ear yield in 2014 compared to 2013 may be due to rainfall distribution (Table C2). Fe and Zn deficiency symptoms were only observed at the V6-stage in all treatments in both years, but recovered as the plants grew over the growing season.

Ahmad et al. (2009) showed that N fertilizer had a significant positive correlation with Zn concentration in sweet corn kernels, which may indicate that N fertilizer response indirectly increased Zn concentrations. In our study, a similar positive relationship was found between kernel N and Zn concentrations in both years (Table C13), although treatment effects on leaf kernel N and Zn concentrations were not significant.

3.4.2 Feather meal decreased kernel Fe concentration and dry weight

The feather meal treatment had a higher percentage of N applied as NH_4^+-N (Table C4), which may have decreased kernel Fe concentration. Decreased leaf and kernel dry weights in the feather meal treatment may have been due to the slow-release properties and low rate of N mineralization of feather meal (Hadas and Kautsky, 1994). The kernel Fe concentration in the blood meal treatment was greater than that of feather meal (Table C11). It may be possible that methionine in the blood meal fertilizer (Akhter et al., 2008) induced greater Fe kernel concentration in blood meal compared to other fertilizers (Table 11). Aciksoz et al. (2011) reported that methionine was secreted in the leaves and may be possibly transported into roots to accumulate Fe concentration in wheat grain.

3.5 Conclusion

Cyano-fertilizer, fish emulsion, and blood meal increased Fe concentration in sweet corn compared to feather meal. The amounts of NO_3^--N, Fe, and Zn applied in organic N fertilizers were positively correlated with kernel Fe concentration, while the amount of NH_4^+-N applied was negatively correlated with kernel Fe concentration. There was no N rate or treatment effect on leaf or kernel N concentrations in sweet corn. Local organic growers could consider either blood meal, fish emulsion, or cyano-fertilizer as one of the organic N fertilizer sources to maintain an adequate level of Fe concentration in sweet corn grown in calcareous soils.

TABLES

Table C1. Initial soil properties of the 0-30 cm soil depth of the sweet corn experimental site.

Soil properties	2013	2014
pH[¶]	7.5	7.7
Electrical conductivity[¶] (dS m^{-1})	0.6	0.6
Cation exchange capacity, CEC[ǂ] (meq 100 g^{-1})	29	30
Organic matter, OM[#] (%)	2.5	2.6
NH_4^+-N[##] (ppm)	2.5	2.7
NO_3^--N[##] (ppm)	1.9	2.1
P[§] (ppm)	30	32
K[♯] (ppm)	463	456
Ca[♯] (ppm)	4560	4650
Mg[♯] (ppm)	616	622
Fe[♯] (ppm)	6	7
Zn[♯] (ppm)	1.4	1.6

[¶] pH and electrical conductivity were determined in water (1:1).
[ǂ] CEC was determined using ammonium acetate (1N $NH_4C_2H_3O_2$, pH 7.0) extraction.
[#] OM was determined by the loss on ignition method.
[##] Samples were extracted using 2M KCl.
[§] Sample was extracted with 0.5M $NaHCO_3$.
[♯] Samples were extracted using ammonium bicarbonate diethylenetriamine pentaacetate (AB-DTPA).

Table C2. Average monthly precipitation and the amount of water applied during growing season in 2013 and 2014 in sweet corn.

	2013	2014
Planting	July 2	July 1
Harvest	September	September 19
Precipitation (mm)		
July	39	69
August	13	26
September	119	9
Amount of water applied (mm)		
Irrigation	729	737
Fertigation	414	414
Total (mm)	1314	1257

[ǂ]Total amount of water applied over the growing season is the combination of precipitation, irrigation, and fertigation. All plots received the same amount of water applied via irrigation. When liquid fertilizers were applied, the control and solid fertilizer treatments were irrigated with the same amount of water as that used to fertigate the liquid fertilizer treatments.

Table C3. Nutrient analysis of fertilizer samples.

	N[ǂ]	P[♯]	K[♯]	Fe[♯]	Ca[♯]	Mg[♯]	Zn[♯]
	%	----------mg kg^{-1}----------					
Cyano-fertilizer	0.2	6	0.1	6	10	18	0.1
Fish emulsion	5	1600	20510	131	725	921	18
Feather meal	13	640	1776	62	1466	575	18
Blood meal	13	32	366	118	904	283	14

[ǂ]Samples were analyzed using CN analyzer.
[♯]Samples were digested with HNO_3 and H_2O_2 and analyzed using ICP-OES.

Table C4. Inorganic N concentration and percentage of N applied as inorganic-N of fertilizers.

	NH_4^+-N¶	NO_3^--N¶	N as NH_4^+-N	N as NO_3^--N
	----------mg kg^{-1}----------		----------%----------	
Cyano-fertilizer	4.7	0.01	0.24	0.0005
Fish emulsion	23.7	0.12	0.05	0.0002
Feather meal	1232	2.30	0.95	0.002
Blood meal	27.7	8.40	0.02	0.006

¶Samples were extracted using 2M KCl and were analyzed using autoanalyzer.

Table C5. Amount of iron (Fe) and zinc (Zn) applied at 56 and 112 kg ha^{-1} over the course of growing season.

	Fe		Zn	
	56	112	56	112
	----------kg ha^{-1}----------		----------kg ha^{-1}----------	
Cyano-fertilizer	3.4×10^{-4}	6.8×10^{-4}	5.6×10^{-6}	1.1×10^{-5}
Fish emulsion	7.3×10^{-3}	1.5×10^{-2}	3.2×10^{-7}	6.4×10^{-7}
Feather meal	3.5×10^{-3}	7.0×10^{-3}	3.2×10^{-7}	6.4×10^{-7}
Blood meal	6.6×10^{-3}	1.3×10^{-2}	7.8×10^{-4}	1.6×10^{-3}

Table C6. Analysis of variance (ANOVA) of sweet corn ear yield, kernel, and leaf dry weight, nutrient concentrations and content in 2013 and 2014.

	Marketable ear yield		**Kernel dry weight**		**Leaf dry weight**	
	2013	2014	2013	2014	2013	2014
	P > F	P > F	P > F	P > F	P > F	P > F
N rate	0.0333*	<.0001*	0.1151	0.3269	0.2220	0.2321
Treatment	<.0001*	0.0008*	0.0243*	0.1658	0.0056*	0.3652
N rate* Treatment	0.0209*	0.0025*	0.3616	0.3620	0.8179	0.3589
	Leaf N concentration		**Leaf Fe concentration**		**Leaf Zn concentration**	
N rate	0.2916	0.5341	0.5415	0.4176	0.1801	0.6935
Treatment	0.2105	0.9119	0.4499	0.8736	0.1281	0.4492
N rate* Treatment	0.2633	0.1448	0.6330	0.1587	0.1820	0.1412
	Kernel N concentration		**Kernel Fe concentration**		**Kernel Zn concentration**	
N rate	0.6234	0.8434	0.0821*	0.4880	0.8719	0.3271
Treatment	0.2341	0.1258	0.0037*	0.6427	0.5790	0.3113
N rate* Treatment	0.2712	0.6480	0.5348	0.6122	0.6143	0.8680
	Leaf N content		**Leaf Fe content**		**Leaf Zn content**	
N rate	0.2044	0.3412	0.2130	0.6443	0.2213	0.4404
Treatment	0.0063*	0.9352	0.0351*	0.9105	0.1459	0.5146
N rate* Treatment	0.5080	0.3967	0.2466	0.2569	0.1788	0.4105
	Kernel N content		**Kernel Fe content**		**Kernel Zn content**	
N rate	0.2140	0.2828	0.9456	0.3066	0.2272	0.1437
Treatment	0.0578*	0.1880	0.0144*	0.4030	0.0185*	0.1998
N rate*Treatment	0.3840	0.3196	0.4844	0.4580	0.2326	0.2037

*P-values are significantly different at $P < 0.1$.

Table C7. Leaf and kernel dry weights in 2013. Means with the same letter are not significantly different at P < 0.1 using Tukey's Studentized Range (HSD) test of mean separation.

Treatment	Leaf dry weight	Kernel dry weight
	---g plant^{-1}---	---g plant^{-1}---
Cyano-fertilizer	96 a	77 a
Feather meal	72 b	47 b
Fish emulsion	114 a	74 a
Blood meal	92 a	73 a
Control	47 c	25 c

Table C8. Leaf N concentration and content in 2013. Means with the same letter are not significantly different at P < 0.1 using Tukey's Studentized Range (HSD) test of mean separation.

Treatment	Leaf N concentration	Leaf N content
	-----%-----	------g N plant^{-1} ------
Cyano-fertilizer	2.6 a	2.5 a
Feather meal	2.8 a	2.0 b
Fish emulsion	2.6 a	2.9 a
Blood meal	2.8 a	2.6 a
Control	2.7 a	1.2 c

Table C9. Leaf Fe concentration and content in 2013. Means with the same letter are not significantly different at P < 0.1 using Tukey's Studentized Range (HSD) test of mean separation.

Treatment	Leaf Fe concentration	Leaf Fe content
	-----mg Fe kg^{-1}-----	------mg Fe plant^{-1} ------
Cyano-fertilizer	475.2 a	43.7 a
Feather meal	436.5 a	31.4 b
Fish emulsion	404.3 a	45.1 a
Blood meal	495.2 a	45.6 a
Control	488.3a	22.9 c

Table C10. Kernel N concentration and content in 2013. Means with the same letter are not significantly different at P < 0.1 using Tukey's Studentized Range (HSD) test of mean separation.

Treatment	Kernel N concentration	Kernel N content
	----------%----------	-------g N plant^{-1} --------
Cyano-fertilizer	2.7 a	2.1 a
Feather meal	2.6 a	1.2 b
Fish emulsion	2.8 a	2.1 a
Blood meal	2.7 a	1.9 a
Control	2.6 a	0.7 c

Table C11. Kernel Fe concentration and content in 2013. Means with the same letter are not significantly different at P < 0.1 using Tukey's Studentized Range (HSD) test of mean separation.

Treatment	Kernel Fe concentration	Kernel Fe content
	-----mg Fe kg^{-1}-----	------mg Fe plant^{-1} ------
Cyano-fertilizer	61 b	4.8 a
Feather meal	52 c	2.5 c
Fish emulsion	68 b	5.1 a
Blood meal	86 a	6.3 a
Control	42 c	1.1 c

Table C12**.** Kernel Zn concentration and content in 2013.

Treatment	Kernel Zn concentration	Kernel Zn content
	-----mg Zn kg^{-1}-----	------mg Zn plant^{-1} ---
Cyano-fertilizer	34.7 a	2.7 a
Feather meal	36.1 a	1.7 b
Fish emulsion	33.7 a	2.5 a
Blood meal	38.5 a	2.8 a
Control	35.3 a	0.9 c

Table C13. Pearson correlation coefficients of N, Zn, and Fe leaf and kernel concentrations and yield in sweet corn in 2013 and 2014.

	2013		**2014**	
	r	P-value	r	P-value
Ear yield & Kernel Fe conc.	0.52	0.0446*	0.29	0.2054
Kernel N conc. & Kernel Zn conc.	0.64	0.0100*	0.62	0.0034*
Leaf N conc. & Leaf Fe conc.	0.53	0.0433*	0.13	0.5819
Leaf Zn conc. & Leaf Fe conc.	0.39	0.3214	0.63	0.0169*

*P-values are significantly different at $P < 0.1$.

Table C14. Pearson correlation coefficients of Fe kernel concentration and ear yield with inorganic-N (NH_4^+-N and NO_3^--N) and micronutrients (Fe and Zn) applied in organic N fertilizers in 2013.

	Kernel Fe concentration		Ear yield	
	r	P-value	r	P-value
NO_3^--N app	0.68	0.0060*	-0.58	0.0186*
NH_4^+-N app	-0.49	0.0416*	-0.69	0.0026*
Fe app	0.59	0.0413*	-0.54	0.0298*
Zn app	0.79	0.0024*	-0.22	0.4140

P-values are significantly different at $P < 0.1$.

FIGURES

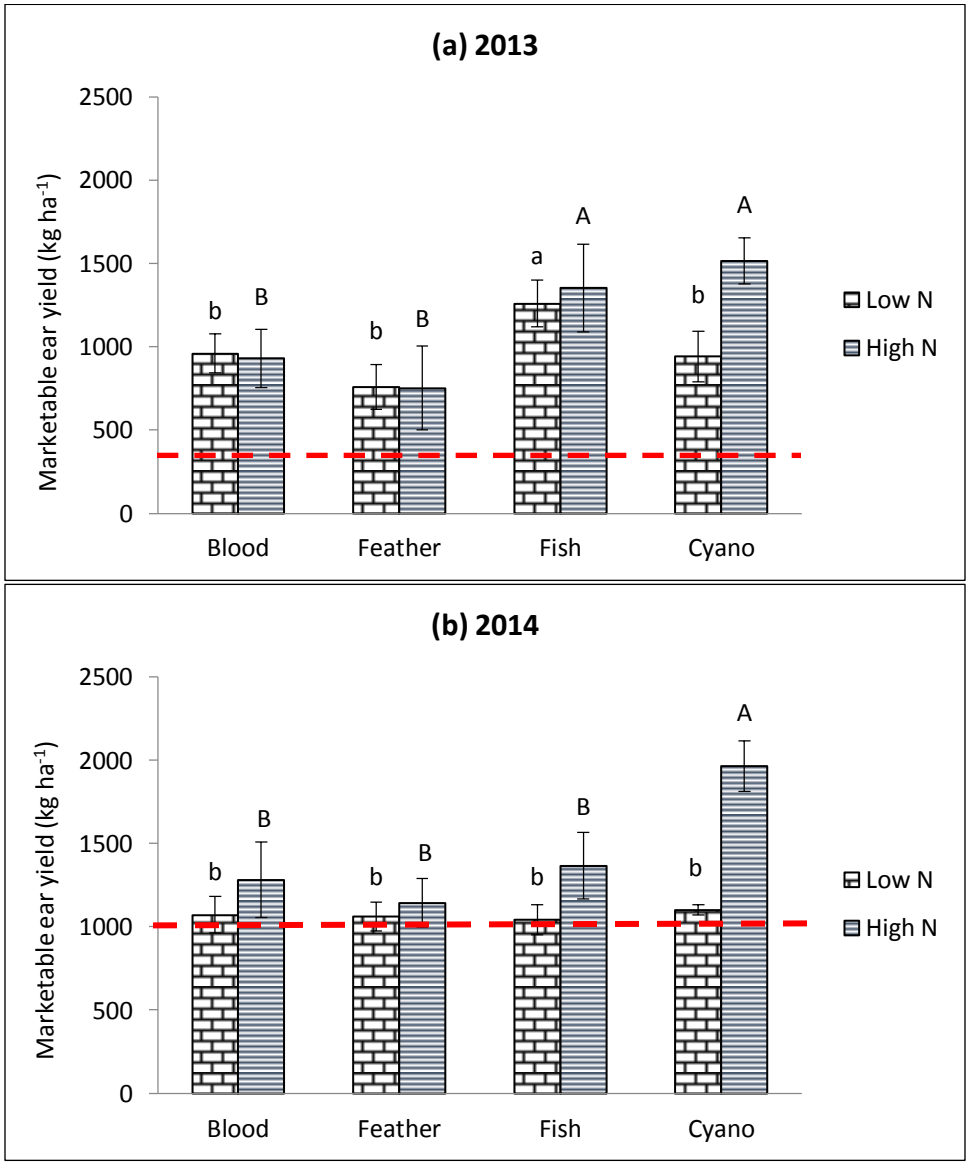

Figure C1**.** Effects of different organic fertilizers on sweet corn marketable ear yield in (a) 2013 and (b) 2014. Fertilizers were applied at 56 kg N ha^{-1} (Low N) and 112 kg N ha^{-1} (High N). Bars represent standard deviation of mean. Means with the same letter are not significantly different at $P < 0.1$ using Tukey's Studentized Range (HSD) test of mean separation. Upper case letters indicate significant differences within mean at the high N rate, while lower case letters indicate significant differences within mean at the low N rate. The dashed line represents control. In 2013, all treatments were significantly different from control at $P < 0.1$. In 2014, the cyano-fertilizer treatment was significantly different from control at the high N rate at $P < 0.1$. Blood = Blood meal; Feather = Feather meal; Fish = Fish emulsion; Cyano = Cyano-fertilizer.

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CHAPTER 4. ORGANIC NITROGEN FERTILIZERS INFLUENCED WATER USE EFFICIENCY IN DRIP IRRIGATED SWEET CORN

4.1 Introduction

Most of Colorado receives less than 50 cm of precipitation per year, and drip irrigation is a practice that reduces water use and increases water use efficiency. Drip irrigation interests vegetable growers because of potential improvements in irrigation efficiency (Doerge et al., 1989).
Water use efficiency (WUE) can be measured on various scales from the leaf to the field (Sinclair and Ludlow, 1984). WUE is a measure of C assimilated per unit of water transpired by the plant (Stanhill, 1986). It can be measured either using a leaf gas exchange (Bunce, 2010) or field-scale approach (Taylor et al., 1983).

Field water use efficiency (fWUE) is defined as the yield produced per unit of water used. Methods of measuring instantaneous WUE (iWUE) using the leaf gas exchange approach are derived from direct measurements of photosynthesis and transpiration as long as plants have similar leaf to atmosphere water vapor concentration gradients under field conditions (Ehleringer et al., 1986).

Fertilizer type has potential to influence WUE. Nitrogen (N) plays a crucial role in crop growth and yield development of sweet corn (*Zea mays*), and its application affects WUE. Organic growers often use commercial organic animal-based fertilizers which vary in nutrient composition, forms of available N (NH_4^+-N and NO_3^--N), and have high transportation costs to meet the N demand of crops. Organic agriculture often relies on fertilizers and soil amendments from off-farm sources to meet crop N demand (Gaskell et al., 2006). There are two main classes of organic N fertilizers, which are solid (compost, animal manure, blood meal, and feather meal) and liquid (fish emulsion). In organic agriculture, solid fertilizers are often incorporated into the soil before planting, while liquid fertilizers are generally applied season-long through irrigation.

All of these organic materials are rich in slow-releasing organic N. While these organic fertilizers are commercially available, many of them are produced off-farm and distributed widely throughout the United States. Farmers usually rely on off-farm sources for additional N needed during the growing season. However, there are other opportunities for fertilizer options being developed that allow farmers to produce N on-farm, such as cultivation of cyanobacteria as cyano-fertilizer. Cyanobacteria have unique dual properties because they can both fix N from the atmosphere and photosynthesize to produce N fertilizer without fossil fuels.

Different organic N fertilizers have different chemical properties that influence plant growth and development and plant-water realtions. Macronutrients; including calcium (Ca) and magnesium (Mg) and micronutrients; including iron (Fe) and zinc (Zn) in organic N fertilizers influenced stomatal regulation, photosynthesis, and osmoregulation in plants (Welch and Shuman, 1995; O'Carrigan et al., 2014). Phytohormones in organic N fertilizers can also influence plant growth and development. Phytohormones play a significant role in regulating plant-water relations (Kang et al., 2012; Gururani et al., 2015). Auxin in the form of Indole-3-acetic acid (IAA) was associated with the increased growth and development of photosynthetic pigments in corn and wheat (Wang and Li, 2007; Agami and Mohamed, 2013). In a study conducted by Balraj et al. (2014), IAA caused a wider and circular structure of eggplants xylem vessels which subsequently increased water transport.

A phenolic compound phytohormone, salicylic acid (SA) increased WUE in rice, mustard, and corn (Moussa and Khodary, 2004; Yusuf et al., 2008; Li et al., 2008; Nazar et al., 2015). The effect of exogenous application of salicylic acid (SA) on growth, photosynthesis, stomatal regulation, and plant water relations have been discussed mostly in corn and soybeans (Barkosky and Einhelling, 1993; Khan et al., 2003; Hayat et al., 2010). However, most of the exogenous application of SA to plants was in the form of foliar application. SA may be related to the induction of antioxidant response and protective role of membranes that increase the tolerance of plant in regulating water use (Yildrim et al., 2008). WUE of broccoli plants increased with SA application by enhancing cell division in broccoli (Mirdad, 2014). Overall, effects of SA in plants depend on its concentration and plant species (Salehi et al., 2011).

