INTEGRATING NITROGEN FERTILIZER STRATEGIES, MODELS, AND COVER CROPS TO OPTIMIZE CORN PRODUCTION AND SOIL HEALTH

By

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ABSTRACT

INTEGRATING NITROGEN FERTILIZER STRATEGIES, MODELS, AND COVER CROPS TO OPTIMIZE CORN PRODUCTION AND SOIL HEALTH

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Degradation of Great Lakes water quality and weather variability emphasize improved nitrogen (N) management strategies that synchronize N availability with corn (Zea mays L.) N uptake. Field studies were conducted at either Lansing or Richville, Michigan from 2014 to 2016 and included investigations of cover crops (radish [Raphanus sativus (L.) var. The Buster], forage oats [Avena sativa (L.) var. Magnum], or no cover crop), and N management strategies (starter N as in-furrow [IF] or sub-surface banded 5-cm beside and 5-cm below the seed furrow [5x5] followed by sidedress [SD] at V4, V10, or split 50/50, pre-plant incorporated N with or without enhanced efficiency N, poultry litter [PL] followed by V11 SD, and zero N). Assessments included cover crop biomass quantity and quality, soil NO3-N levels, corn grain production and profitability, soil basal CO2 respiration, and soil bacterial community composition (16S rRNA sequencing). Radish and oat cover crops (CC) seeded in August reduced autumn soil NO3-N levels 78 – 84% from the no CC control. The following year CC residues reduced N availability to a corn cash crop and reduced effectiveness of 5x5 starter N to maintain yield potential when full SD was applied at V11. Sidedress N delayed from V4 to V11 resulted in similar or reduced yield and profitability. Soil bacterial diversity at R1 was inversely proportional to corn grain yield and suggested bacterial relative abundance may increase indication of yield rather than diversity.

Dedicated to my family and friends who have provided encouragement along the way.

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CHAPTER 1

LITERATURE REVIEW

Michigan Corn Production

In 2016 corn (*Zea mays* L.) production accounted for 27% of total United States crop acres and 32% of total Michigan (MI) crop acres (USDA-NASS, 2017). Corn grain production is Michigan's second largest cash receipt commodity (>1,000 million US$) and was ranked 13th (2016) in average United States production (Mg^{-1}) (https://www.nass.usda.gov/Quick_Stats/Ag_Overview/stateOverview.php?state=MICHIGAN; verified 15 Oct. 2017). Crop nitrogen (N) fertilization represents 59% of major U.S. applied plant nutrients with corn accounting for >45% of total N fertilizer applied at a mean rate of 157 and 137 kg N ha^{-1} in the U.S. and MI, respectively (USDA-ERS, 2016).

Tissue Nitrogen

Nitrogen is a constituent of chlorophyll molecules, proteins, enzymes, nucleic acids, a component of energy transfer compounds such as adenosine diphosphate (ADP) and adenosine triphosphate (ATP), and influences carbohydrate utilization during photosynthesis (Havlin et al., 2014). Nitrogen is often the most limiting nutrient in grain cropping and natural systems due to its large plant elemental composition (1 – 6%) (Fernández et al., 2009; Robertson and Vitousek, 2009). Like many crops fertilizer N is applied to corn to supplement native soil N supplies. Corn N requirements are a function of physiological requirements for biomass yield and tissue N content (Cassman et al., 2002). Corn grain has been found to remove 11.6, 4.8, and 3.5 kg m^{-3} of N, P_2O_5, and K_20, respectively, and a corn yield of 10,000 kg ha^{-1} may remove 190 kg N ha^{-1}

(Cassman et al., 2002; Warncke et al., 2009). Corn yield potential is affected by interacting factors including agronomic practices [e.g., fertility management], cultivars, and the environment (Evans and Fischer, 1999). Corn production will require accurate estimates of soil N supply and corn requirements across environments when determining fertilizer N application strategies.

Climate Variability

Recent studies project more heat waves (5°C above climatic normal) and increasing air temperatures (1.5 to 2.0°C) over the next 30 years may reduce early spring frost dates (1 wk) in the northern hemisphere in addition to increased precipitation frequency in winter and spring months with more intense events [> 50.8 mm in 48 hours] (Schwartz et al., 2006; Tebaldi et al., 2006; Hayhoe et al., 2007; Karl et al., 2009). Warming spring temperatures are likely to move corn planting dates earlier than currently optimal [1 and 7 May] (Lauer et al., 1999). In lieu of mounting concerns over Great Lakes Basin surface- and ground-water quality, weather variability may increase N loss opportunities and require further refining of N management strategies in response to changing climates (Scharf et al., 2002; Scavia et al., 2014; Dove and Chapra, 2015).