Different N forms in fertilizer can induce various effects on plant growth and physiology. Inorganic N forms in fertilizer have been shown to affect WUE of French bean (Guo et al., 2002) and N assimilation in wheat (Duan et al., 2003). Inorganic N forms, particularly NH_4^+-N, decreased WUE and net photosynthetic rate of tea plants (Du et al., 2015), while high NO_3^--N concentration in fertilizers decreased leaf transpiration rate in corn (Wilkinson et al., 2007). NO_3^--N contributes changes to stomatal aperture, increased dry matter accumulation and WUE in tomato compared to NH_4^+-N being used as fertilizer source (Claussen, 2002). When high concentration NH_4^+-N used as the source of fertilizer, leaf area, fresh weight, and WUE of artichoke was lower compared to NO_3^--N (Elia et al., 1996) because of interferences with photosynthetic production of ATP, metabolism of carbohydrates, and amino acid synthesis (Haynes and Goh, 1978).

Many WUE studies have been conducted on N fertilizer effects (Doerge et al., 1989; Du et al., 2015; Guo et al., 2002; Wilkinson et al., 2007), but none of them have compared cyano-fertilizer with other organic fertilizers on sweet corn. There is also no literature describing how inorganic N forms and phytohormone concentrations in fertilizers affect WUE of sweet corn. We hypothesized that (i) phytohormones in organic fertilizers (IAA and SA) will increase WUE and ear yield, (ii) a high percentage of N applied as NH_4^+-N will reduce WUE (fWUE and iWUE) and ear yield of sweet corn, (iii) a high percentage N applied as NO_3^--N will increase transpiration rate and reduce WUE (fWUE and iWUE), and (iv) macronutrients (Ca and Mg) and micronutrients (Fe and Zn) in organic N fertilizers may influence gas exchange components in sweet corn. The overall aim of this study is to assess whether phytohormones and forms of inorganic N in organic fertilizers affect WUE (fWUE and iWUE) and leaf gas exchange components of drip irrigated sweet corn under field conditions.

4.2 Materials and Methods

4.2.1 Experimental site

Field experiments were carried out at the Colorado State University Horticulture Research Center (CSU HRC), Fort Collins, CO. Previously, the field area was planted with buckwheat followed by winter cover crops, rye (*Secale cereal*) and turnips (*Brassica rapa*) . The experimental site is in a semi-arid zone

with clay loam soils. The soil was classified as a fine, smectitic, mesic Aridic Argiustoll of the Nunn series (NRCS, 1980).

4.2.2 Soil analysis

Soil samples were collected to a depth of 30 cm from a representative area of the field area (15 m x 30 m) and analyzed by the Soil, Water, and Plant Testing Laboratory at Colorado State University. Particle size distribution was determined using the hydrometer method based on Stoke's law (Gee and Bauder, 1979). Chemical analyses included soil pH and electrical conductivity (EC) measured in supernatant suspension of 1:1 soil to water using a Mettler Toledo pH/EC meter (Thermo Fischer Scientific, Waltham, MA). Cation exchange capacity (CEC) was determined by summation of the exchangeable bases (Ca^{2+}, K^{+}, Mg^{2+}, Na^{+}) plus hydrogen (H^{+}) using ammonium acetate (1N $NH_4C_2H_3O_2$, pH 7.0) as described by Hajek et al. (1972). Organic matter (OM) content was determined by the loss on ignition method (Blume et al., 1990). Soil inorganic N (NH_4^{+}-N and NO_3^{-}-N) was extracted using 2M KCl, and the filtered extract was analyzed using an Alpkem Flow Solution IV Auto Analyzer (OI Analytical, College Station, TX). Phosphorus (P) was extracted with 0.5M sodium bicarbonate ($NaHCO_3$) solution, which was developed by Olsen et al. (1954) and analyzed using a Perkin Elmer Inductively Coupled Plasma/Optical Emission Spectrometer (ICP-OES) (Perkin Elmer, Waltham, MA). Major cations (K, Ca, and Mg) and micronutrients (Fe and Zn) were extracted with ammonium bicarbonate–diethylene triaminepentaacetic acid (AB-DTPA) (Jones, 2001) and analyzed using a Perkin Elmer Inductively Coupled Plasma/Optical Emission Spectrometer (ICP-OES) (Perkin Elmer, Waltham, MA). The results of soil physical, chemical, and nutrient analyses are presented in Table D1.

4.2.3 Experimental design and plot layout

Treatments consisting of four different types of N fertilizer and control (no fertilizer) were arranged in a Randomized Complete Block Design with four replications. The plot size was 76 cm x 457 cm.

4.2.4 Planting materials

Luscious se^{+} sweet corn seeds were purchased from Johnny's Seeds (Johnny's Selected Seed, Waterville, ME). Seeds were planted at a depth of approximately 2.5 cm. Sweet corn seeds (*Zea mays* var. Luscious F1 se+) were hand planted on July 1, 2013 and July 2, 2014 with an in-row spacing of 12 cm in rows spaced 75 cm apart.

4.2.5 Irrigation

The amount of precipitation was obtained from a nearby CoAgMet weather station (http://www.coagmet.colostate.edu/). Water was applied daily using an automated drip irrigation system. Two lines of John Deere® drip tape (Deere & Co., Moline, IL) with a flow rate of 125 L hour^{-1} 100 m^{-1} were stretched across each row spaced 30 cm apart with a double header in each plot. The drip system used for this study comprised of laterals (15 mm diameter) and emitters were 30 cm apart. Irrigation was applied for 15 min day^{-1} at the initial stage and was changed to 30 min day^{-1} after plants reached the V6-stage (Table D2).

4.2.6 Fertilizer treatments

The fertilizers compared in this study were liquid fertilizers [Alaska® fish emulsion (Planet Natural, Bozeman, MO) and cyano-fertilizer], and solid fertilizers [blood meal and feather meal (Down To Earth Inc., Eugene, OR)]. For this study, N-fixing cyanobacteria (*Anabaena* sp.*)* was cultured from local soils and inoculated into nutrient-supplemented raceways according to the method by Barminski (2014).

4.2.7 Methods of fertilizer application

Both the fish emulsion and cyano-fertilizer were in liquid form and were supplied in four split applications over the growing season, while the blood meal and feather meal were in dry powdered form and incorporated into the soil prior to planting. Blood meal and feather meal were applied 6 cm from the plants and 6 cm deep through band application using a hoe. All fertilizers were applied at 112 kg N ha^{-1}.

4.2.8 Fertilizer analysis

Fertilizer samples were sent to the Soil, Water, and Plant Testing Laboratory at CSU and analyzed for N, P, K, Ca, Mg, Fe, and Zn (Table D3). Fertilizer samples were digested with nitric acid (HNO_3) and hydrogen peroxide (H_2O_2). Major cations (K, Ca, and Mg) and micronutrients (Fe and Zn) were analyzed using a Perkin Elmer Inductively Coupled Plasma/Optical Emission Spectrometer (ICP-OES) (Perkin Elmer, Waltham, MA). Solid fertilizers were extracted using 2M KCl, and the filtered extracts were analyzed for NH_4^+-N and NO_3^--N. The filtered extracts of solid and liquid fertilizers were analyzed using an Alpkem Flow Solution IV Auto Analyzer (OI Analytical, College Station, TX) (Table D4).

4.2.9 Phytohormone analysis

Phytohormone analyses were conducted at the Proteomics and Metabolomics Facility, CSU. Fertilizer samples were adjusted to pH 7.0 with 1N NaOH and extracted three times with water-saturated n-butanol followed by vacuum drying (Pan et al., 2010). The extracts obtained were filtered through membrane filters (pore size 0.45 µm). Supernatants were harvested by centrifugation at 5,000 g for 20 minutes at 4^{o}C. Supernatants were homogenized in liquid nitrogen using a cold mortar and pestle. The resulting powder was extracted using 80% methanol containing 10 mg L^{-1} butylated hydroxytoluene at 4^{o}C. Samples were methylated with diazomethane and dissolved in heptane. Gas chromatography-mass spectrometry (GC-MS) analyses were performed according to Edlund et al (1995). The amount of phytohormones applied over the growing season for each fertilizer treatment is presented in Table D5.

4.2.10 Measurements

4.2.10.1 Leaf gas exchange measurements

Leaf gas exchange measurements of the uppermost, fully expanded leaf from three plants in each plot were carried out during tasseling using a portable open photosynthesis system, LI- 6400XT (LI-COR Inc., Lincoln, NE). The leaf gas exchange components, such as leaf transpiration rate, leaf vapor pressure deficit (VPD), net photosynthetic rate, and leaf temperature were only measured in 2014. Measurements were taken between 10:00 and 15:00 at ambient CO_2 (380 µl L^{-1}), leaf temperature inside the cuvette was

set to 26°C, and photon flux density was 2000 µmol m^{-2} s^{-1}. Relative humidity was monitored and ranged between 50 and 60% during the day. Each leaf was allowed to reach a steady state of CO_2 uptake in the LI-6400XT leaf chamber before measurements were taken.

4.2.10.2 Ear yield, ear dry weight, and kernel number

All above-ground plant parts from each treatment were hand harvested and separated into leaves, stems, and ears at 79 days after planting. The separated plant parts were dried at 70°C for 72 hours and weighed to determine the total above-ground biomass. One ear per treatment of a high degree of kernel filling was selected for kernel number measurement. Kernel number per ear was determined manually by counting the number of kernels per ear. Kernel number was only counted in 2014.

4.2.10.3 Water use efficiency

Field water use efficiency (fWUE) was calculated using ear yield divided by amount of water applied over the growing season (precipitation + irrigation). Instantaneous water use efficiency (iWUE) was only measured in 2014 and calculated as the ratio of net photosynthetic rate to transpiration rate and expressed in µmol CO_2 $mmol^{-1}$ H_2O.

4.2.11 Statistical analysis

Data were analyzed using SAS version 9.3 (SAS Institute Inc., Cary, NC). The Univariate and Boxplot procedures were used to evaluate the normality of data distribution. Analysis of variance (ANOVA) was performed on the data by using the GLM procedure. The Tukey's Studentized Range (HSD) test of mean separation value was calculated from the obtained mean square errors to determine whether main effects or interactions were significant ($P < 0.1$). The relationships between parameters were assessed by linear correlation using the CORR procedure. PROC REG with the stepwise selection method was used to select the best combination of independent variables (gas exchange components, IAA, SA, NH_4^+-N, NO_3^--N, Ca, Mg, Fe, and Zn applied in organic N fertilizers) to predict the dependent variables (fWUE and iWUE).

4.3 Results

4.3.1 Ear yield, dry weight components, and kernel number

Fish emulsion and cyano-fertilizer treatments had a higher fresh yield compared to other treatments and control in 2013 (P = 0.0032; Figure D1a). Only the cyano-fertilizer treatment had a higher yield compared with other treatments and control in 2014 (P <.0001; Figure D1b). No fertilizer effect was observed in the dry weight of leaves or stems (data not shown). A significant fertilizer effect was observed in number of kernels per ear. The feather meal treatment had a lower kernel number compared with other treatments including control in 2014 (Table D6).

4.3.2 fWUE, iWUE, and leaf gas exchange components

The fish emulsion and cyano-fertilizer treatments had a higher fWUE compared with control in 2013 (P = 0.0002; Figure D2a). The cyano-fertilizer treatment had a higher fWUE compared with blood meal and feather meal in 2013 (Figure D2a) and higher than feather meal and fish emulsion treatments in 2014 (P <.0001; Figure D2b). The cyano-fertilizer treatment had a higher iWUE compared with other treatments including control in 2014 (P <.0001; Figure D3).

The blood meal and control treatments had a higher leaf transpiration rate (P <.0001; Figure D4) and leaf VPD (P <.0001; Figure D5) compared with other treatments. The feather meal treatment had a lower net photosynthetic rate (P <.0001; Figure D6) and higher leaf temperature compared with other treatments including control (P = 0.003; Figure D7). Negative correlation was found between amount of IAA applied in organic N fertilizers and leaf VPD (r = -0.49; P = 0.0163; Table D7), which may explain the lowest leaf temperature observed in the fish emulsion treatment (Figure D7). Leaf VPD (r = 0.54; P = 0.0071) and transpiration rate (r =0.53; P = 0.0057) were positively correlated with amount of Fe applied in organic N fertilizers and negative correlated with WUE (Table D7). SA applied in organic N fertilizers was negatively correlated with stomatal conductance (r = -0.79; P = 0.0059). Amount of Ca applied in organic N fertilizers was positively correlated with leaf temperature (r = 0.53; P =0.0082) but negatively correlated with net photosynthetic rate (r = -0.74; P = 0.0056).

The amounts of SA, NH_4^+-N, Ca, and Fe applied in organic N fertilizers were correlated with both iWUE and fWUE in 2014 (Table D7). iWUE was positively correlated with yield (r = 0.61; P = 0.0153). Among other independent variables, including IAA, SA, NH_4^+-N, NO_3^--N, Ca, Mg, and Fe applied in organic N fertilizers, only SA can be used to predict both fWUE and iWUE (Table D8).

4.4 Discussion

4.4.1 Amount of salicylic acid applied influenced fWUE and iWUE

Greater amounts of SA applied in the cyano-fertilizer treatment compared to other treatments (Table D5) influenced fWUE and iWUE (Tables D7 and D8). SA enhances the distribution of photoassimilates and has been shown to increase iWUE in grape (Wang and Li, 2007). SA induced stomatal closure, improved membrane integrity, regulated plant water use and increased iWUE in barley (El-Tayeb, 2005), corn (Rao et al., 2012; Saruhan et al., 2012), and wheat (Kang et al., 2012). Sharafizad et al. (2012) found that SA increased wheat yield due to the role of SA in enhancing cell division and ion transport (Harper and Balke, 1981; Argueso et al., 2012). Ear yield in the cyano-fertilizer treatment was positively correlated with SA applied in organic N fertilizers (r = 0.73; P = 0.0067), thus influencing fWUE.

Among variables analyzed (amount of IAA, SA, NH_4^+-N, NO_3^--N, Ca, Mg, Fe, Zn applied in the fertilizers), SA application and stomatal conductance were the two independent variables influencing iWUE, and only SA application in organic N fertilizers influenced fWUE of sweet corn (Table D8). Although SA has been found to induce stomatal closure in regulating plant water use (Larque-Saavedra 1978; Larque-Saavedra 1979; Nazar et al., 2015; Sharafizad et al., 2012; Yusuf et al., 2008), there was no treatment effect on stomatal conductance (data not shown), although the correlation between SA application and stomatal conductance (r = -0.71; P=0.0059) was significant. Most studies conducted on SA applied to plants evaluated foliar applications. In our study, the cyano-fertilizer was applied directly to the soil through fertigation. Due to different methods of fertilizer application, the mechanism of stomatal closure from SA application may not necessarily be the same, and it may vary across species.

4.4.2 Amount of Ca applied in organic N fertilizers correlated with leaf temperature and net photosynthetic rate

Higher Ca application in the feather meal treatment compared to other fertilizer treatments (Table D4b) may have contributed to the low net photosynthetic rate (Figure D6) and lower kernel number per ear (Table D6) because kernel formation is dependent on leaves as the source of carbohydrates, thus affecting C assimilation, yield and fWUE.

The feather meal treatment had the lowest net photosynthetic rate (Figure D6) and the highest leaf temperature (Figure D7), which may have been due to the higher amount of Ca applied in the feather meal treatment compared to other treatments (Table D4b). Amount of Ca applied in organic N fertilizers was positively correlated with leaf temperature ($r = 0.53$; $P = 0.0082$) and negatively correlated with net photosynthetic rate ($r = -0.74$; $P = 0.0056$), iWUE ($r = -0.62$; $P = 0.0305$), and fWUE ($r = -0.75$; $P = 0.0050$).

A positive correlation between Ca applied in organic N fertilizers with leaf temperature may have been due to the function of Ca in the regulation of stomatal closing in response to external stimuli (Zhang et al., 2014), which influences leaf temperature. Increased leaf temperature in the feather meal treatment may have been due to stomatal closing during the time of measurements between 10:00 to 15:00. If a plant closes its stomata, CO_2 absorption can be inhibited, which in turn can cause decreases in net photosynthetic rate. The net photosynthetic rate was negatively correlated with leaf temperature ($r = -0.65$; $P = 0.0087$). As stomata close to reduce excessive water loss, transpiration is reduced, thus plants cannot dissipate much heat, causing internal leaf temperature to rise, as observed in our study in the feather meal treatment, where an increase in temperature decreased net photosynthetic rate.

4.4.3 Amounts of NH_4^+-N and Fe applied in organic N fertilizers with WUE

Amounts of NH_4^+-N and Fe applied in organic N fertilizers decreased both iWUE and fWUE (Table D7). Fertilizer with high NH_4^+-N concentration decreases fWUE and has been shown to decrease net photosynthetic rate in corn (Lewis et al., 1989; Foyer et al., 1994) due to interferences with photosynthetic production of ATP (Haynes and Goh, 1978; Tabatabaei et al., 2006). It may be possible

that a higher percentage of N applied as NH_4^+-N in the feather meal treatment compared to other fertilizer treatments (Table D4a) decreased water transport in the xylem conduit. Schulze-Till et al. (2009) found that water flow in the xylem conduit was lower in NH_4^+-N (5 mM) treated common bean due to a reduced number of functional xylem conduits.

The amount of Fe applied in organic N fertilizers was positively correlated to leaf VPD ($r = 0.54$; $P = 0.0071$) and transpiration rate ($r = 0.53$; $P = 0.0057$; Table D7), thus decreasing iWUE and fWUE. Among other fertilizer treatments, the blood meal treatment had the highest transpiration rate (Figure D4) and leaf VPD (Figure D5), similar to control. Although the amount of Fe applied in the blood meal treatment was similar to the fish emulsion treatment (Table D4b), the fish emulsion had a lower transpiration and leaf VPD, which may have been due to higher indole-3-acetic acid (IAA) applied in the fish emulsion treatment, since IAA application was negatively correlated with leaf VPD ($r = -0.49$; $P = 0.0163$; Table D7). Increased Fe applied in organic N fertilizers has been shown to increase transpiration rate of peach leaves due to physical alterations of stomatal opening (Eichert et al., 2010). Borowski and Michalek (2011) found that increased Fe application in organic N fertilizers resulted in increased transpiration rate and Fe translocation in plants, which affected photosynthetic pigments and gas exchange rates of French bean.

4.5 Conclusion

In conclusion, we found that the amounts of phytohormones, Ca, and Fe applied in organic N fertilizers may have affected fWUE, iWUE, kernel number, and leaf gas exchange components of sweet corn. We conclude that organic growers could increase WUE by fertigating their crops with cyano-fertilizer. Cyano-fertilizer apparently had a higher WUE due to the high amount of SA applied. A positive relationship was observed between the amount of SA applied and both iWUE and fWUE. The amount of Fe applied in organic N fertilizers showed a positive correlation with leaf VPD and transpiration rate. The amount of Ca applied in the feather meal treatment may have contributed to increasing leaf temperature and decreasing net photosynthetic rate. The amount of NH_4^+-N and Ca applied in the feather meal treatments were negatively correlated with both iWUE and fWUE. Further research needs to be

conducted to understand the mechanisms through which phytohormone, macronutrients, and micronutrients in organic N fertilizers regulate water transport in plants, particularly horticultural crops.

TABLES

Table D1. Initial soil properties of the 0-30 cm soil depth of the sweet corn experimental site.

Soil properties	2013	2014
pH[¶]	7.5	7.7
Electrical conductivity[¶] (dS m^{-1})	0.6	0.6
Cation exchange capacity, CEC[ǂ] (meq 100 g^{-1})	29	30
Organic matter, OM[#] (%)	2.5	2.6
NH_4^+-N[##] (ppm)	2.5	2.7
NO_3^--N[##] (ppm)	1.9	2.1
P[§] (ppm)	30	32
K[♯] (ppm)	463	456
Ca[♯] (ppm)	4560	4650
Mg[♯] (ppm)	616	622
Fe[♯] (ppm)	6	7
Zn[♯] (ppm)	1.4	1.6

[¶]pH and electrical conductivity were determined in water (1:1).
[ǂ]CEC was determined using ammonium acetate (1N $NH_4C_2H_3O_2$, pH 7.0) extraction.
[#]OM was determined by the loss on ignition method.
[##]Samples were extracted using 2M KCl.
[§]Sample was extracted with 0.5M $NaHCO_3$.
[♯]Samples were extracted using ammonium bicarbonate diethylenetriamine pentaacetate (AB-DTPA).

Table D2. Average monthly precipitation and the amount of water applied during growing season in 2013 and 2014 in sweet corn.

	2013	2014
Planting	July 2	July 1
Harvest	September	September 19
Precipitation (mm)		
July	39	69
August	13	26
September	119	9
Amount of water applied (mm)		
Irrigation	729	737
Fertigation	414	414
Total (mm)	1314	1257

[ǂ]Total amount of water applied over the growing season is the combination of precipitation, irrigation, and fertigation. All plots received the same amount of water applied via irrigation. When liquid fertilizers were applied, the control and solid fertilizer treatments were irrigated with the same amount of water as that used to fertigate the liquid fertilizer treatments.

Table D3. Nutrient analysis of fertilizer samples.

	N[ǂ]	P[♯]	K[♯]	Fe[♯]	Ca[♯]	Mg[♯]	Zn[♯]
	%	----------mg kg^{-1}----------					
Cyano-fertilizer	0.2	6	0.1	6	10	18	0.1
Fish emulsion	5	1600	20510	131	725	921	18
Feather meal	13	640	1776	62	1466	575	18
Blood meal	13	32	366	118	904	283	14

[ǂ]Samples were analyzed using CN analyzer.
[♯]Samples were digested with HNO_3 and H_2O_2 and analyzed using ICP-OES.

Table D4a. Inorganic N concentration and percentage of N applied as inorganic-N of fertilizers.

	NH_4^+-N[¶]	NO_3^--N[¶]	N as NH_4^+-N	N as NO_3^--N
	----------mg kg^{-1}----------		----------%----------	
Cyano-fertilizer	4.7	0.01	0.24	0.0005
Fish emulsion	23.7	0.12	0.05	0.0002
Feather meal	1232	2.30	0.95	0.002
Blood meal	27.7	8.40	0.02	0.006

[¶]Samples were extracted using 2M KCl and were analyzed using autoanalyzer.

Table D4b. Amount of Ca, Mg, Fe, and Zn applied in the fertilizers over the course of growing season.

	Ca	Mg	Fe	Zn
	----------kg ha^{-1}----------			
Cyano-fertilizer	1.1×10^{-3}	2.0×10^{-3}	6.7×10^{-4}	1.1×10^{-5}
Fish emulsion	8.1×10^{-2}	1.0×10^{-1}	1.5×10^{-2}	2.0×10^{-3}
Feather meal	1.6×10^{-1}	6.4×10^{-2}	6.9×10^{-3}	2.0×10^{-3}
Blood meal	1.0×10^{-1}	3.2×10^{-2}	1.3×10^{-2}	1.6×10^{-3}

Table D5. Amount of phytohormones applied in the fertilizer over the growing season.

	Indole-3-acetic acid (IAA)[‡]	Salicylic acid (SA)[‡]
	----------kg ha^{-1}----------	----------kg ha^{-1}----------
Cyano-fertilizer	4.4×10^{-5}	4.8×10^{-3}
Fish emulsion	2.4×10^{-3}	1.2×10^{-4}
Feather meal	1.5×10^{-4}	1.5×10^{-4}
Blood meal	3.9×10^{-5}	3.4×10^{-5}

[‡]Amount of phytohormone applied was calculated as the mass of fertilizer applied over the growing season multiplied by the phytohomone concentration analyzed in fertilizers. Fertilizer samples were extracted in 80% methanol containing 10 mg L^{-1} butylated hydroxytoluene at 4°C.

Table D6. Kernel number of sweet corn at harvest in 2014. Means with the same letter are not significantly different at $P < 0.1$ using Tukey's Studentized Range (HSD) test of mean separation.

	Kernel number (ear^{-1})
Control	652 a
Blood meal	739 a
Feather meal	582 b
Fish emulsion	711 a
Cyano-fertilizer	654 a

Table D7**.** Pearson correlation coefficients on amount of Fe, Zn, Ca, Mg, indole-3-acetic acid (IAA), and salicylic acid (SA) applied in the fertilizers (blood meal, feather meal, fish emulsion, and cyano-fertilizer) with gas exchange rates components, instantaneous water use efficiency (iWUE), and field-scale water use efficiency (fWUE) in 2014.

	Lf temp.	Lf VPD	gs	Tr	Pn	iWUE	fWUE
Lf temp.	1.0000	0.019	-0.033	-0.69	-0.65	-0.73	-------
Lf VPD	0.019	0.9720 1.0000	0.9507 0.98	0.1226 0.54	0.0087* 0.39	0.0994 -0.53	-------
gs	0.9720 -0.033	0.98	0.0005* 1.0000	0.0041* 0.44	0.1058 -0.91	0.0440* -0.25	-------
Tr	0.9507 -0.69	0.0005* 0.34	0.44	0.3812 1.0000	0.0118* -0.039	0.6271 0.074	-------
Pn	0.1226 -0.24	0.5041 0.39	0.3812 -0.91	-0.039	0.9419 1.0000	0.8888 0.24	-------
iWUE	0.6420 -0.73	0.2058 -0.23	0.0118* -0.25	0.9419 0.074	0.24	0.6406 1.0000	0.52
IAAapp	0.0994 -0.14	0.6540 -0.49	0.6271 -0.42	0.8888 0.42	0.6406 0.70	-0.32	0.0476* -0.32
SAapp	0.7959 0.078	0.0163* 0.14	0.4080 -0.79	0.4051 0.090	0.1204 0.074	0.5306 0.71	0.2208 0.68
NO3app	0.8841 -0.022	0.7807 -0.57	0.0059* -0.51	0.1204 0.29	0.8894 0.74	0.0126* -0.37	0.0040* 0.64
NH4app	0.9668 0.59	0.2328 -0.59	0.3063 -0.52	0.5839 -0.46	0.0932 0.41	0.4727 -0.49	0.0082* -0.65
Caapp	0.2112 0.53	0.2166 0.02	0.2936 0.17	0.3602 0.08	0.4159 -0.74	0.0212* -0.62	0.0066* -0.75
Mgapp	0.0082* -0.35	0.9912 -0.20	0.5995 -0.08	0.8123 -0.28	0.0056* -0.34	0.0305* -0.23	0.0050* -0.75
Feapp	0.2612 -0.42	0.5369 0.54	0.8134 0.43	0.5650 0.53	0.2938 0.12	0.4679 -0.64	0.0046* -0.69
Znapp	0.1729 0.04	0.0071* 0.09	0.1569 0.19	0.0057* 0.14	0.7018 -0.48	0.0238* -0.31	0.0122* -0.38
	0.9012	0.7721	0.5329	0.6812	0.2134	0.2339	0.3002

*P-values are significantly different at $P < 0.1$.

Lf temp. = leaf temperature; Lf VPD = leaf vapor pressure deficit; gs = stomatal conductance; Tr = leaf transpiration rate; Pn = net photosynthetic rate; iWUE = instantaneous water use efficiency; IAAapp = Indole-3-acetic acid application; SAapp = Salicylic acid application; NO3app = NO_3^--N application; NH4Napp = NH_4^+-N application, Caapp = Calcium application, Mgapp = Magnesium application, Feapp = Iron application, and Znapp = Zinc application. The symbol -------- indicates that no leaf gas exchange data was recorded with field scale WUE.

Table D8. A stepwise regression model of field water use efficiency (fWUE) and instantaneous water use efficiency (iWUE). Variables analyzed were amount of IAA, SA, NH_4^+-N, NO_3^--N, Ca, Mg, Fe, and Zn applied in the fertilizers to predict fWUE and iWUE. All variables left in the model are significant at the 0.05 level.

	Estimate	P-value	Model
Field water use efficiency (fWUE)			fWUE = 32.7 + 1652SA
r^2 =0.46			
Intercept	32.7	<.0001	
Salicylic acid application, SA	1652	0.0040	
Instantaneous water use efficiency			iWUE = 18.4 -54gs + 642SA
r^2 = 0.84			
Intercept	18.4	<.0001	
Stomatal conductance, gs	-54	0.0035	
Salicylic acid application, SA	642	0.0044	

FIGURES

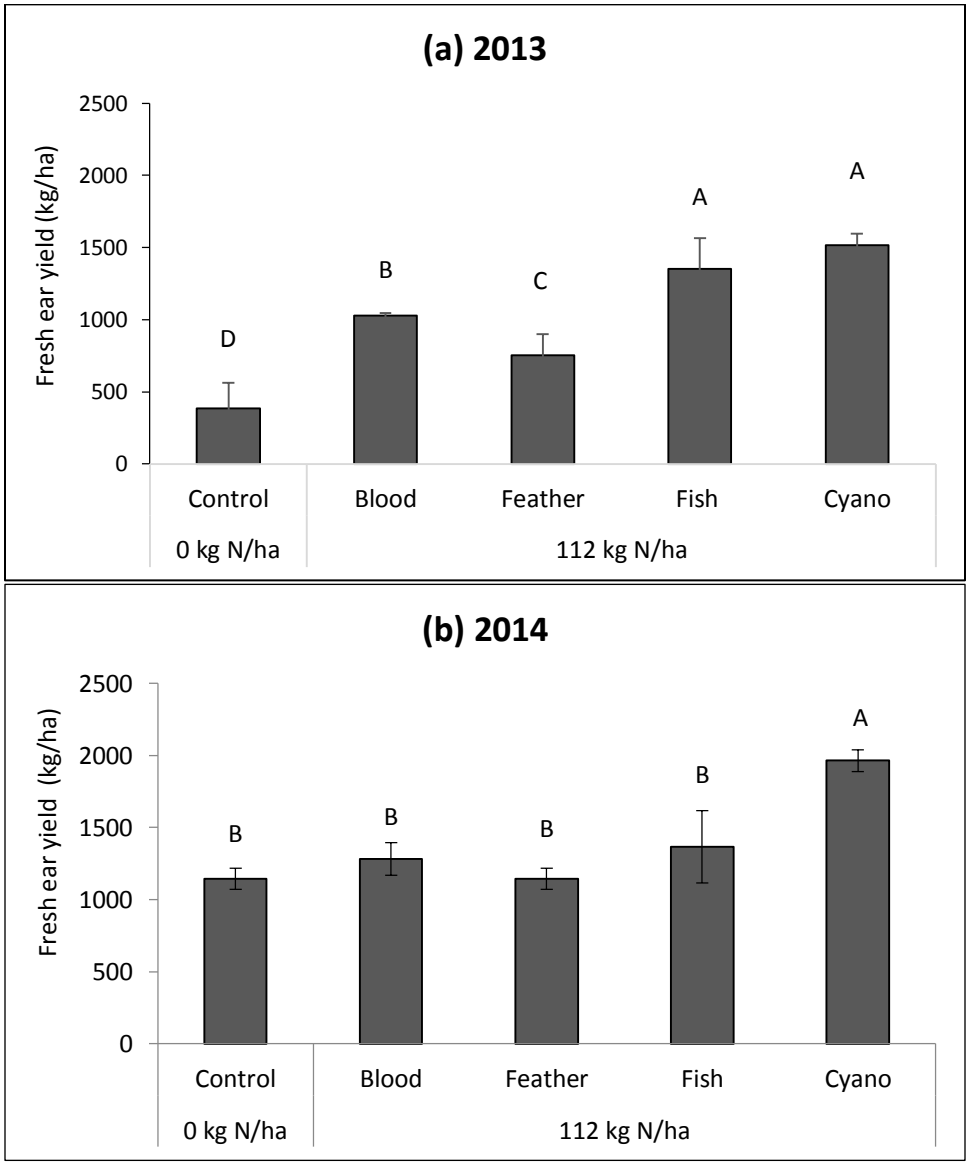

Figure D1. Effects of different organic fertilizers on fresh ear yield of sweet corn at harvest in (a) 2013 and (b) 2014. Bars represent the standard error of mean. Means with the same letter are not significantly different at $P < 0.05$ using Tukey's Studentized Range (HSD) test of mean separation. Blood = Blood meal; Feather = Feather meal; Fish = Fish emulsion; Cyano = Cyano-fertilizer.

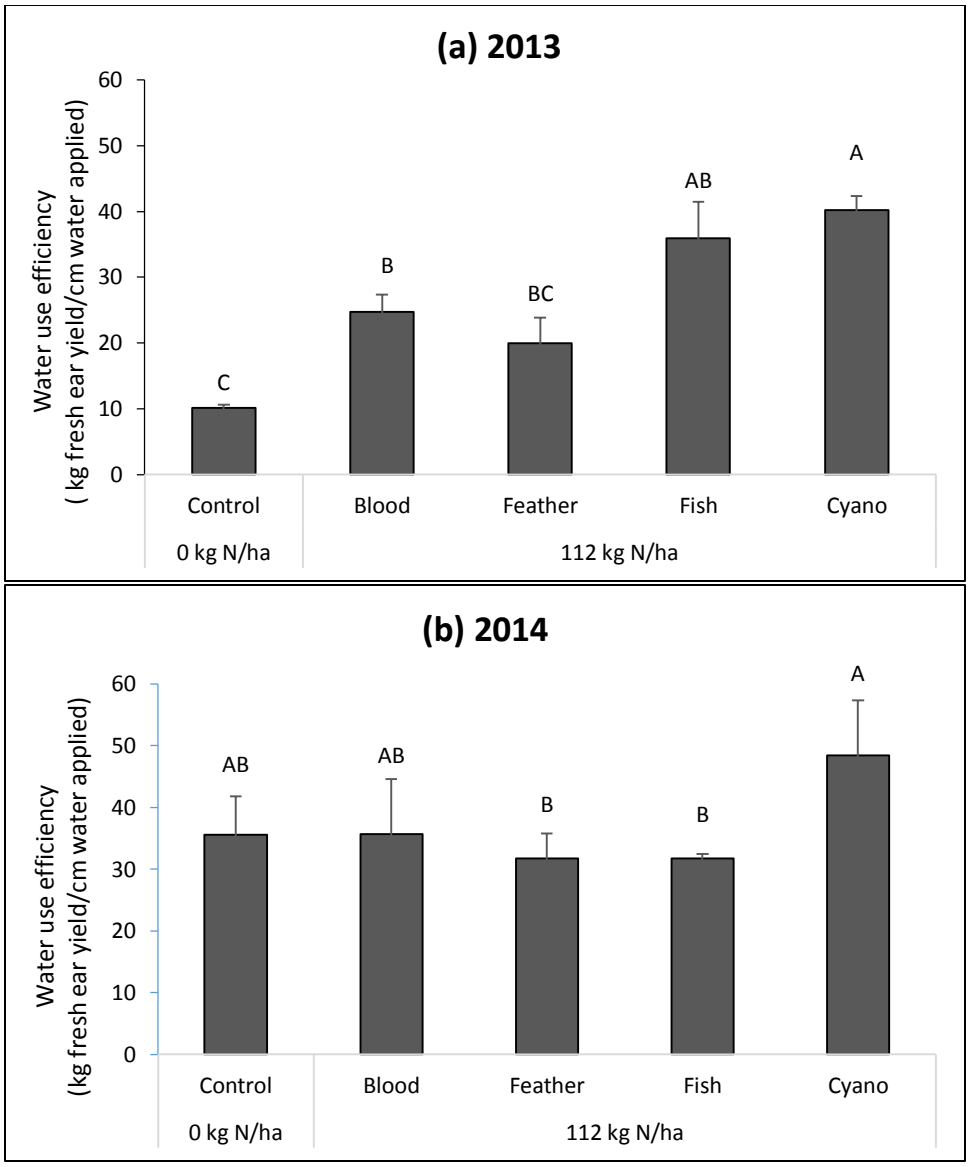

Figure D2. Effects of different organic fertilizers on field-scale water use efficiency (fWUE) at harvest in (a) 2013 and (b) 2014. Bars represent the standard error of mean. Means with the same letter are not significantly different at $P < 0.05$ using Tukey's Studentized Range (HSD) test of mean separation. Blood = Blood meal; Feather = Feather meal; Fish = Fish emulsion; Cyano = Cyano-fertilizer.

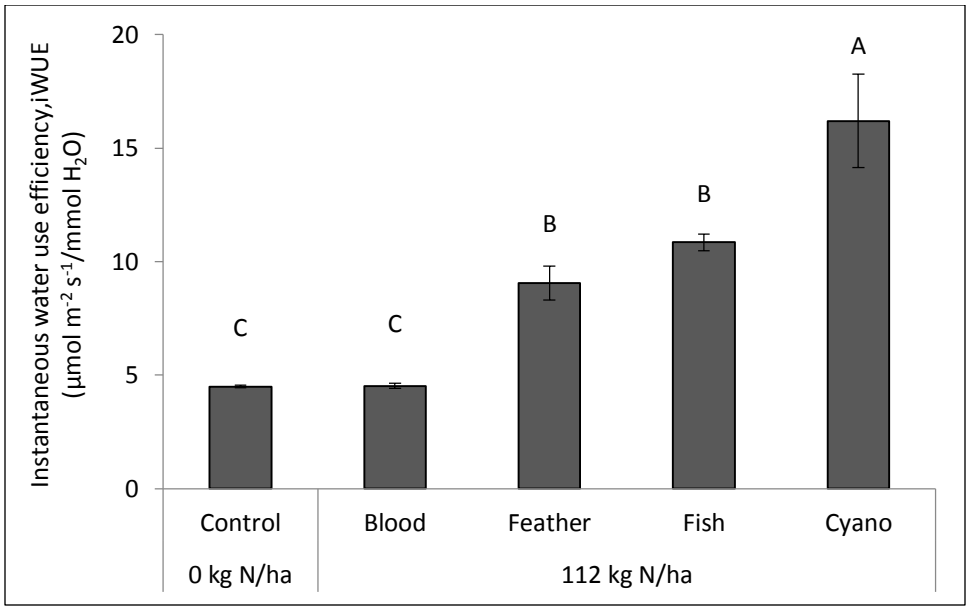

Figure D3. Effects of different organic fertilizers on instantaneous water use efficiency (iWUE) at tasseling in 2014. Bars represent the standard error of mean. Means with the same letter are not significantly different at $P < 0.05$ using Tukey's Studentized Range (HSD) test of mean separation. Blood = Blood meal; Feather = Feather meal; Fish = Fish emulsion; Cyano = Cyano-fertilizer.

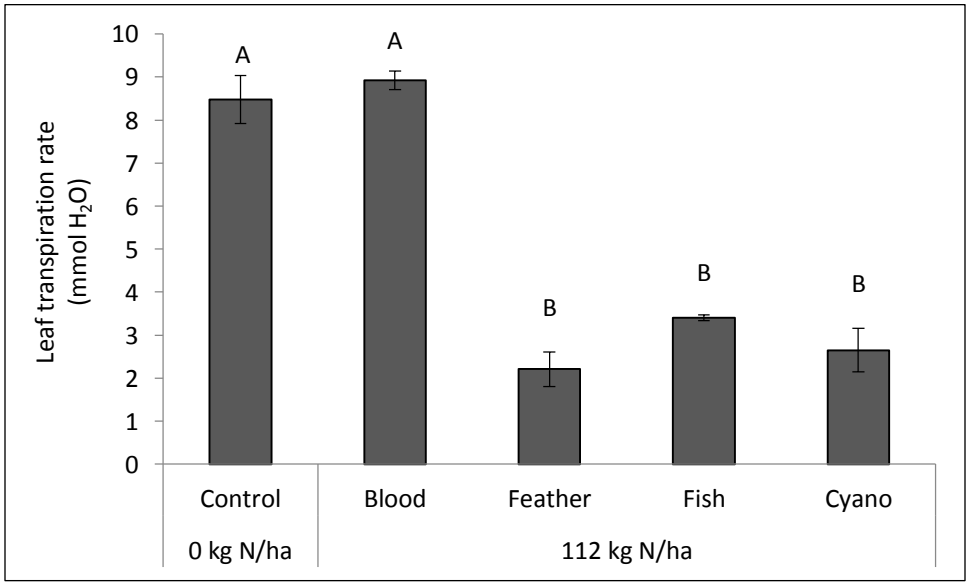

Figure D4. Effects of different organic fertilizers on leaf transpiration rate at tasseling in 2014. Bars represent the standard error of mean. Means with the same letter are not significantly different at $P < 0.05$ using Tukey's Studentized Range (HSD) test of mean separation. Blood = Blood meal; Feather = Feather meal; Fish = Fish emulsion; Cyano = Cyano-fertilizer.

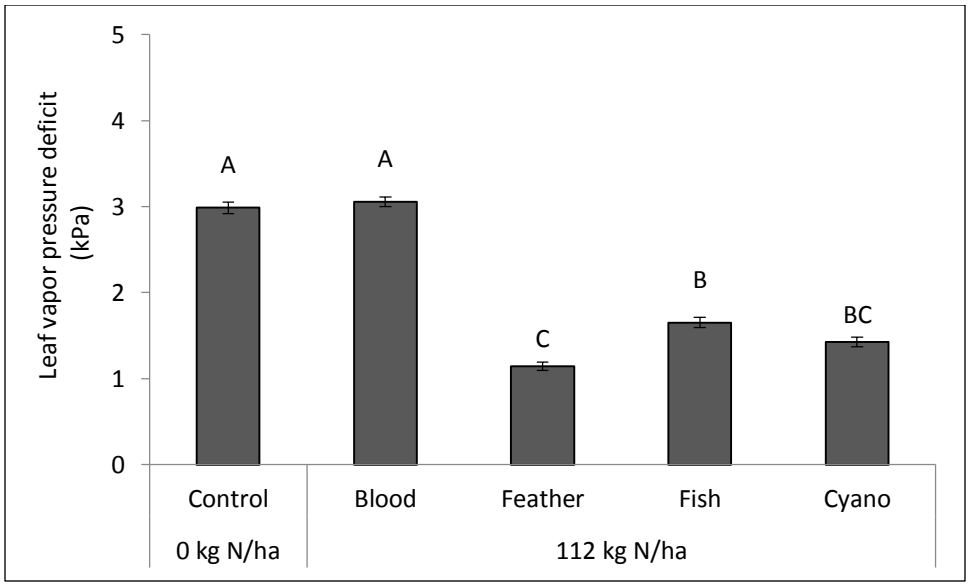

Figure D5. Effects of different organic fertilizers on leaf vapor pressure deficit (VPD) at tasseling in 2014. Bars represent the standard error of mean. Means with the same letter are not significantly different at $P < 0.05$ using Tukey's Studentized Range (HSD) test of mean separation. Blood = Blood meal; Feather = Feather meal; Fish = Fish emulsion; Cyano = Cyano-fertilizer.

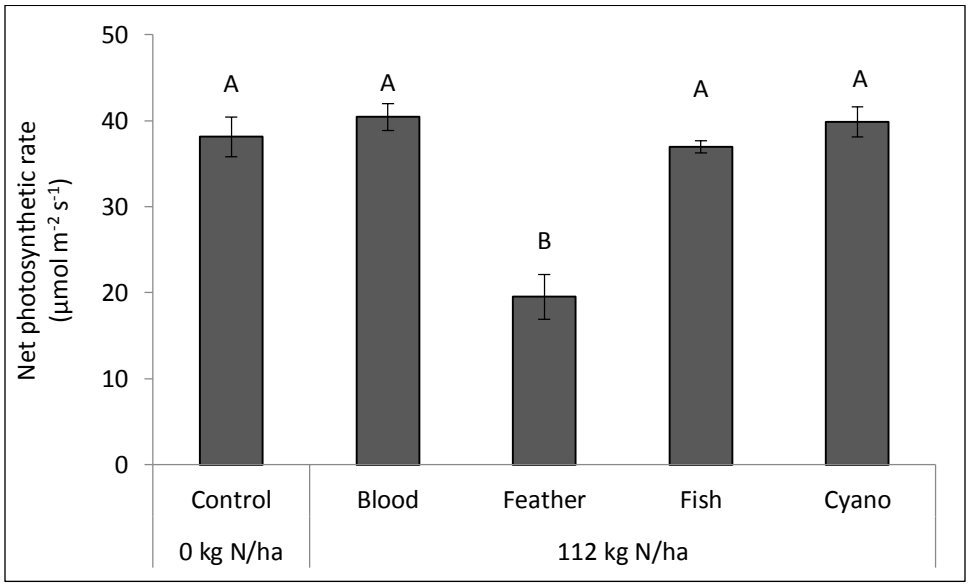

Figure D6. Effects of different organic fertilizers on net photosynthetic rate at tasseling in 2014. Bars represent the standard error of mean. Means with the same letter are not significantly different at $P < 0.05$ using Tukey's Studentized Range (HSD) test of mean separation. Blood = Blood meal; Feather = Feather meal; Fish = Fish emulsion; Cyano = Cyano-fertilizer.

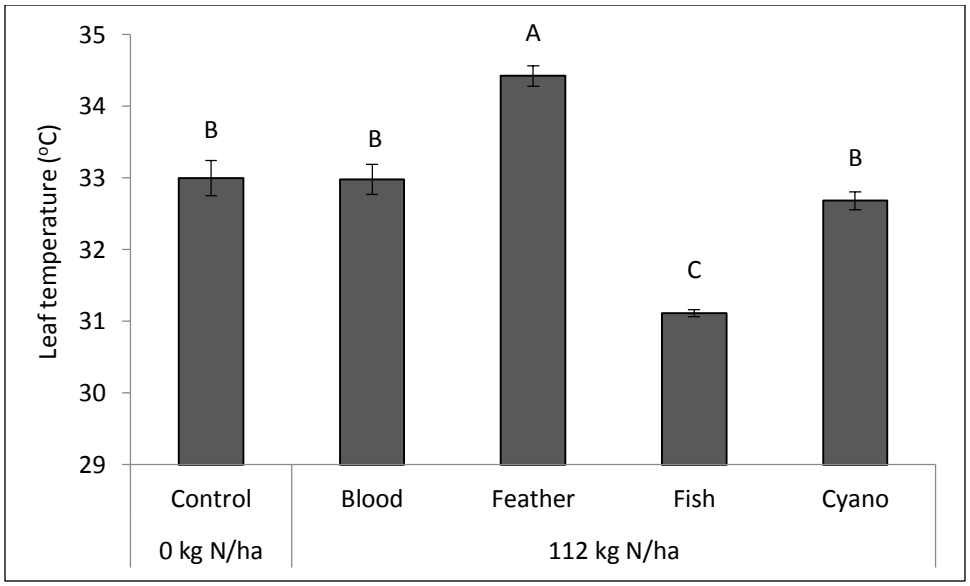

Figure D7. Effects of different organic fertilizers on sweet corn on leaf temperature at tasseling in 2014. Bars represent the standard error of mean. Means with the same letter are not significantly different at P < 0.05 using Tukey's Studentized Range (HSD) test of mean separation. Blood = Blood meal; Feather = Feather meal; Fish = Fish emulsion; Cyano = Cyano-fertilizer.

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CHAPTER 5. ORGANIC NITROGEN FERTILIZER APPLICATION RATE AFFECTS MARKETABLE YIELD, NITROGEN USE EFFICIENCY, AND RESIDUAL SOIL NO_3^--N OF DRIP IRRIGATED LETTUCE

5.1 Introduction

Organic vegetable production relies on fertilizers such as compost, fish emulsion, blood meal, and feather meal to meet crop N demand (Gaskell et al., 2010). There are two main classes of N fertilizers, solid and liquid. In organic agriculture, solid fertilizers are often incorporated into the soil before planting, while liquid fertilizers are generally applied over the growing season through irrigation. In North America, increasing fertilizer costs increase the economic need to maximize NUE and avoid over application of N fertilizers (Hong et al., 2007), thus also leading to reduced environmental risk.

When applied N fertilizer plus mineralized soil N exceed crop needs, this may lead to the accumulation of residual soil NO_3^-N (Mitsch et al., 2001). Post-harvest residual soil NO_3^-N is affected by N rate, timing of application, and precipitation. These factors influence the potential N loss of NO_3^-N within the soil profile. Improper management of N fertilizers can contaminate ground water with NO_3^--N (Aneja et al., 2003).

Optimizing N use efficiency (NUE) is important to reduce N losses to groundwater and to supply an adequate amount of plant-available N for crops (Thorup-Kristensen, 2001), especially shallow-rooted crops such as lettuce. Lettuce (*Lactuca sativa*) ranks second in cultivation among all vegetables produced for consumption in the United States (Coelho et al., 2005). Lettuce responds to abiotic and biotic factors within its growing environment even though lettuce has a shallow root zone (Knight et al., 2013). The rooting zone of lettuce is commonly concentrated within the 0-15 cm depth (Coelho et al., 2005; Althaus et al., 2009).

Adoption of drip irrigation systems minimizes water consumption for lettuce cultivation. Lettuce is irrigated frequently with small volumes of water applied on a daily basis to maintain the shallow root

zone in a moist condition (Althaus et al., 2009). The small volume of water applied could reduce the problem of deep drainage within the soil profile, thus potentially reducing NO_3^--N leaching below the root zone.

Many studies have been conducted on N response of crops to organic fertilizers, but none of them have compared cyano-fertilizer with commonly-used, animal-based organic fertilizers. The main objectives of this study were to evaluate the effects of N fertilizer source and rate on marketable yield, N uptake, NUE, and residual soil inorganic-N under field conditions. We hypothesized that the amount of N applied as NO_3^--N in organic N fertilizers would influence residual soil NO_3^--N in deeper depths.

5.2 Materials and Methods

5.2.1 Experimental site

Field experiments were carried out at the Colorado State University Horticulture Research Center, Fort Collins, CO. Previously, the field area was planted with winter cover crops of rye (*Secale cereal*) and turnips (*Brassica rapa*). The experimental site is in a semi-arid zone with clay loam soils. The soil at the research area was classified as a fine, smectitic, mesic Aridic Argiustoll (NRCS, 1980).

5.2.2 Soil analysis

Soil samples were collected to a depth of 30 cm from the representative area of the field area (15 m x 30 m) and analyzed by the Soil, Water, and Plant Testing Laboratory at Colorado State University. Particle size distribution was determined using the hydrometer method based on Stoke's law (Gee and Bauder, 1979). Chemical analyses included soil pH and electrical conductivity (EC) measured in supernatant suspension of 1:1 soil to water using a Mettler Toledo pH/EC meter (Thermo Fischer Scientific, Waltham, MA). Cation exchange capacity (CEC) was determined by summation of the exchangeable bases (Ca^{2+}, K^+, Mg^{2+}, Na^+) plus hydrogen (H^+) using ammonium acetate (1N $NH_4C_2H_3O_2$, pH 7.0) as described by Hajek et al. (1972). Organic matter (OM) content was determined by the loss on ignition method (Blume et al., 1990). Soil inorganic-N (NH_4^+-N and NO_3^--N) was extracted using 2M KCl, and the filtered extract was analyzed using an Alpkem Flow Solution IV Auto Analyzer (OI Analytical, College Station, TX). Phosphorus (P) was extracted with 0.5M sodium bicarbonate ($NaHCO_3$)

solution, which was developed by Olsen et al. (1954) and analyzed using a Perkin Elmer Inductively Coupled Plasma/Optical Emission Spectrometer (ICP-OES) (Perkin Elmer, Waltham, MA). Major cations (K, Ca, and Mg) and micronutrients (Fe and Zn) were extracted with ammonium bicarbonate–diethylene triaminepentaacetic acid (AB-DTPA) (Jones, 2001) and analyzed using a Perkin Elmer Inductively Coupled Plasma/Optical Emission Spectrometer (ICP-OES) (Perkin Elmer, Waltham, MA) (Table E1).

5.2.3 Experimental design and plot layout

Treatments consisted of four different types of N fertilizer at two N rates and a no fertilizer control. Thirty-six experimental plots were arranged in a Randomized Complete Block design with four replications. The plot size for each treatment was 76 cm x 457 cm. The entire experimental area was 15 m x 30 m.

5.2.4 Planting materials

Lactuca sativa var. 'Concept' seeds (Johnny's Selected Seeds, Waterville, ME) were planted in 72-cell trays containing a well-mixed Sunshine® organic potting soil mix (SunGro Horticulture, Agawam, MA) in the first week of May 2013 and the first week of May 2014. All seeds were started at the Plant Environmental Research Center's (PERC) greenhouse facility on the CSU campus. After four weeks, seedlings were transplanted to the field in the first week of June of 2013 and 2014.

5.2.5 Irrigation

Two drip tapes (John Deere® & Co., Moline, IL) with a flow rate of 125 L $hour^{-1}$ 100 m^{-1} were stretched across each plot spaced 30 cm apart with a double header in each plot. Water was applied daily at 08:00 to 08:30 using an automated drip irrigation system. The drip system used for this study comprised of laterals (15 mm diameter) with a spacing of 30 cm between inline emitters. Irrigation was applied for 15 min day^{-1} at the initial stage and was changed to 30 minutes day^{-1} at 4^{th} leaf stage. The amount of precipitation and amount of irrigation water applied over the growing season is presented in Table E2. Weather data was obtained from a nearby CoAgMet weather station (http://www.coagmet.colostate.edu/).

5.2.6 Fertilizer treatments

The fertilizers compared in this study were liquid fertilizers [Alaska® fish emulsion (Planet Natural, Bozeman, MO) and cyano-fertilizer], and solid fertilizers [blood meal and feather meal (Down To Earth Inc., Eugene, OR)]. The N-fixing cyanobacteria (*Anabaena* sp.*)* was cultured from local soils and grown in nutrient-supplemented raceways according to the method by Barminski (2014).

5.2.7 Methods of fertilizer application

Fertilizers were applied at 56 and 112 kg N ha^{-1} in 2013, while rates of 28 and 56 kg N ha^{-1} were applied in 2014. Both the fish emulsion and cyano-fertilizer were in liquid form and were supplied in four split applications via fertigation over the growing season. The blood meal and feather meal were in dry powdered form and were incorporated into the soil prior to planting. Blood meal and feather meal were applied 6 cm from the rooting zone x 6 cm depth through sub-surface band application next to treatment rows using a hoe.

5.2.8 Fertilizer analysis

Fertilizer samples were sent to the Soil, Water, and Plant Testing Laboratory at CSU and analyzed for N, P, K, Ca, Mg, Fe, and Zn (Table E3). Fertilizer samples were digested with nitric acid (HNO_3) and hydrogen peroxide (H_2O_2). Major cations (K, Ca, and Mg) and micronutrients (Fe and Zn) were analyzed using a Perkin Elmer Inductively Coupled Plasma/Optical Emission Spectrometer (ICP-OES) (Perkin Elmer, Waltham, MA). Solid fertilizers were extracted using 2M KCl, and the filtered extracts were analyzed for NH_4^+-N and NO_3^--N. The filtered extracts of solid fertilizers and the filtered liquid fertilizers were analyzed using an Alpkem Flow Solution IV Auto Analyzer (OI Analytical, College Station, TX) (Table E4).

5.2.9 Measurements

5.2.9.1 Marketable yield

Lettuce was harvested 39 days in 2013 and 57 days in 2014 after transplanting. The lettuce crops were damaged by a hailstorm on June 22, 2014 resulting in a setback and subsequent longer growing period. Once harvested, brown leaves were stripped and removed to obtain a fresh green marketable yield

and the marketable lettuce heads were weighed. All harvested samples were washed with deionized water before oven-drying for elemental analysis. The harvested samples from each subplot were oven-dried at the Agriculture Research Development & Education Center (ARDEC) at 72^{o}C for 72 hours. The dried samples were weighed and then ground to pass a 1.0-mm screen (20 mesh) using a plant tissue grinder (Thomas Wiley®, Swedesboro, NJ). Sub-samples were then taken from each sample for further analysis.

5.2.9.2 Leaf N concentration, N content, and N use efficiency

Total N of plant tissues was measured using a LECO CN analyzer (Leco Corp., St. Joseph, MI). Samples were digested with nitric acid (HNO_3) and hydrogen peroxide (H_2O_2). Elemental measurements were made using a Perkin-Elmer Inductively Coupled Plasma/Optical Emission Spectrometer (ICP-OES) (Perkin-Elmer, Waltham, MA). Tissue nutrient concentrations were multiplied by leaf dry weights to determine the N content. Nitrogen use efficiency (NUE) was calculated by dividing marketable yield by the amount of N fertilizer applied.

5.2.9.3 Residual soil inorganic-N

Residual soil inorganic-N was sampled in the 0-30 cm depth in 2013, and sampling was extended to include the 30-60 cm depth in 2014. After harvest, all sub-plots were soil sampled at two depths (0-30 cm and 30-60 cm) using a Giddings soil sampler drill rig (Purple Wave, Inc., Windsor, CO). Soil samples were dried for two weeks in a walk-in cooler to reduce NH_4^+-N volatilization. Once dried, inorganic-N was extracted using 2 M KCl at a 1:10 ratio (2 g of soil in 20 mL of 2M KCl). The samples were then filtered and run through an Alpkem Flow Solution IV Auto Analyzer (OI Analytical, College Station, TX) for NH_4^+-N and NO_3^--N.

5.2.10 Statistical analysis

Data were analyzed using SAS version 9.3 (SAS Institute Inc., Cary, NC). Univariate and Boxplot procedures were used to evaluate the normality of data distribution. Analysis of variance (ANOVA) was performed on the data with the MIXED procedure ($P < 0.1$). Fertilizer treatment, soil depth, and N rate were categorized as fixed effect and replication was categorized as random effect. When

treatment or interaction effects were significant, means were separated by Tukey's Studentized Range (HSD) test.

5.3 Results

5.3.1 Residual soil NO_3^--N

Soil NO_3^-N was affected by fertilizer treatment in 2013 (P=0.0159; Table E5), although soil NH_4^+-N was not affected. The blood meal treatment had a higher residual soil NO_3^--N across N rates compared with other treatments including control in 2013 (Table E6). In 2014, residual soil NO_3^-N at the 0-30 cm depth was higher than the 30-60 cm depth (Table E7).

5.3.2 Marketable yield and N use efficiency

Effect of N rate was observed in marketable yield and NUE of lettuce in 2013 and 2014 (Table E8). Marketable yield and NUE were not affected by fertilizer treatment in either year. No treatment or N rate effects were observed in leaf N concentration or N content in either year. Higher marketable yield compared to control was observed at 112 kg N ha^{-1} in 2013 ($P < 0.1$) and at 28 kg N ha^{-1} in 2014 (Table E9). The marketable yield in 2013 and 2014 was between the range of 900-1050 kg ha^{-1} and 700-1500 kg ha^{-1}, respectively (Table E9). In both years, higher NUE was observed at the lower N rate than the higher N rate ($P < 0.0001$; Table E9). NUE and marketable yield were 1.4 times higher in 2014 than 2013 when applied at 56 kg N ha^{-1}.

5.4 Discussion

5.4.1 Residual soil NO_3^--N and marketable yield

In 2014, higher residual soil NO_3^--N in the top 0-30 cm depth (Table E7) is probably due to the direct contact of fertilizers applied and the role of drip irrigation. In a study conducted on lettuce by Althaus et al. (2009), residual soil NO_3^--N concentration decreased with increasing soil depth, similarly to our study (Table E7). Althaus et al. (2009) reported that deep drainage occurred in lettuce grown under drip irrigation systems during the course of the growing season and that NO_3^--N might have leached below the lettuce root zone.

Lower marketable yield in the control plot in 2014 may have been due to a localized hailstorm on June 22, 2014 resulting in reduced marketable yield harvested in the control treatment in 2014 compared to 2013. The hailstorm occurred when lettuce was at the four to five leaf growth stage, and regrowth was observed within two weeks. Hence, the higher marketable yield observed at 56 kg N ha^{-1} in 2014 compared to 2013 may have been due to a doubling in precipitation in June and July compared to 2013 (Table E2).

5.4.2 N application rate and N use efficiency

In both years, higher NUE was observed at the lower N rate compared with the higher N rate (Table E10). Dobermann (2005) reported that NUE declines with increasing N fertilizer rate; this has been reported for many crops. Annual crops usually produce less than 50 kg of fresh yield per kg of N fertilizer applied (Cassman and Dobermann, 2002), which is in range with the NUE results in our study. Montemurro and Maiorana (2007) reported that lettuce had a higher economic yield at 56 kg N ha^{-1}, although it could produce higher yield at 112 kg N ha^{-1}. The corresponding increase in yield at the high N rate was not economical based on the cost spent for fertilizer application and the value of the crop.

5.5 Conclusion

In both years, N rate effect was only observed in marketable yield and NUE. Blood meal fertilizer had a higher percentage of N applied as NO_3^--N and had a higher residual soil NO_3^--N concentration in 2013. Greater residual soil NO_3^--N was observed in the 0-30 cm depth compared to the 30-60 cm depth in 2014. In vegetable production systems, organic growers commonly apply fertilizers at higher rates than those needed for optimum yield. Organic growers could achieve higher marketable yield and NUE when applying fertilizers at rates between 28 kg N ha^{-1} and 56 kg N ha^{-1} compared with 112 kg N ha^{-1} when growing lettuce on soils with organic matter of above 2%. Further research is required to relate residual NO_3^--N with the depth of lettuce rooting zone and water movement under drip irrigation to be able to investigate NO_3^--N movement through the soil profile throughout the course of a growing season.

TABLES

Table E1. Initial soil properties of the 0-30 cm soil depth of the experimental site in lettuce.

Soil properties	2013	2014
pH[‡]	7.3	7.5
Electrical conductivity[‡] (dS m^{-1})	0.5	0.6
Cation exchange capacity[§] (meq 100 g^{-1})	30	29
Organic matter [#](%)	2.9	2.7
NH_4^+-N[¶] (ppm)	1.9	2.4
NO_3^--N[¶] (ppm)	5.2	4.0
P[‖] (ppm)	26	31
K[ʃ] (ppm)	432	473
Ca[ʃ] (ppm)	4411	4513
Mg[ʃ] (ppm)	622	634
Fe[ʃ] (ppm)	6.5	6.3
Zn[ʃ] (ppm)	1.5	1.6

[‡]pH and electrical conductivity were determined in water (1:1).
[§]CEC was determined using $NH_4C_2H_3O_2$ extraction.
[#]OM was determined by the loss on ignition method.
[¶]Samples were extracted using 2M KCl
[‖]Samples were extracted using 0.5M $NaHCO_3$.
[ʃ]Samples were extracted using ammonium bicarbonate diethylenetriamine pentaacetate (AB-DTPA).

Table E2. Average monthly precipitation and the amount of water applied during growing season in 2013 and 2014 in lettuce.

	2013	2014
Planting	July 1	June 9
Harvest	August 9	August 4
Precipitation (mm)		
June	--	34
July	39	69
August	9	0.3
Water applied (mm)		
Irrigation	210	221
Fertigation	230	230
Total (mm)	488	554

[‡]Total amount of water applied over the growing season is the combination of precipitation, irrigation, and fertigation. All plots received the same amount of water applied via irrigation. When liquid fertilizers were applied, the control and solid fertilizer treatments were irrigated with the same amount of water as that used to fertigate the liquid fertilizer treatments.

Table E3. Nutrient analysis of fertilizer samples.

	N[‡]	P[#]	K[#]	Fe[#]	Ca[#]	Mg[#]	Zn[#]
	%	----------mg kg^{-1}----------					
Cyano-fertilizer	0.2	6	0.1	6	10	18	0.1
Fish emulsion	5	1600	20510	131	725	921	18
Feather meal	13	640	1776	62	1466	575	18
Blood meal	13	32	366	118	904	283	14

[‡]Samples were analyzed using CN analyzer.
[#]Samples were digested with HNO_3 and H_2O_2 and analyzed using ICP-OES.

Table E4. Inorganic N concentration and percentage of N applied as inorganic-N of fertilizers.

	NH_4^+-N¶	NO_3^--N¶	N as NH_4^+-N	N as NO_3^--N
	----------mg kg^{-1}----------		----------%----------	
Cyano-fertilizer	4.7	0.01	0.24	0.0005
Fish emulsion	23.7	0.12	0.05	0.0002
Feather meal	1232	2.30	0.95	0.002
Blood meal	27.7	8.40	0.02	0.006

¶Samples were extracted using 2M KCl and were analyzed using autoanalyzer.

Table E5. Analysis of variance (ANOVA) of soil NO_3^--N in 2013 and 2014.

	2013‡		2014¶	
	F	P > F	F	P > F
Depth	--	--	16.98	0.0001*
N rate	0.50	0.4917	0.33	0.5688
Trt	4.09	0.0159*	2.06	0.1176
Depth x Trt	--	--	3.00	0.1392
N rate x Trt	0.89	0.1244	0.24	0.8697
Depth x N rate x Trt	--	--	0.31	0.8216

‡Soils were sampled from one depth (0-30 cm) in 2013. Soil NH_4^+-N and total soil inorganic ANOVA models were not significant at $P < 0.1$.
¶Soils were sampled from two depths (0-30 cm and 30-60 cm) in 2014.
*P-values are significantly different at $P < 0.1$.

Table E6. Treatment effect on residual soil NO_3^--N in 2013. Means with the same letter are not significantly different at $P < 0.1$ using Tukey's Studentized Range (HSD) test of mean separation.

	Residual soil NO_3^--N (mg kg^{-1})
Cyano-fertilizer	10.3 b
Fish emulsion	8.7 b
Feather meal	17.1 b
Blood meal	33.6 a
Control	5.4 c

Table E7. Depth effect on residual soil NO_3^--N in lettuce in 2014. Means with the same letter are not significantly different at P < 0.1 using Tukey's Studentized Range (HSD) test of mean separation.

	Residual soil NO_3^--N (mg kg^{-1})	
Depth	0-30 cm	30-60 cm
	7.8 a	5.6 b

Table E8. Analysis of variance (ANOVA) of marketable yield and N use efficiency (NUE).

	2013				2014			
Effect	Marketable yield		N use efficiency		Marketable yield		N use efficiency	
	F	P > F	F	P > F	F	P > F	F	P > F
N rate	4.56	0.0508*	2.87	<.0001*	4.34	0.0364*	9.58	<.0001*
Trt	1.75	0.2026	1.15	0.3622	2.00	0.1416	2.14	0.1251
N rate x Trt	0.56	0.6488	0.29	0.8308	1.25	0.3126	1.52	0.2379

*P-values are significantly different at P < 0.1.

Table E9. Lettuce marketable yield and nitrogen use efficiency (NUE) of lettuce in 2013 and 2014. Means within year with the same letter are not significantly different at P < 0.1 using Tukey's Studentized Range (HSD) test of mean separation.

	2013‡	2014¶	2013‡	2014¶
N rate	Marketable yield		Nitrogen use efficiency (NUE)	
(kg ha^{-1})	----------kg ha^{-1}----------		----------kg $yield^{-1}$ kg N applied-------	
0	958 b	779 c	--	--
28	--	1483 a	--	53 a
56	939 b	1269 b	17 a	23 b
112	1018 a	--	9 b	--

‡Fertilizers were applied at 56 and 112 kg N ha^{-1} in 2013.
¶ Fertilizers were applied at 28 and 56 kg N ha^{-1} in 2014.

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CHAPTER 6: INFLUENCE OF ORGANIC NITROGEN FERTILIZER ON RESIDUAL SOIL NO_3^--N AND MARKETABLE YIELD OF DRIP IRRIGATED SWEET CORN

6.1 Introduction

Organic vegetable production relies on fertilizers such as compost, fish emulsion, blood meal, and feather meal to meet crop nitrogen (N) demand (Gaskell and Smith, 2010). There are two main classes of N fertilizers, solid and liquid. In organic agriculture, solid fertilizers are often incorporated into the soil before planting, while liquid fertilizers are generally applied over the growing season through irrigation. The variability in soil N supply is rarely accounted for in current N fertilizer management practices for horticultural crops. Increasing fertilizer costs increase the economic need to avoid over application of N fertilizers (Hong et al., 2007).

When applied N fertilizer plus mineralized soil N exceed crop needs, this may lead to the accumulation of residual soil NO_3^-N (Mitsch et al., 2001). Post-harvest residual soil NO_3^-N is affected by N rate, timing of application, and precipitation. These factors influence the potential N loss of NO_3^-N movement within the soil profile. Improper management of N fertilizer can contaminate groundwater with NO_3^--N (Aneja et al., 2003). Optimizing N use efficiency (NUE) is important to reduce NO_3^--N losses to groundwater and to supply an adequate amount of plant available-N needed for crops (Thorup-Kristensen, 2001). N rates influence crop N uptake demand (Snyder et al., 2010). Synchronizing soil N availability with crop N demand is a particularly challenging with organic N sources because crop N demand is minimal early in the growing season and increases several weeks after emergence (Olson and Kurtz, 1982).

Drip irrigation provides controlled application of water and fertilizer by supplying water and nutrients near the crop roots. Drip irrigation can be used to maintain optimum soil moisture within the crop root zone which may result in better utilization of applied N and improved yield (Ramireddy et al., 1982). Sweet corn root development changes in response to water distribution within the soil profile

(Coelho and Or, 1999). Generally, corn extracts most of its water within the 0-30 cm depth (Panda et al., 2004), as supported by Klepper (1990) who found that roots extract water preferentially from the upper soil layers.

Sweet corn is usually grown for the fresh market, and yield is reported on a fresh weight basis (Heckman, 2007). To achieve optimum yield, sweet corn obtains available-N from the soil at planting and throughout the growing season (Steele et al., 1982). Information on optimum yield of a crop can be used to compare the profitability of fertilizer types and reduce fertilizer production costs. Choosing the type of fertilizer and selecting an optimum N rate over the course of a growing season is important to achieve the best economic returns (Bodnar and Hagerman, 2000).

Many studies have been conducted on N response of crops to organic fertilizers, but none of them have compared cyano-fertilizer with commonly used animal-based organic fertilizers (blood meal, feather meal, and fish emulsion). In this study, our goal was to quantify N response from organic N fertilizers applied to sweet corn. The main objective of this study was (i) to evaluate the effects of N fertilizer source and N application rates on marketable ear yield, residual soil NO_3^--N, N content, and NUE of drip irrigated sweet corn in order to improve fertilizer recommendations for organic sweet corn growers. We hypothesized that amount of N applied as NO_3^--N in organic N fertilizers influences residual soil NO_3^--N and higher residual NO_3^--N observed in deeper depths in sweet corn.

6.2 Materials and Methods

6.2.1 Experimental site

Field experiments were carried out at the Colorado State University Horticulture Research Center (CSU HRC), Fort Collins, CO. Previously, the field area was planted with buckwheat followed by winter cover crops, rye (*Secale cereal*) and turnips (*Brassica rapa*). The experimental site is in a semi-arid zone with clay loam soils. The soil was classified as a fine, smectitic, mesic Aridic Argiustoll of the Nunn series (NRCS, 1980).

6.2.2 Soil analysis

Soil samples were collected to a depth of 30 cm from the representative area of the field area (15 m x 30 m) and analyzed by the Soil, Water, and Plant Testing Laboratory at Colorado State University. Chemical analyses included soil pH and electrical conductivity (EC) measured in supernatant suspension of 1:1 soil to water using a Mettler Toledo pH/EC meter (Thermo Fischer Scientific, Waltham, MA). Cation exchange capacity (CEC) was determined by summation of the exchangeable bases (Ca^{2+}, K^{+}, Mg^{2+}, Na^{+}) plus hydrogen (H^{+}) using ammonium acetate (1N $NH_4C_2H_3O_2$, pH 7.0) as described by Hajek et al. (1972). Organic matter (OM) content was determined by the loss on ignition method (Blume et al., 1990). Soil inorganic-N (NH_4^{+}-N and NO_3^{-}-N) was extracted using 2M KCl, and the filtered extract was analyzed using an Alpkem Flow Solution IV Auto Analyzer (OI Analytical, College Station, TX). Phosphorus (P) was extracted with 0.5M sodium bicarbonate ($NaHCO_3$) solution (Olsen et al., 1954) and analyzed using a Perkin Elmer Inductively Coupled Plasma/Optical Emission Spectrometer (ICP-OES) (Perkin Elmer, Waltham, MA). Major cations (K, Ca, and Mg) and micronutrients (Fe and Zn) were extracted with ammonium bicarbonate–diethylene triaminepentaacetic acid (AB-DTPA) (Jones, 2001) and analyzed using a Perkin Elmer Inductively Coupled Plasma/Optical Emission Spectrometer (ICP-OES) (Perkin Elmer, Waltham, MA). The results of soil physical, chemical, and nutrient analyses are presented in Table F1.

6.2.3 Experimental design and plot layout

Treatments consisted of four different types of N fertilizer and control arranged in a Randomized Complete Block Design with four replications. The plot size was 76 cm x 457 cm.

6.2.4 Planting materials

Luscious se^{+} sweet corn seeds were purchased from Johnny's Seeds (Johnny's Selected Seed, Waterville, ME). Seeds were planted at a depth of approximately 2.5 cm. Sweet corn seeds (*Zea mays* var. Luscious F1 se+) were hand planted on July 1, 2013 and July 2, 2014 with an in-row spacing of 12 cm in rows spaced 75 cm apart.

6.2.5 Irrigation

Two drip tapes (John Deere®, Deere & Co., Moline, IL) with a flow rate of 125 L hour^{-1} 100 m^{-1} were stretched across each row spaced 30 cm apart with a double header in each plot. The drip system used for this study comprised of laterals (15 mm diameter), with emitters 30 cm apart. Irrigation was applied at 8 am for 15 min day^{-1} at the initial stage and was changed to 30 min day^{-1} after plants reached the V6-stage. The amount of precipitation was obtained from a nearby CoAgMet weather station (http://www.coagmet.colostate.edu/) (Table F2).

6.2.6 Fertilizer treatments

The fertilizers compared in this study were liquid fertilizers [Alaska® fish emulsion (Planet Natural, Bozeman, MO) and cyano-fertilizer], and solid fertilizers [blood meal and feather meal (Down To Earth Inc., Eugene, OR)]. For this study, N-fixing cyanobacteria (*Anabaena* sp.*)* was cultured from local soils and inoculated into nutrient-supplemented raceways according to the method of Barminski (2014).

6.2.7 Methods of fertilizer application

Both the fish emulsion and cyano-fertilizer were in liquid form and were supplied in four split applications over the growing season, while the blood meal and feather meal were in dry powdered form and incorporated into the soil prior to planting. Blood meal and feather meal were applied 6 cm from the row and 6 cm deep through band application using a hoe. All fertilizers were applied at 56 and 112 kg N ha^{-1}.

6.2.8 Fertilizer analysis

Fertilizer samples were sent to the Soil, Water, and Plant Testing Laboratory at CSU and analyzed for N, P, K, Ca, Mg, Fe, and Zn (Table F3). Total N concentration was analyzed with a LECO CN analyzer (Leco Corp., St. Joseph, MI). Fertilizer samples were digested with nitric acid (HNO_3) and hydrogen peroxide (H_2O_2). Major cations (P, K, Ca, and Mg) and micronutrients (Fe and Zn) were analyzed using a Perkin Elmer Inductively Coupled Plasma/Optical Emission Spectrometer (ICP-OES) (Perkin Elmer, Waltham, MA). Solid fertilizers were extracted using 2M KCl, and the filtered extracts were analyzed for NH_4^+-N and NO_3^--N. The filtered extracts of solid fertilizers and the filtered liquid

fertilizers were analyzed using an Alpkem Flow Solution IV Auto Analyzer (OI Analytical, College Station, TX) (Table F4).

6.2.9 Measurements

6.2.9.1 Marketable ear yield and ear dry weight

All above-ground plant parts from each treatment were hand harvested and separated into leaves, stems, and marketable ears on September 20, 2013 and September 19, 2014, both at 79 days after planting. The separated plant parts were dried at 70^{o}C for 72 hours and weighed to determine the total above-ground biomass. The dried kernels from each ear sample were removed and then weighed for dry matter and then ground for analysis using a plant tissue grinder (Thomas Willey®, Swedesboro, NJ). Sub-samples were then taken from each sample for further analysis.

6.2.9.2 Leaf and kernel N concentrations, N content, and N use efficiency

All harvested leaf samples were washed with deionized water before oven-drying for elemental analysis. Total N of plant tissues was measured using a LECO CN analyzer (Leco Corp., St. Joseph, MI). After digestion, other elements were measured using a Perkin-Elmer Inductively Coupled Plasma/Optical Emission Spectrometer (ICP-OES) (Perkin-Elmer, Waltham, MA). Leaf and kernel N concentrations were multiplied by leaf and kernel dry weights, respectively to determine the N uptake. Nitrogen use efficiency (NUE) was calculated by dividing marketable ear yield by the amount of N fertilizer applied.

6.2.9.3 Residual soil inorganic-N

Soils were sampled from 0-30 cm deep in 2013 to 120 cm deep in 30 increments in 2014. After harvest, all sub-plots were soil sampled using a Giddings soil sampler drill rig (Purple Wave, Inc., Windsor, CO). Soil samples were dried for two weeks in a walk-in cooler. Once dried, inorganic-N was extracted using 2 M KCl at a 1:10 ratio (2 g of soil in 20 mL of 2M KCl). The samples were then filtered and run through the Alpkem Flow Solution IV Auto Analyzer (OI Analytical, College Station, TX) for NH_4^+-N and NO_3^--N.

6.2.10 Statistical analysis

Data were analyzed using SAS version 9.3 (SAS Institute Inc., Cary, NC). Univariate and Boxplot procedures were used to evaluate the normality of data distribution. Analysis of variance (ANOVA) was performed on the data with the MIXED procedure ($P < 0.1$). Fertilizer treatment, soil depth, and N rate were categorized as fixed effects, and replication was categorized as a random effect. When treatment or interaction effects were significant, means were separated by Tukey's Studentized Range (HSD) test. The relationships between parameters were assessed by linear correlation using the CORR procedure.

6.3 Results

6.3.1 Residual soil NO_3^--N

A significant effect of soil depth was observed in residual soil NO_3^--N in 2014 (Table F5). Only depth was significant in 2014, but not in 2013. Higher residual soil NO_3^--N was observed at 0-30 cm depth compared to 30-120 cm depth ($P < 0.0001$), and residual soil NO_3^--N decreased with increasing depth (Table F6). N rate and treatment effects were significant in 2013 but not in 2014. Higher residual soil NO_3^--N was observed at 112 kg N ha^{-1} compared to 56 kg N ha^{-1} and control (Table F7). The feather meal and fish emulsion treatments had a higher residual soil NO_3^--N compared with other treatments (Table F8).

6.3.2 Marketable ear yield, N use efficiency, and N content

An interaction effect between N rate and treatment was observed in marketable ear yield and NUE in both years (Table F9). N rate and treatment effects on leaf and kernel N contents were observed in 2013 (Table F10), but not in 2014. In fact, no significant effect was observed in leaf or kernel N concentrations in either year.

The fish emulsion and cyano-fertilizer treatments had a higher marketable ear yield at the high N rate compared with other treatments, while only fish emulsion had a higher yield compared to other treatments at the low N rate in 2013 (Figure F1a). The marketable ear yield in the cyano-fertilizer treatment was higher at the high N rate compared with other fertilizers in 2014 (Figure F1b). Marketable

ear yield differed with N application rate ($P < 0.0001$) in both years (Table F9). About 38% and 30% of the change in ear yield can be explained by different N application rate in 2013 and 2014, respectively (Figure F2).

Higher NUE was observed at the low N rate compared to the high N rate ($P < 0.0001$) in both years. The fish emulsion and cyano-fertilizer treatments had a higher NUE compared to feather meal at the high N rate in 2013 (Figure F3a). The cyano-fertilizer had a higher NUE compared with other treatments at the high N rate in 2014 (Figure F3b). The feather meal treatment had a lower leaf and kernel N contents compared to other fertilizer treatments, and both leaf and kernel N contents were different from control in 2013 (Table F11).

6.4 Discussion

6.4.1 Feather meal decreased leaf and kernel N contents

Higher residual soil NO_3^--N at 0-30 cm depth may be due to the direct contact of soil with fertilizers applied. The residual soil NO_3^--N was lower than feather meal, which may probably be due to higher leaf and kernel N contents in the blood meal treatment compared to feather meal in 2013 (Table F11).

Hermanson et al. (2000) reported that N content is affected by the forms of N supplied by the soil or added as fertilizer. Among fertilizer treatments, lower leaf and kernel N contents were observed in the feather meal treatment (Table F11), which may be due to low N mineralization of the feather meal N (Hadas and Kautsky, 1994) to meet crop N demand from V6 to tasseling stage over a short growing season of 79 days. However, N mineralization of feather meal, generally increases from 70 days onwards (Hadas and Kautksy, 1994). Therefore, higher residual soil NO_3^--N was observed in the feather meal treatment after harvest (Table F8).

Feather meal may not be a good choice of fertilizer to be used by organic sweet corn growers to meet sweet corn N demand at tasseling (60 to 65 days after planting). Feather meal needs a longer period (70 days after incubation under optimum conditions at 25^{o}C) to release available-N (Hadas and Kautsky, 1994). Feather meal is a byproduct of the poultry industry and consists of 90% keratin, which is resistant

to rapid decomposition due to its structural rigidity (Hadas and Kautsky, 1994; Mazotto et al., 2011). Higher keratin in the feather meal treatment may contribute to decreased leaf and kernel N contents, and lower yields in the feather meal treatment.

6.4.2 N use efficiency and N application rate

In most cases, an increase in residual soil NO_3^--N when N is applied at rates greater than 100 kg N ha^{-1} decreases NUE (Zhang et al., 2002). Annual crops usually produce less than 50 kg yield per amount of kg N applied (Cassman and Dobermann, 2002), which is in range with the NUE results in our study.

Zhang et al. (2002) reported that N fertilizer rate of 56 kg N ha^{-1} was required for optimum sweet corn marketable ear yield. In a study conducted by Zhang and his colleagues, a good marketable ear quality was produced when N rate was applied between 56 to 112 kg N ha^{-1}, similarly as reported in our results, where leaf and N contents were higher at 56 and 112 kg N ha^{-1} (Table F11). N fertilizer rate above the requirement for optimum yield of sweet corn could pose a threat to the environment. In addition, increasing fertilizer costs increases the economic need to avoid over application of N fertilizer.

6.5 Conclusion

The feather meal and fish emulsion treatments had a higher residual soil NO_3^--N compared with other treatments. The fish emulsion, cyano-fertilizer, and blood meal had a higher leaf and kernel N contents at both N rates. Higher NUE was observed in the fish emulsion and cyano-fertilizer treatments compared to the blood meal and feather meal treatments at 112 kg N ha^{-1} in 2013. The cyano-fertilizer treatment had a higher marketable ear yield and NUE compared with other treatments at 112 kg N ha^{-1} in 2014. Blood meal could be a good source of N fertilizer due to leaf and N contents compared to feather meal, although marketable ear yield was the same as in the feather meal treatment. Cyano-fertilizer and blood meal could be a good source of N fertilizer for organic sweet corn growers due to its low residual NO_3^--N concentration compared to fish emulsion and feather meal.

TABLES

Table F1. Initial soil properties of the 0-30 cm soil depth of the sweet corn experimental site.

Soil properties	2013	2014
pH[¶]	7.5	7.7
Electrical conductivity[¶] (dS m^{-1})	0.6	0.6
Cation exchange capacity, CEC[ǂ] (meq 100 g^{-1})	29	30
Organic matter, OM[#] (%)	2.5	2.6
NH_4^+-N[##] (ppm)	2.5	2.7
NO_3^--N[##] (ppm)	1.9	2.1
P[§] (ppm)	30	32
K[♯] (ppm)	463	456
Ca[♯] (ppm)	4560	4650
Mg[♯] (ppm)	616	622
Fe[♯] (ppm)	6	7
Zn[♯] (ppm)	1.4	1.6

[¶] pH and electrical conductivity were determined in water (1:1).
[ǂ] CEC was determined using ammonium acetate (1N $NH_4C_2H_3O_2$, pH 7.0) extraction.
[#] OM was determined by the loss on ignition method.
[##] Samples were extracted using 2M KCl.
[§] Sample was extracted with 0.5M $NaHCO_3$.
[♯] Samples were extracted using ammonium bicarbonate diethylenetriamine pentaacetate (AB-DTPA).

Table F2. Average monthly precipitation and the amount of water applied during growing season in 2013 and 2014 in sweet corn.

	2013	2014
Planting	July 2	July 1
Harvest	September	September 19
Precipitation (mm)		
July	39	69
August	13	26
September	119	9
Amount of water applied (mm)		
Irrigation	729	737
Fertigation	414	414
Total (mm)	1314	1257

[‡]Total amount of water applied over the growing season is the combination of precipitation, irrigation, and fertigation. All plots received the same amount of water applied via irrigation. When liquid fertilizers were applied, the control and solid fertilizer treatments were irrigated with the same amount of water as that used to fertigate the liquid fertilizer treatments.

Table F3. Nutrient analysis of fertilizer samples.

	N[‡]	P[#]	K[#]	Fe[#]	Ca[#]	Mg[#]	Zn[#]
	%	----------mg kg^{-1}----------					
Cyano-fertilizer	0.2	6	0.1	6	10	18	0.1
Fish emulsion	5	1600	20510	131	725	921	18
Feather meal	13	640	1776	62	1466	575	18
Blood meal	13	32	366	118	904	283	14

[‡]Samples were analyzed using CN analyzer.
[#]Samples were digested with HNO_3 and H_2O_2 and analyzed using ICP-OES.

Table F4. Inorganic N concentration and percentage of N applied as inorganic-N of fertilizers.

	NH_4^+-N¶	NO_3^--N¶	N as NH_4^+-N	N as NO_3^--N
	----------mg kg^{-1}----------		----------%----------	
Cyano-fertilizer	4.7	0.01	0.24	0.0005
Fish emulsion	23.7	0.12	0.05	0.0002
Feather meal	1232	2.30	0.95	0.002
Blood meal	27.7	8.40	0.02	0.006

¶Samples were extracted using 2M KCl and were analyzed using autoanalyzer.

Table F5. Analysis of variance (ANOVA) of residual soil NO_3^--N in sweet corn in both years.

	2013	2014
	P > F	P > F
Depth	0.2028	<.0001*
N rate	<.0001*	0.9773
Treatment	<.0001*	0.9688
Depth*Nrate	0.1233	0.4492
Nrate*Treatment	0.1269	0.7175
Depth*Nrate*Treatment	0.2102	0.4383

*P-values are significantly different at P < 0.1.

Table F6. Residual soil NO_3^--N in 2014 at four different depths in sweet corn. Means with the same letter are not significantly different at P < 0.1 using Tukey's Studentized Range (HSD) test of mean separation.

	Residual soil NO_3^--N (mg kg^{-1})
Depth (cm)	
0-30	10.7 a
30-60	4.9 b
60-90	3.4 b
90-120	3.3 b

Table F7. N rate effect on residual soil NO_3^--N after harvest in 2013. Means with the same letter are not significantly different at P < 0.1 using Tukey's Studentized Range (HSD) test of mean separation.

N rate (kg N ha^{-1})	Residual soil NO_3^--N (mg kg^{-1})
0	4.3 b
56	9.9 b
112	23.1 a

Table F8. Treatment effect on residual soil NO_3^--N after harvest in 2013. Means with the same letter are not significantly different at P < 0.1 using Tukey's Studentized Range (HSD) test of mean separation.

	Residual soil NO_3^--N (mg kg^{-1})
Treatment	
Cyano-fertilizer	11.8 b
Fish emulsion	23.6 a
Feather meal	19.6 a
Blood meal	11.2 b
Control	6.9 c

Table F9. Analysis of variance (ANOVA) of marketable ear yield and N use efficiency (NUE).

	2013		2014	
	Ear yield	NUE	Ear yield	NUE
	P > F	P > F	P > F	P > F
N rate	0.0333*	<.0001*	<.0001*	<.0001*
Treatment	<.0001*	0.0002*	0.0008*	0.0048*
N rate*Treatment	0.0209*	0.0043*	0.0025*	0.0228*

*P-values are significantly different at P < 0.1.

Table F10. Analysis of variance (ANOVA) of leaf and kernel N contents in sweet corn in both years.

	2013		2014	
	Leaf N content	Kernel N content	Leaf N content	Kernel N content
	P > F	P > F	P > F	P > F
N rate	0.0299*	0.0582*	0.9352	0.1880
Treatment	0.0508*	0.2164	0.3412	0.2828
N rate*Treatment	0.7451	0.3857	0.3967	0.3196

*P-values are significantly different at P < 0.1.

Table F11. Treatment and N rate effects on leaf and kernel N contents in 2013. Means with the same letter are not significantly different at P < 0.1 using Tukey's Studentized Range (HSD) test of mean separation.

	Leaf N content	Kernel N content
Treatment	----------kg ha^{-1}----------	
Cyano-fertilizer	7.2 a	5.9 a
Fish emulsion	8.6 a	5.7 a
Feather meal	5.8 b	3.7 b
Blood meal	7.3 a	5.5 a
Control	3.7 c	1.9 c
N rate (kg N ha^{-1})		
0	3.7 b	1.9 b
56	6.6 a	4.8 a
112	7.8 a	5.6 a

FIGURES

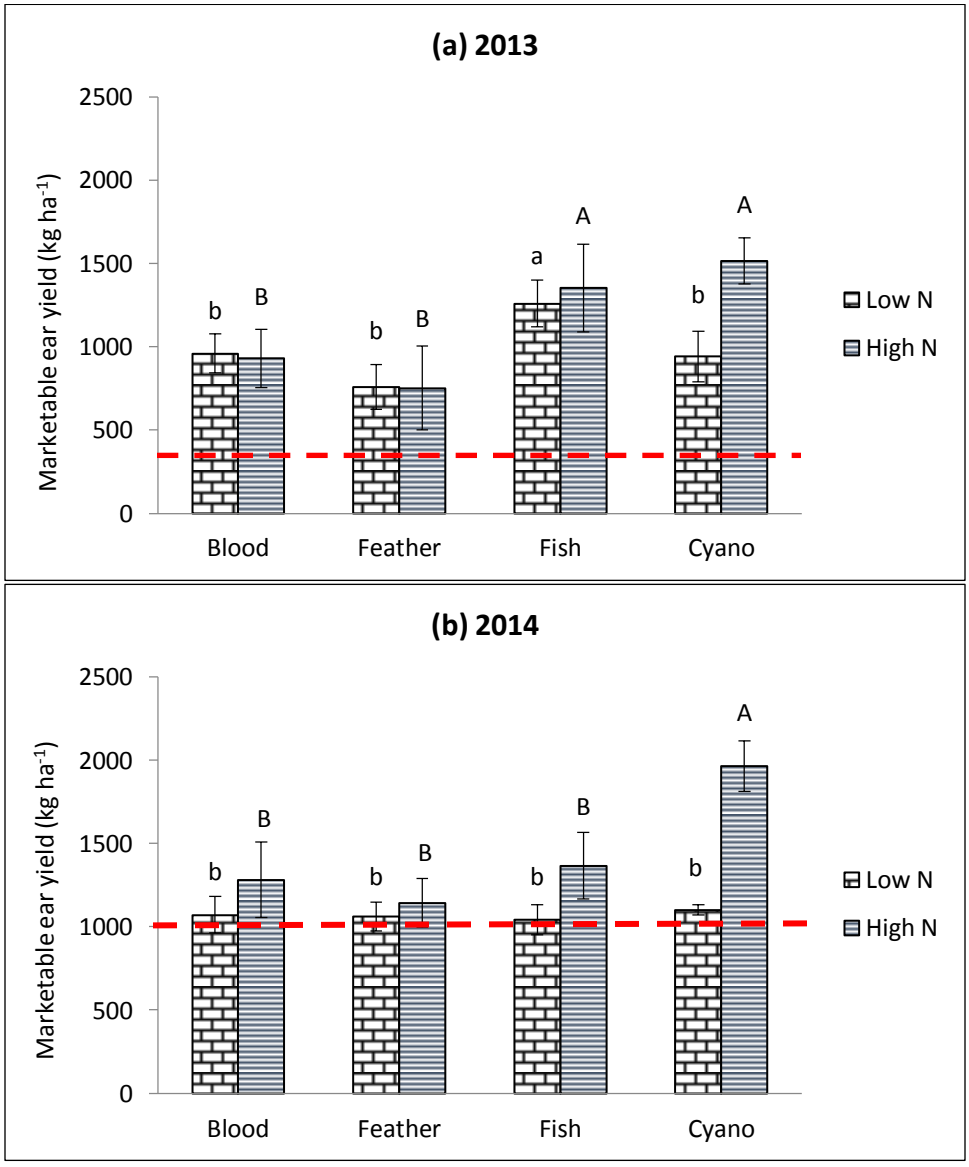

Figure F1. Effects of different organic fertilizers on sweet corn marketable ear yield in (a) 2013 and (b) 2014. Fertilizers were applied at 56 kg N ha^{-1} (Low N) and 112 kg N ha^{-1} (High N). Bars represent standard deviation of mean. Means with the same letter are not significantly different at $P < 0.1$ using Tukey's Studentized Range (HSD) test of mean separation. Upper case letters indicate significant differences within mean at the high N rate, while lower case letters indicate significant differences within mean at the low N rate. The dashed line represents control. All treatments at the high N rate are significantly different from control, except feather meal at $P < 0.1$. Blood = Blood meal; Feather = Feather meal; Fish = Fish emulsion; Cyano = Cyano-fertilizer.

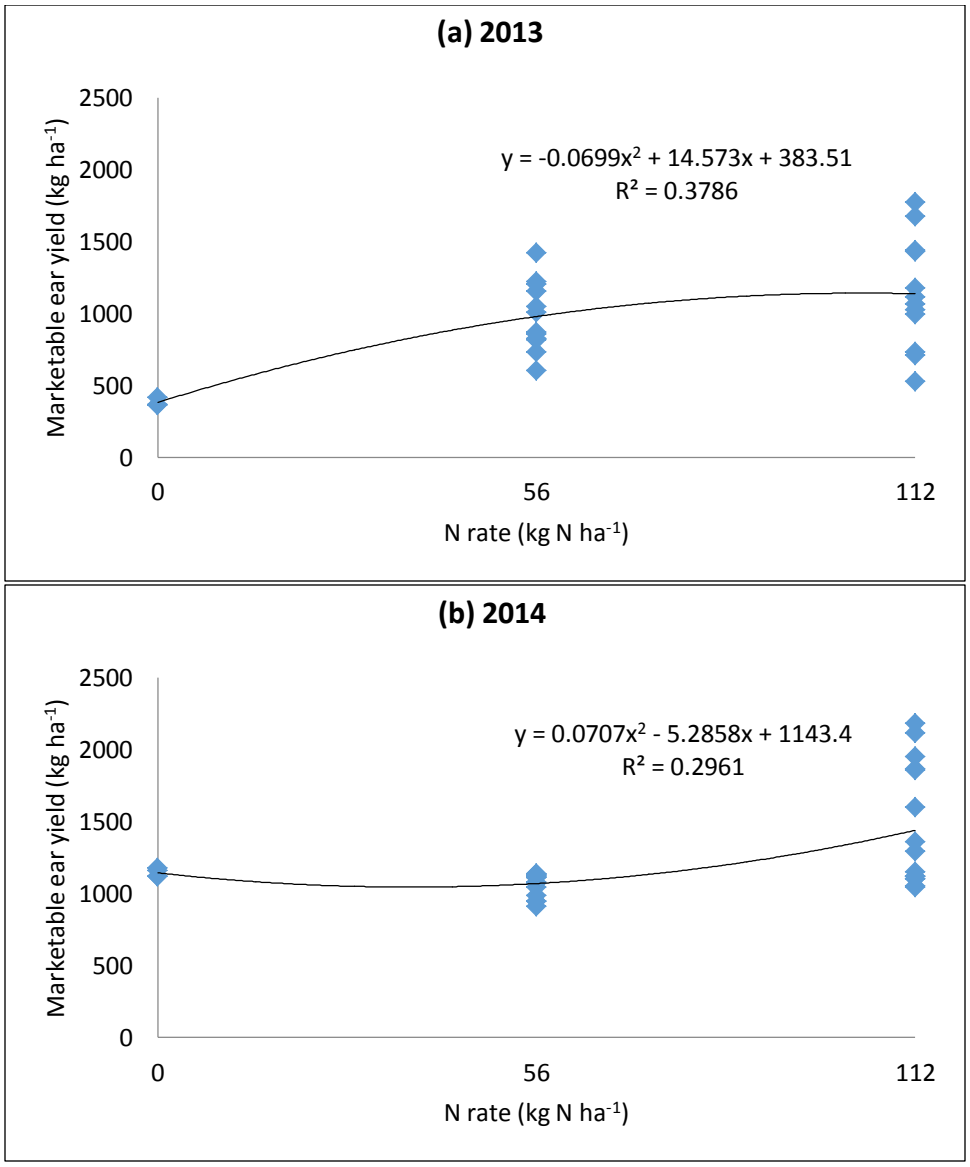

Figure F2. Relationships between sweet corn marketable ear yield and N application rate in (a) 2013 and (b) 2014.

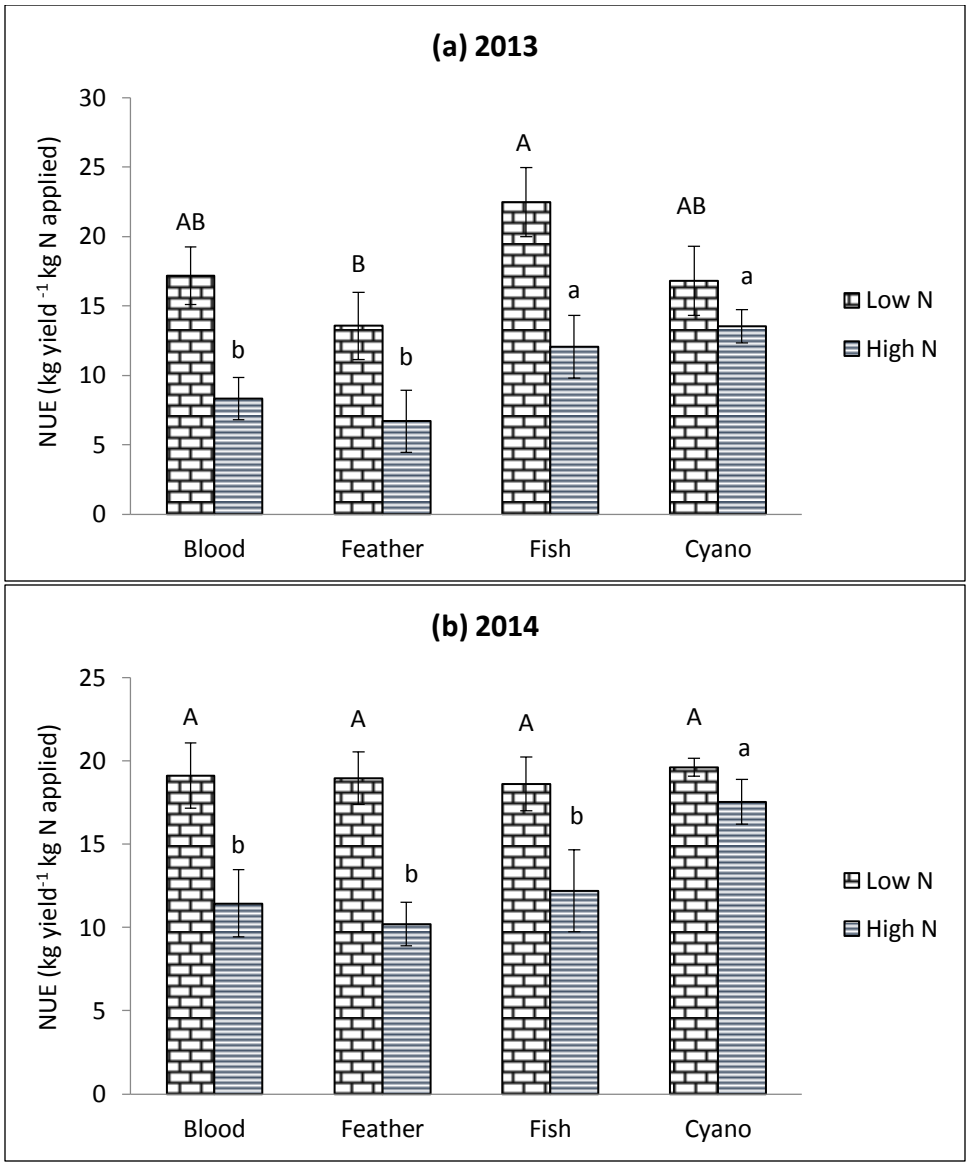

Figure F3. Effects of different organic fertilizers on nitrogen use efficiency (NUE) in (a) 2013 and (b) 2014. Fertilizers were applied at 56 kg N ha^{-1} (Low N) and 112 kg N ha^{-1} (High N). Bars represent standard deviation of mean. Means with the same letter are not significantly different at $P < 0.1$ using Tukey's Studentized Range (HSD) test of mean separation. Upper case letters indicate significant differences within mean at the high N rate, while lower case letters indicate significant differences within mean at the low N rate. Blood = Blood meal; Feather = Feather meal; Fish = Fish emulsion; Cyano = Cyano-fertilizer.

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CHAPTER 7. ORGANIC NITROGEN FERTILIZER APPLICATION INFLUENCED SOIL PERMANGANATE OXIDIZABLE CARBON IN SWEET CORN AND LETTUCE

7.1 Introduction

Soil N availability is regulated by fertilizer inputs and microbially-mediated processes. The quantity and quality of soil C that is readily available to soil microbes, also referred to as labile C, can therefore influence soil N dynamics. Labile C pool has a shorter residence time in soils of several weeks to months or years compared to recalcitrant C pools (Paul et al., 2001). Blair et al. (1995) proposed that permanganate oxidizable carbon (POXC) is a method that quantifies biologically labile C using potassium permanganate ($KMnO_4$), an oxidizing agent that reacts with the labile C pool (Blair et al., 1995).

POXC fraction includes labile humic material and polysaccharides C that has been proven to be sensitive to soil management practices (Blair et al., 1995). Soil oxidizable C is a component of the organic fraction which is decomposed by microorganisms and may provide an indication of the energy supply available to soil microorganisms and used to decompose organic N (Ghani et al., 2003; Zou et al., 2005). Soil oxidizable C concentration can correlate with organic N decomposition, thus potentially reflects the amount of plant-available N (Ford and Greenland, 1968).

It is important to understand the distributions of soil oxidizable C and available-N concentrations in response to N fertilization throughout the soil profile. Various C inputs through decomposition of organic matter from organic N fertilizer, root production, and root exudates could influence the quantity of labile C pools (Benbi et al., 2015). Rooting depth could influence the soil oxidizable C concentrations through rhizodeposition of C-rich compounds. Trumbore (2000) found that soil microorganisms utilized C release from crop roots in the upper soil layer to activate soil microbial communities during organic N decomposition. Sharma et al. (2014) reported that higher amount of POXC concentration in the soil surface of 0-15 cm in horticultural and agricultural systems. The soil surface had optimum conditions for

soil microorganisms to utilize C due to more optimum soil temperature and moisture conditions than at deeper depths.

A number of reports on soil POXC concentrations have been published for agronomic crops such as corn (Culman et al., 2013) and corn-sugarbeet-soybean (Awale et al., 2013) using conventional fertilizers, but few have compared organic N fertilizers on horticultural crops, such as sweet corn (*Zea mays*) or lettuce (*Lactuca sativa*). Organic vegetable production relies on N fertilizers such as compost, fish emulsion, blood meal, and feather meal to meet crop N demand (Gaskell et al., 2010). There are two main classes of N fertilizers, solid and liquid. In organic agriculture, solid fertilizers are often incorporated into the soil before planting, while liquid fertilizers are generally applied over the growing season through irrigation.

Organic animal-based fertilizers (fish emulsion, blood meal, feather meal, compost) vary in nutrient composition and can have high transportation costs to meet the N demand of crops. Several types of organic N fertilizers are commercially available and distributed widely throughout the United States. Farmers usually rely on off-farm sources for additional N needed during the growing season. However, there are other opportunities for fertilizer options being developed that allow farmers to produce N on-farm, such as cultivation of cyanobacteria for use as cyano-fertilizer. Cyanobacteria have unique dual properties because they can both fix N from the atmosphere and photosynthesize to produce fixed N without fossil fuels.

In this study, our objectives were to compare the impact of organic N fertilizers and N rates on soil POXC concentration at different depths in sweet corn and lettuce and to determine whether soil POXC concentration could be a sensitive indicator in organic crop production. We hypothesized that (i) greater soil POXC concentration and residual soil NO_3^--N will be observed in the upper depths compared to the lower depths in sweet corn and lettuce, (ii) Soil POXC concentration may be correlated with leaf N concentration, N content, and marketable yield, (iii) higher total amount of root C input in sweet corn relative to lettuce will increase soil POXC concentration in the upper depths, and (iv) either soil POXC concentration or residual soil NO_3^--N may correlate with the marketable yield of sweet corn and lettuce.

7.2 Materials and Methods

7.2.1 Experimental site

Field experiments were carried out in a certified organic field in 2014 at the Colorado State University Horticulture Research, Fort Collins, CO. Previously, the field area was planted with buckwheat followed by winter cover crops, rye (*Secale cereal*) and turnips (*Brassica rapa*). The experimental site was located in a semi-arid zone with clay loam soils. The soil was classified as a fine, smectitic, mesic Aridic Argiustoll of the Nunn series (NRCS, 1980).

7.2.2 Soil analysis

Soil samples were collected prior to the experiment to a depth of 30 cm from the field area (15 m x 30 m) and analyzed by the Soil, Water, and Plant Testing Laboratory at Colorado State University. Chemical analyses included soil pH and electrical conductivity (EC) measured in supernatant suspension of 1:1 soil to water using a Mettler Toledo pH/EC meter (Thermo Fischer Scientific, Waltham, MA). Cation exchange capacity (CEC) was determined by summation of the exchangeable bases (Ca^{2+}, K^{+}, Mg^{2+}, Na^{+}) plus hydrogen (H^{+}) using ammonium acetate (1N $NH_4C_2H_3O_2$, pH 7.0) as described by Hajek et al. (1972). Organic matter (OM) content was determined by the loss on ignition method (Blume et al., 1990). Soil inorganic N (NH_4^{+}-N and NO_3^{-}-N) was extracted using 2M KCl, and the filtered extract was analyzed using an Alpkem Flow Solution IV Auto Analyzer (OI Analytical, College Station, TX). Phosphorus (P) was extracted with 0.5M sodium bicarbonate ($NaHCO_3$) solution, which was developed by Olsen et al. (1954) and analyzed using a Perkin Elmer Inductively Coupled Plasma/Optical Emission Spectrometer (ICP-OES) (Perkin Elmer, Waltham, MA). Major cations (K, Ca, and Mg) and micronutrients (Fe and Zn) were extracted with ammonium bicarbonate–diethylene triaminepentaacetic acid (AB-DTPA) (Jones, 2001) and analyzed using a Perkin Elmer Inductively Coupled Plasma/Optical Emission Spectrometer (ICP-OES) (Perkin Elmer, Waltham, MA). The results of soil physical, chemical, and nutrient analyses are presented in Table G1.

7.2.3 Experimental design and plot layout

The experimental units were arranged in a Randomized Complete Block Design with four replications. The sub-plot size was 76 cm x 457 cm.

7.2.4 Planting materials

Lactuca sativa var. 'Concept' seeds (Johnny's Selected Seeds, Waterville, ME) were planted in 72-cell trays containing a well-mixed Sunshine® organic potting soil mix (SunGro Horticulture, Agawam, MA) in the first week of May 2014. All seeds were started at the Plant Environmental Research Center's (PERC) greenhouse facility on the CSU campus. After four weeks, seedlings were transplanted to the field on June 9, 2014.

Zea mays var. 'Luscious se^{+}' sweet corn seeds were purchased from Johnny's Seeds (Johnny's Selected Seed, Waterville, ME). Seeds were hand planted at a depth of approximately 2.5 cm on July 1, 2014 with an in-row spacing of 12 cm within rows spaced 75 cm apart.

7.2.5 Irrigation

Irrigation was applied every day and adjusted for precipitation. The amount of precipitation and irrigation over the growing season is presented in Table G2. The amount of precipitation was obtained from a nearby CoAgMet weather station (http://www.coagmet.colostate.edu/). Water was applied daily using an automated drip irrigation system. Two drip tapes (John Deere®, Deere & Co., Moline, IL) with a flow rate of 125 L hour^{-1} 100 m^{-1} were stretched across each row spaced 30 cm apart with a double header in each plot. The drip system used for this study comprised of laterals (15 mm diameter). Emitters were 30 cm apart. Irrigation was applied at 08:00 to 08:30 for 30 min day^{-1} at the initial stage and was changed to 60 min day^{-1} at 4th leaf stage for lettuce and 6th leaf stage for sweet corn until harvest.

7.2.6 Fertilizer treatments

The fertilizers compared in this study were liquid fertilizers [Alaska® fish emulsion (Planet Natural, Bozeman, MO) and cyano-fertilizer], and solid fertilizers [blood meal and feather meal (Down To Earth Inc., Eugene, OR)]. For this study the N-fixing cyanobacteria (*Anabaena* sp.*)* was cultured from

local soils and inoculated into nutrient-supplemented raceways according to the method by Barminski (2014). The culture was grown for approximately 14 d cycles before each application.

7.2.7 Methods of fertilizer application

Both the fish emulsion and cyano-fertilizer were in liquid form and were supplied in four split applications (approximately two weeks apart from each application) over the growing season, while the blood meal and feather meal were in dry powdered form and incorporated into the soil prior to planting. Blood meal and feather meal were applied 6 cm from the root zone and 6 cm deep through band application using a hoe. All fertilizers were applied at 28 and 56 kg N ha^{-1} for lettuce and 56 and 112 kg N ha^{-1} for sweet corn.

7.2.8 Fertilizer analysis

Fertilizer samples were sent to the Soil, Water, and Plant Testing Laboratory at CSU and analyzed for N, P, K, Ca, Mg, Fe, and Zn (Table G3a). Fertilizer samples were digested with nitric acid (HNO_3) and hydrogen peroxide (H_2O_2). Major cations (P, K, Ca, and Mg) and micronutrients (Fe and Zn) were analyzed using a Perkin Elmer Inductively Coupled Plasma/Optical Emission Spectrometer (ICP-OES) (Perkin Elmer, Waltham, MA). Total C and N concentrations of fertilizer samples were analyzed with a LECO CN analyzer (Leco Corp., St. Joseph, MI) and the total C input for each fertilizer treatments was calculated (Table G3b).

7.2.9 Measurements

7.2.9.1 Marketable yield

All above-ground plant parts of sweet corn from each treatment were hand harvested and separated into leaves, stems, and ears 79 days after planting. Lettuce was harvested 39 days after transplanting. Lettuce heads were harvested from each plot and weighed for fresh weights.

7.2.9.2 Leaf N concentration and N content

The oldest most expanded lettuce leaves were harvested at 39 days after transplanting, while sweet corn leaves were sampled at tasseling. All harvested samples were washed with deionized water before oven-drying at the CSU Agriculture Research Development & Education Center (ARDEC) at 72^{o}C

for 72 hours. The dried samples were weighed and then ground to pass a 1.0-mm screen (20 mesh) using a plant tissue grinder (Thomas Wiley®, Swedesboro, NJ). Sub-samples were then taken from each sample for further analysis.

Total N of plant tissues of sweet corn and lettuce were measured using a LECO CN analyzer (Leco Corp., St. Joseph, MI). Samples were digested with nitric acid (HNO_3) and hydrogen peroxide (H_2O_2). Elemental measurement was made using a Perkin-Elmer Inductively Coupled Plasma/Optical Emission Spectrometer (ICP-OES) (Perkin-Elmer, Waltham, MA). Tissue nutrient concentrations were multiplied by leaf dry weights to determine the N content.

7.2.9.3 Soil sampling

Soils were sampled in October 19, 2014 after harvest from four different soil depths (0-30 cm, 30-60 cm, 60-90 cm, 90-120 cm) for sweet corn and two depths (0-30 cm and 30-60 cm) for lettuce. The collected soil samples were air-dried at room temperature. Soils were sieved with a 2.0 mm sieve to obtain a uniform particle size for residual soil NO_3^--N and POXC analysis.

7.2.9.4 Residual soil inorganic-N

Residual soil inorganic-N was sampled at 0-30 cm and 30-60 cm depths in lettuce and 0-30, 30-60 cm, 60-90 cm, and 90-120 cm in sweet corn. After harvest, all sub-plots were soil sampled using a Giddings soil sampler drill rig (Purple Wave, Inc., Windsor, CO). Soil samples were air-dried for two weeks in a walk-in cooler to reduce NH_4^+-N volatilization. Once dried, inorganic-N was extracted using 2 M KCl at a 1:10 ratio (2 g of soil in 20 mL of 2M KCl). The samples were then filtered and run through the Alpkem Flow Solution IV Auto Analyzer (OI Analytical, College Station, TX) for NH_4^+-N and NO_3^- N.

7.2.9.5 Permanganate oxidizable C extraction

Sub-samples of 2.5 g of air-dried soil were weighed into 50-mL centrifuge tubes. Eighteen mL of deionized water and 2 mL of 0.02M $KMnO_4$ were added to each tube. All tubes were shaken for 2 min at 240 oscillations per minute on an oscillating shaker. Tubes were removed from the shaker and allowed to

settle for 10 min. Then, 0.5 mL of the supernatant were transferred into a 50-mL centrifuge tube and mixed with 49.5 mL of deionized water.

7.2.9.6 Permanganate oxidizable C determination

An aliquot of 200 µL from each tube was loaded into a cuvette containing four standard stock solutions (0.00005, 0.0001, 0.00015, and 0.0002 mol L^{-1} $KMnO_4$) including a blank of deionized water, a soil standard, and a solution standard based on the laboratory reference samples. Sample absorbance was read with DR3900 Benchtop VIS Spectrophotometer (Hach®, Loveland, CO) at 550 nm. POXC analysis was determined following Weil et al. (2003) and Culman et al. (2012):

POXC (mg kg^{-1} soil) = [0.02 mol L^{-1}-(a + b x Abs)] x (9000 mg C mol^{-1})(0.02 L solution Wt^{-1}).

where,

0.02 mol L^{-1} = concentration of the initial $KMnO_4$ solution,

a = intercept,

b = slope of the standard curve,

Abs = absorbance of the sample,

9000 mg = amount of C oxidized by 1 mol of $KMnO_4$ (from Mn^{7+} to Mn^{4+}),

0.02 L = volume of $KMnO_4$ solution reacted,

Wt = mass of soil (kg) used in the reaction.

7.2.10 Statistical analysis

Data were analyzed using SAS version 9.3 (SAS Institute Inc., Cary, NC). Univariate and Boxplot procedures were used to evaluate the normality of data distribution. Analysis of variance (ANOVA) was performed on the data with the MIXED procedure ($P < 0.1$). Fertilizer treatment, soil depth, and N rate were categorized as fixed effects, and replication was categorized as a random effect. Means were separated by Tukey's Studentized Range (HSD) test for mean separation. Means separation test on interaction effects were simplified within N rate of one factor using the SLICE option. The relationships between parameters (marketable yield, leaf N concentration, N content, soil POXC concentration, and residual soil NO_3^--N) were assessed by linear correlation using the CORR procedure.

7.3 Results

7.3.1 Sweet corn

Treatment effect was observed in soil POXC concentration (P = 0.0344; Table G4a). Soil POXC concentration in the cyano-fertilizer treatment was higher than control and blood meal treatments in sweet corn (Table G5).

A significant interaction between N rate and treatment was observed in soil POXC concentration (P = 0.0013) and the marketable ear yield of sweet corn (P = 0.0025; Table G4b). An increase in the marketable ear yield of sweet corn was significantly correlated with soil POXC concentration (r = 0.3839; P = 0.0208). The cyano-fertilizer treatment had a higher marketable ear yield (Figure G2) at 112 kg N ha^{-1} compared with other treatments. Sweet corn had a higher marketable ear yield at 112 kg N ha^{-1} compared to 56 kg N ha^{-1} and control (Table G6).

Soil POXC concentration had a positive relationship with marketable ear yield (r = 0.34; P = 0.0043), but not with residual soil NO_3^--N. Depth effect was observed in residual soil NO_3^--N, where higher residual soil NO_3^--N was observed at 0-30 cm compared to deeper depths (P = 0.0032; Table G7). There was no treatment or N rate effect on leaf N concentration or N content of sweet corn.

7.3.2 Lettuce

Significant depth and treatment effects on soil POXC concentration were observed in lettuce (Table G4a). Soil POXC concentration in the control and blood meal treatments were higher than the cyano-fertilizer treatment in lettuce (P = 0.0454; Table G5).

Marketable yield of lettuce only differed by N rate (P = 0.0364; Table G4b), but not by treatment. Soil POXC concentration at 0-30 cm depth was higher than at the 30-60 cm depth (Table G5). The highest marketable yield was observed at 28 kg N ha^{-1} compared to 56 kg N ha^{-1} (Table G6).

Higher residual soil NO_3^--N was observed at 0-30 cm compared to deeper depths (Table G7). No significant relationship observed among N content, leaf N, and residual soil NO_3^--N with soil POXC concentration.

7.4 Discussion

7.4.1 Amount of C input from different fertilizer treatments

In our study, there are two main factors which may affect soil POXC concentration, quantity of total C inputs from fertilizer treatments and crop root inputs. The cyano-fertilizer treatment had a higher soil POXC concentration than control in sweet corn (Table G5), was likely not due to total C applied in organic N fertilizers compare to others (Table G3b). Lower C: N ratio in the cyano-fertilizer treatment may have speed up the decomposition rate during the short growing season. Halder and Kheroar (2003) reported that cyanobacterial biomass application to soils improved soil labile C after 45 days of incorporation up to 75 days of incubation, which is within range with the growing period of sweet corn.

Over the course of the growing season, a biweekly split application of cyano-fertilizer through fertigation may have provided fresh C inputs to the soils over the growing season, which enhance the microbial decomposition, thus increased labile C. Gregorich et al. (2013) reported that fresh C inputs in maize-cropped soils enhanced soil microbial C decomposition over the growing season. The blood meal treatment had a higher total C input compared to other fertilizer treatments (Table G3b). Decreased soil POXC concentration in the blood meal treatment in sweet corn (Table G5) may be due to low energy gained by soil microorganisms to decompose higher organic C compounds, resulting in reduced soil POXC concentration. Although the C: N ratio and amount of total C input in the feather meal treatment is similar to blood meal, but the slow-release properties of feather meal due to its high keratin content may reduce the decomposition rate (Hadas and Kautsky, 1994). Geraei et al. (2016) reported that higher organic C compounds are less degraded and may need longer time to respond to soil management practices.

7.4.2 Relationship between crop root C inputs and soil POXC concentration across depths

Addition of organic matter to soils through fertilizer application and root C deposition generally occurs at the surface. Soil labile C tend to decrease with soil depth due to higher root C inputs and C inputs from fertilizer application in surface layers. A decrease in root-derived C reduced soil labile C accumulation due to low soil microbial decomposition activity (O'Brien et al., 2010; Chen et al., 2015).

In corn, 26-34% of ^{14}C was released as respiration from the corn roots as a source of energy for soil microorganisms (Liljeroth et al., 1994).

Corn plants usually have low root: shoot ratio, resulting in low root C input compared to the aboveground part, resulting in low labile C across depths (Rasse et al., 2005). Therefore, no significant effect was observed in soil POXC concentration across depth in sweet corn. This result indicates that sweet corn has much larger and deeper roots than lettuce. More relative roots with depth likely contributed to the lack of a soil POXC concentration decline with depth in sweet corn.

Other than C inputs from the fertilizer treatments, differences in plant rooting depth can shift soil C concentrations across the soil profile (Kramer and Gleixner, 2006). Depth effect of soil POXC concentration was observed only in lettuce, but not in sweet corn, may be due to rooting depth influencing root-derived C input. In our study, while collecting soil samples, we observed that lettuce rooting depth was generally between 0-30 cm depth. It may be possible that lettuce root deposition contributed to higher soil POXC concentrations at 0-30 cm depth compared to 30-60 cm depth. In our study, soil POXC concentration at 0-30 cm depth in lettuce (Table G5) was within a similar range as reported by Culman et al. (2012) and Wang et al. (2014). Belowground, roots are a major source of soil C input (Rasse et al., 2005). In fact, roots release C as a source of energy to soil microorganisms (Neumann, 2006). We expect that the limited amount of lettuce root below 30 cm, reduced soil POXC concentration in the 30-60 cm compared to 0-30 cm depth.

Maynard and Hochmuth (2007) documented that sweet corn rooting depth was between 0-60 cm, while lettuce rooting depth is commonly concentrated in the 0-30cm depth (Althaus et al., 2009). Trumbore (2000) found that labile C concentration declines with increasing soil depth. Zhong et al. (2015) found that soil POXC concentration were highest at 0-20 cm depth and gradually decreased as soil depth increased. At deeper soil depths, roots are protected by cooler temperatures and less aeration (Rasse et al., 2005), resulting in slower decomposition rates.

7.4.3 Soil POXC concentration response to N application rate

Different crop species affected soil POXC concentration and sweet corn marketable yield at the same N rate applied. The soil POXC concentration of sweet corn at the low N rate (56 kg N ha^{-1}) was higher by 2.8% compared to lettuce at the high N rate (56 kg N ha^{-1}) (Table G5). We expect that the longer growing periods of sweet corn compared to lettuce (Table G2) may result in higher soil POXC concentration compared to lettuce after harvest.

Lower soil POXC concentration in lettuce at 56 kg N ha^{-1} compared to sweet corn at 56 kg N ha^{-1} was likely due to both higher application rates and lower root C inputs in lettuce compared to sweet corn. The quantity of ^{14}C allocation from lettuce roots into the soil was two to five times lower than in corn (Kuzyakov et al., 2002). In sweet corn, no significant N rate effect between the low N rate and control treatments in soil POXC concentration (Table G5) may be due to the small amount of recalcitrant C pools at 56 kg N ha^{-1}, which is difficult to detect over short periods of growing season compared to the high N rate (Song et al., 2014).

Other than difference in the total C inputs from fertilizer treatments, we expect that sweet corn and lettuce differ in root C inputs and rooting depth, which may affect the opposite trend of soil POXC concentration in sweet corn and lettuce. C inputs provide an energy source for soil microbial growth and activity (Tassara et al., 2008). In our study, the different pattern observed in soil POXC concentration between crop species may be due to root-derived C difference between crop species, as reported by Neumann (2006). In lettuce, higher soil POXC concentration was observed in the control than the cyano-fertilizer treatment, while the opposite trend was found in sweet corn.

Soil POXC concentration had no relationship with N content, leaf N, and residual soil NO_3^--N in sweet corn and lettuce. Awale et al. (2013) found no significant correlation between soil POXC concentration and residual NO_3^--N within corn-sugarbeet-soybean rotation. Awale and his colleagues claimed that their result might be due to short duration of growing season since the fertilizers were applied.

7.4.4 Yield response to N application rate

The cyano-fertilizer treatment increased soil POXC concentration and marketable ear yield at 112 kg N ha^{-1} in sweet corn, but not in lettuce, which may be due to the low N rate applied compared to sweet corn. Between 50 kg N ha^{-1} and 80 kg N ha^{-1} of N application rate is needed for lettuce to maximize lettuce yield in an organic system (Stopes et al., 1989). It was reported that sweet corn requires between 112 kg N ha^{-1} and 240 kg N ha^{-1} (Bravo et al., 1995) for yield development. In general, no significant difference in the marketable ear yield of sweet corn in the control treatment (0 kg N ha^{-1}) and 56 kg N ha^{-1} (Table G6), which may be due to the long term compost application history at the research site. The slow organic N decomposition associated with composts could improve nutrient uptake over time.

7.5 Conclusion

In conclusion, amount of C inputs and crop species may have affected soil POXC concentration in a single season study from 39 to 79 days of growing season. Soil POXC concentration was higher in the cyano-fertilizer treatment compared to the control treatment in sweet corn, while the opposite trend was found in lettuce. Depth effect was observed in soil POXC concentration at 0-30 cm compared to 30-60 cm in lettuce, but not in sweet corn. Soil POXC concentration was higher at 112 kg N ha^{-1} compared to 56 kg N ha^{-1} in sweet corn, but there was no N rate effect in lettuce. Greater soil POXC concentration and marketable ear yield of sweet corn were observed in the cyano-fertilizer treatment compared to others at 112 kg N ha^{-1}. Higher residual soil NO_3^--N was observed at 0-30 cm compared to deeper depths in sweet corn and lettuce. However, no significant relationship observed among residual soil NO_3^--N, leaf N concentration, and N content with soil POXC concentration in sweet corn and lettuce. Overall, our results indicate that soil POXC concentration was responsive to N fertilizer application over a short-term growing season of horticultural crops.

TABLES

Table G1. Initial soil properties of the 0-30 cm depth of the experimental sites.

	Sweet corn	Lettuce
pH[§]	7.5	7.5
Electrical conductivity[§] (dS m^{-1})	0.6	0.6
Cation exchange capacity (cmol kg^{-1})	29	29
Organic matter (%)	2.5	2.7
NH_4^+-N[ǂ] (ppm)	2.5	2.4
NO_3^--N[ǂ] (ppm)	1.9	4.0
P[¶] (ppm)	30	31
K[♯] (ppm)	463	473
Ca[♯]	4560	4513
Mg[♯]	616	634
Fe[♯]	6.0	6.3
Zn[♯]	1.4	1.6

[§] pH and electrical conductivity were determined in water (1:1).
[ǂ] Samples were extracted for inorganic N (NH_4^+-N and NO_3^--N) using 2M KCl.
[¶] P content was extracted with the Olsen P extractant ($NaHCO_3$).
[♯] Samples were extracted using a ammonium bicarbonate diethylenetriamine pentaacetate (AB-DTPA).

Table G2. Amount of precipitation, irrigation, and fertigation over the growing season in 2014.

	Sweet corn	Lettuce
Planting date	July 1, 2014	June 9, 2014
Harvest date	September 19, 2014	August 4, 2014
Precipitation	----------mm----------	
June	--	34
July	69	69
August	26	0.3
September	9	--
Water applied	----------mm----------	
Irrigation	737	230
Fertigation	414	460
Total[§]	1257	783

[§]Total amount of water applied over the growing season is the combination of precipitation, irrigation, and fertigation. All plots received the same amount of water applied via irrigation. When liquid fertilizers were applied, the control and solid fertilizer treatments were irrigated with the same amount of water as that used to fertigate the liquid fertilizer treatments.

Table G3a. Nutrient analysis of fertilizer samples.

	P[#]	K[#]	Ca[#]	Mg[#]	Fe[#]	Zn[#]
Fertilizer	----------mg kg^{-1}----------					
Cyano-fertilizer	6	0.1	10	18	6	0.1
Fish emulsion	1600	20510	725	921	131	18
Feather meal	640	1776	1466	575	62	18
Blood meal	32	366	904	283	118	14

[#]Samples were digested with nitric acid (HNO_3) and hydrogen peroxide (H_2O_2).

Table G3b. Total C and N, and C: N ratio of fertilizers samples.

	Total C[§]	Total N[§]	C: N ratio	Total C input		
Fertilizer				28 kg N ha^{-}	56 kg N ha^{-1}	112 kg N ha^{-1}
	----------%----------			----------mg kg^{-1}----------		
Cyano-fertilizer	0.04	0.2	0.20	1.2×10^4	2.3×10^4	4.5×10^4
Fish emulsion	0.07	5	0.01	1.9×10^4	3.9×10^4	7.8×10^4
Feather meal	51	13	3.92	1.5×10^7	2.9×10^7	5.7×10^7
Blood meal	51	13	3.92	1.5×10^7	2.9×10^7	5.7×10^7

[§]Samples were analyzed using CN analyzer.

Table G4a. Analysis of variance (ANOVA) of soil permanganate oxidizable C (POXC) concentration.

	Sweet Corn	Lettuce
	P > F	P > F
Depth	0.4526	0.0179*
N rate	0.0287*	0.1650
Treatment	0.0344*	0.0454*
Depth x Treatment	0.9442	0.4730
N rate x Treatment	0.0013*	0.5141
Depth x N rate x Treatment	0.9997	0.6238

*P-values are significantly different at $P < 0.1$.

Table G4b. Analysis of variance (ANOVA) of marketable yield of sweet corn and lettuce.

	Sweet corn	Lettuce
Whole model	$r^2 = 0.532$, $P < 0.0013$, n=36	$r^2 = 0.439$, $P < 0.0028$, n=36
	P > F	P > F
N rate	0.0001*	0.0364*
Treatment	0.0038*	0.1416
N rate x Treatment	0.0025*	0.3126

*P-values are significantly different at $P < 0.1$.

Table G5. Mean separation of soil permanganate oxidizable C (POXC) concentration in sweet corn and lettuce.

	Sweet	Lettuce[§]
Depth	----------mg kg^{-1} soil----------	
0-30 cm	1253 a	1167 a
30-60 cm	1246 a	1154 b
60-90 cm	1239 a	--
90-120 cm	1231 a	--
Treatment		
Control	1194 b	1181 a
Blood meal	1197 b	1177 a
Feather meal	1234 ab	1171 ab
Fish emulsion	1247 ab	1167 ab
Cyano-fertilizer	1254 a	1106 b
N rate[ǂ] (kg N ha^{-1})		
0	1227 b	1155 a
28	--	1176 a
56	1214 b	1181 a
112	1251 a	--

[§]Soil samples were collected at 0-30 cm, 30-60 cm, 60-90 cm, and 90-120 cm depths in sweet corn, while 0-30 cm and 30-60 cm depths in lettuce.

[ǂ] Fertilizers were applied at 56 kg N ha^{-1} and 112 kg N ha^{-1} in sweet corn. In lettuce, fertilizers were applied at 28 kg N ha^{-1} and 56 kg N ha^{-1}.

Table G6. Marketable yield of sweet corn and lettuce at harvest.

	Sweet Corn	Lettuce
N rate[‡] (kg N ha^{-}	----------kg ha^{-1}----------	
0	1043 b	779 c
28	--	1483 a
56	1069 b	1269 b
112	1439 a	--

[‡] Fertilizers were applied at 56 kg N ha^{-1} and 112 kg N ha^{-1} in sweet corn. In lettuce, fertilizers were applied at 28 kg N ha^{-1} and 56 kg N ha^{-1}.

Table G7. Residual soil NO_3^--N of sweet corn and lettuce at harvest.

	Sweet corn[§]	Lettuce[§]
	----------kg N ha^{-1}----------	
Depth (cm)		
0-30	3.3 a	3.9 a
30-60	1.7 b	2.6 b
60-90	1.5 b	--
90-120	1.2 c	--

[§]Soil samples were collected at 0-30 cm, 30-60 cm, 60-90 cm, and 90-120 cm depths in sweet corn, while 0-30 cm and 30-60 cm depths in lettuce.

FIGURES

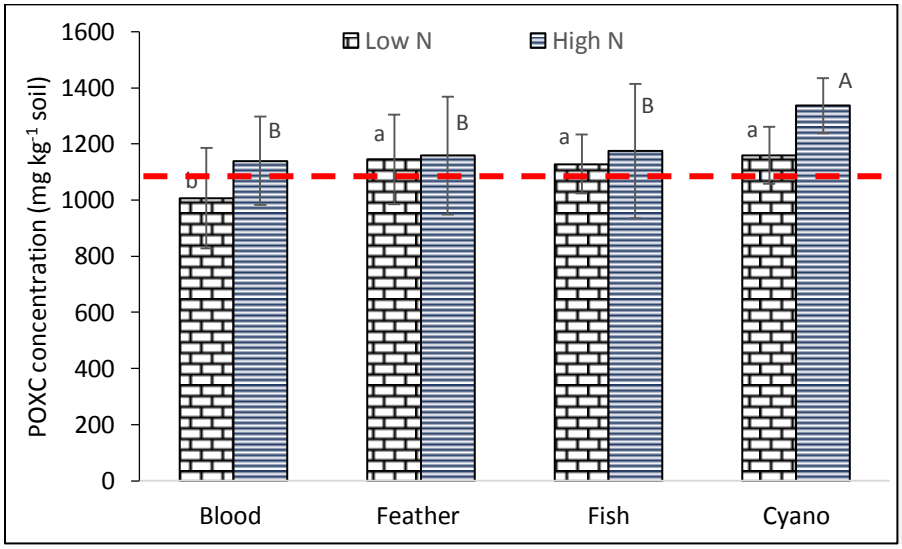

Figure G1. Interaction effects between fertilizer treatments and N rates on sweet corn soil permanganate oxidizable carbon (POXC) concentration in 2014 after harvest. Fertilizers were applied at 56 kg N ha^{-1} (Low N) and 112 kg N ha^{-1} (High N) in 2014. Bars represent standard deviation of mean. Means with the same letter are not significantly different at $P < 0.1$ using Tukey's Studentized Range (HSD) test of mean separation. Upper case letters indicate significant differences within mean at the high N rate (112 kg N ha^{-1}), while lower case letters indicate significant differences within mean at the low N rate (56 kg N ha^{-1}). The dashed line represents control. Cyano-fertilizer treatment at the high N rate was significantly different from control at $P < 0.1$. Blood = Blood meal; Feather = Feather meal; Fish = Fish emulsion; Cyano = Cyano-fertilizer.

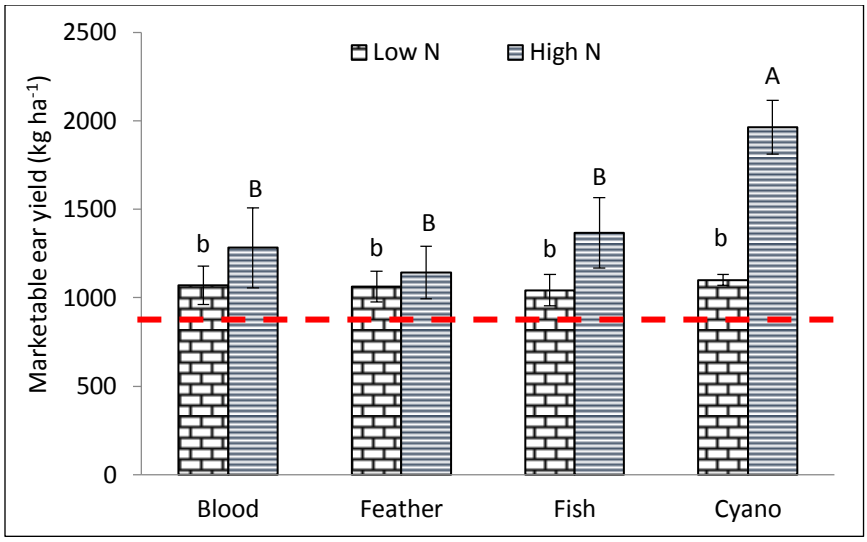

Figure G2. Interaction effects between fertilizer treatments and N rates on sweet corn marketable ear yield in 2014 after harvest. Bars represent standard deviation of mean. Means with the same letter are not significantly different at $P < 0.1$ using Tukey's Studentized Range (HSD) test of mean separation. Upper case letters indicate significant differences within mean at the high N rate (112 kg N ha^{-1}), while lower case letters indicate significant differences within mean at the low N rate (56 kg N ha^{-1}). The dashed line represents control. All fertilizer treatments at the high N rate (112 kg N ha-1) were significantly different from control at $P < 0.1$. Blood = Blood meal; Feather = Feather meal; Fish = Fish emulsion; Cyano = Cyano-fertilizer.

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OVERALL CONCLUSIONS

This study contributes to the fundamental knowledge of lettuce and sweet corn response to organic nitrogen (N) fertilizers in terms of nutritional value (β-carotene, Fe, and Zn), water use efficiency (WUE), residual soil NO_3^--N, N content, NUE, and soil permanganate oxidizable carbon (POXC). Overall, the cyano-fertilizer treatment had a higher yield in sweet corn and lettuce. Higher marketable yield and NUE were observed at 28 and 56 kg N ha^{-1} compared to 112 kg N ha^{-1} in lettuce. Ear yield, N content, and NUE of cyano-fertilizer was on par with fish emulsion at 112 kg N ha^{-1} in sweet corn.

The blood meal treatment had a higher residual soil NO_3^--N compared to other fertilizer treatments after harvest while higher residual soil NO_3^--N was observed in the fish emulsion and feather meal treatments in sweet corn. The result indicates that blood meal is a quick release fertilizer and the response was observed in lettuce, which has shorter growing season compared to sweet corn. The slow-release properties of feather meal fertilizer, which has lower N mineralization compared to blood meal, which showed a treatment effect in residual soil NO_3^--N in sweet corn after harvest (79 days). Blood meal is preferable due to a quick release compared to feather meal, which is known as a slow-release fertilizer due to its high keratin concentration. Feather meal reduced net photosynthetic rate, and dry weight of sweet corn. Blood meal, fish emulsion, and cyano-fertilizer are sources of organic N fertilizer which increase Fe and Zn concentrations compared to feather meal.

Either fish emulsion or cyano-fertilizer could be one of the organic N sources for growing lettuce in clay loam soil due to the effect of phytohormones. Indole-3-acetic acid (IAA) in the fish emulsion fertilizer increased β-carotene. Salicylic acid (SA), which was highest in the cyano-fertilizer treatment, increased lettuce yield components (fresh weight and leaf area). The cyano-fertilizer treatment had a higher WUE possibly due to the high amount of SA applied in the cyano-fertilizer treatments.

The quantity of total C inputs from fertilizer treatments and crop root C inputs may have affected soil labile C. Higher soil POXC concentration was measured in the cyano-fertilizer treatment compared to

the control treatment in sweet corn, while the opposite trend was found in lettuce. Soil POXC concentration could be a key indicator of soil management practices to N fertilizer application over a short-term growing season of horticultural crops.

RECOMMENDATIONS

The blood meal fertilizer would be a better source of solid N fertilizer due to its higher yield compared to feather meal. In organic systems, organic growers usually apply solid fertilizers all at once prior to planting, due to convenience and high labor and fuel costs. However, this practice increases the potential for soil microbial and chemical processes to transform applied organic N into forms of plant-available N, such as NO_3^--N which could leach below the root zone and contaminate groundwater.

For liquid fertilizer, there is potential for cyano-fertilizers to be a source of N fertilizer used by organic growers. The marketable yield, total crop N uptake, and NUE of cyano-fertilizer is on par with fish emulsion. Cyano-fertilizer could be a potential alternative source of N because it can be grown in managed ponds on-farm. thus reducing organic grower's dependence on off-farm fertilizer sources, which often incur high transportation costs and reducing carbon footprint in the atmosphere. We suggest that cyano-fertilizer could be an ideal source of N for organic growers due to its low residual NO_3^--N compared to fish emulsion. Organic growers could apply cyano-fertilizer directly through drip irrigation systems. In our study, no clogging problems of drip emitters were encountered, as long as drip lines were flushed after each fertilizer application.

Further research needs to be conducted to understand the mechanism of phytohormone impact of organic N fertilizers on β-carotene concentration in lettuce. Do inorganic N forms and phytohormones influence enzymes in the carotenoid biosynthesis pathway? If yes, what mechanisms are involved in the biosynthesis process? More research collaboration needs to be done to fully understand the chemical, biological, and genetic regulation mechanisms influencing β-carotene concentration in lettuce.

In our study, phytohormones were only measured in fertilizers. For future research, targeted phytohormones (IAA and SA) could be measured in plant tissues. Phytohormone profiling in plant tissue samples would be a wise step to start with, in order to assess interactions among fertilizer types with phytohormones in plant tissue. In terms of improving Fe and Zn concentrations in lettuce and sweet corn,

siderophores secretion from cyanobacteria in cyano-fertilizer should be measured prior to fertilizer application. Organic acid and siderophore secretion from lettuce and sweet corn roots could be measured in response to organic N fertilizer application in calcareous soils.

Further research needs to be conducted to understand the mechanisms of plant water regulation related to inorganic N forms and phytohormone concentrations in fertilizers. Additional leaf gas exchange measurements could be incorporated to determine whether any influence of organic N fertilizer application on abscisic acid (ABA) in plant tissue in regulating stomatal closure, thus regulating WUE in sweet corn. In our study, leaf gas exchange measurement was conducted only in a C4 crop (sweet corn). It would be interesting to investigate whether WUE from N fertilizer application would have the same effect on other C4 crops (sorghum and proso millet) and C3 crops (lettuce, kale, and spinach).

In this study, soils were sampled at harvest and the pattern of residual soil NO_3^--N could only be observed at harvest. A mini lysimeter study using PVC pipe with perforated holes could be implemented in the greenhouse to track the interaction between root length density and residual soil NO_3^--N at different crop growth stages over the course of the growing season.

A greenhouse study comparing two different cultivation methods (hydroponic vs. soil systems) could be conducted on lettuce and sweet corn to minimize environmental variables such as slow N release from a long term history of compost application at the CSU Horticulture Farm. Root measurements such as root length density and root surface area of lettuce and sweet corn grown in pots would be ideal to add information in improving NUE at harvest.

Other than using POXC as a sensitive indicator to soil management practices, soil microbial biomass activity and root-derived C measurements should be included in future work in order to understand the mechanism of labile C in response to N fertilization. It was reported in other studies that cyanobacteria secrete extracellular polysaccharides and other organic N fertilizers secrete organic acids during decomposition. Polysaccharides and organic acids secreted from the decomposition process could aid in the formation of soil aggregates. Since soil organic matter consists of a heterogenous mixture (labile C fractions, polysaccharides C, organic acids, etc.), quantifying the functional groups of labile C

and root-derived C in response to organic N fertilizer application could be considered in order to know what proportion of labile C represents the C pools under organic agriculture.