Nitrogen Use Efficiency

Nitrogen use efficiency (NUE) has been defined as the ratio of output (biological or economic yield) to N supply, where biological yield consists of total plant dry matter or total plant N, economic yield as grain yield or total grain N (Cassman et al., 2002; Ladha et al., 2005). Nationally the price of urea-N and N-solutions, two commonly used corn N sources, have risen from $159 and $104 per ton in 2000 to $472 and $327 in 2013, respectively (http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1002; verified 14 Nov. 2017). Corn prices received by growers have sharply declined from $6.89 in 2012 to $3.65 in 2014 (https://www.nass.usda.gov/Quick_Stats/Lite/index.php; verified 14 Nov. 2017). Raun and Johnson (1999) reported worldwide NUE for cereal production was only 33%, while the remaining 67% represented a $15.9 billion annual loss of N fertilizer. A regional survey of combined on-farm corn research experiments conducted across Illinois, Michigan, Minnesota, Missouri, Nebraska, and Wisconsin found that 37% of applied N fertilizer was recovered in aboveground plant biomass (Cassman et al., 2002). Previous studies indicate N management strategies that account for N loss opportunities may improve NUE (Raun and Johnson, 1999). Despite a linear trend for increasing NUE from 1980 to 2004, Bundy (2006) suggested that year to year variation of NUE illustrated a need for improved assessment of NUE factors such as N rate selection, accounting for non-fertilizer N sources, and managing applied N. Increased costs of fertilizer N, decreased grain prices, and continued degradation of Great Lakes water quality, suggests further improvement of N management strategies that simultaneously increase NUE and reduce opportunities for N loss (Dove and Chapra, 2015; USEPA, 2017).

Corn Response to N Fertilizer

Soil texture and weather can interact to change N availability in the corn rooting zone and affect corn response to applied N (Tremblay et al., 2012). A 25 mm rainfall has moved nitrate downward 25 mm in clay loam soils and 63.5 mm in sandy soils (Nelson and Huber, 2001). Heavy rainfall events (i.e., > 25.4 mm) in June may reduce corn N availability through denitrification or leaching beyond the active rooting zone (Kyveryga et al., 2010). Rainfall frequency may also impact corn response to N. Infrequent rainfall events may result in dry soil conditions and reduce plant fertilizer N uptake and increase residual inorganic N levels (Gagnon et al., 2012; Jokela and Randall, 1997).

4R Nutrient Management

To provide a framework of site-specific nutrient management decisions, the 4R nutrient stewardship concept has been adopted by the world's fertilizer industry. The 4R concept emphasizes the right 'source' of nutrient application, applied at the right 'rate', at the right 'time', and in the right 'place' to guide best management practices (BMP) that ensure fertilizer uptake is optimized, and environmental loss is minimized (Roberts, 2007). While the 'right' source, placement, and time can help achieve synchrony between N supply and N uptake, correct N rate emphasizes N excess and deficiency. Optimizing the '4R's may help synchronize N availability with crop N demands (Randall and Mulla, 2001). The concept of BMPs is fundamental to efficient and judicious use of nutrients. General fertilizer recommendations can fail to account for external factors such as crop rotation, rainfall, pest pressure, and temperature (Mikkelsen, 2011). In conventional tillage systems spring pre-plant N applications to medium- and fine-textured soils is considered a BMP although spring pre-plant applications of anhydrous ammonia to no-till and strip-till systems may be undesirable to some growers due to delayed planting and compaction of wet soils (Vetsch and Randall, 2004). Growers should consider the ability of the soil to supply N, crop N requirements, climate, and management capabilities when determining 4R requirements (Roberts, 2007).

Corn N application rates

Corn N application rates are based on N demand minus indigenous N soil contributions (Cassman et al., 2002). Biological transformation can result in several mobile forms of N susceptible to leaching, volatilization, surface runoff, gaseous plant emission, denitrification, or erosion making N a dynamic and leaky system (Randall et al., 2008; Millar et al., 2010; Congreves and Eerd, 2015). The optimum N application rate has been regarded as the point where the last increment of N fertilizer results in a profit just large enough to pay for the N (EONR). Unlike phosphorus and potassium plant available N lacks the mechanism for long-term mineral soil storage of weatherable N pools (Robertson and Vitousek, 2009). Applications of N at the EONR can minimize N losses (Hong et al., 2007; Jokela and Randall, 1997; Stevens et al., 2005a). Predicting the optimum rate of N for corn production has both economic and environment implications.

Growers have previously relied on ‘insurance N’ where greater applications were made to ensure sufficient amounts are available for plant N uptake (Schröder et al., 2000). However, rates in excess of crop N uptake may influence the potential for NO_3^- leaching or denitrification, especially during wet and warm conditions (Dinnes et al., 2002). Karlen et al. (1998) found the potential for N loss was greatest when residual soil NO_3^- was present without active crop growth. Andraski et al. (2000) observed increased NO_3^- in groundwater where N rates were in excess of a corn crop EONR (150 kg ha^{-1}). Studies by Steven et al. (2005a; 2005b) determined where excessive corn N (> 201 kg N ha^{-1}) applications were made: 1) mineralization of residual soil N is increased and 2) response of subsequent crops to fertilizer N may decrease due to increased mineralization of newly formed organic N pools and crop residues. Increased mineralization due to excess N application (>201 kg N ha^{-1}) increased the risk of N loss to the environment and emphasized the importance of fertilizing at optimal N levels (Stevens et al., 2005a).

Maximum return to nitrogen: Michigan N recommendations are based on crop N utilization and response to applied N. In 2006 Michigan adopted the Maximum Return to Nitrogen (MRTN) recommendation system. The MRTN is a pre-season general recommendation model (as compared to a field-specific or site-based model) that does not account for individual site variability nor variable in-season weather trends which can affect corn N response. The MRTN system was developed by Iowa State University as a regional guideline approach to N recommendations and has been adopted by Illinois, Michigan, Minnesota, Ohio, and Wisconsin. The MRTN approach assumes a linear increase in N cost and a grain yield plateau. The MRTN system is updated annually and N rate incorporate variability among years. The MRTN approach was preceded by yield-based N recommendations based on Stanford (1973), which utilized a mass-balance approach accounting for dry matter yield goal and non-fertilizer N contributions. Yield goal was multiplied by a 15.4 kg m^{-3} (1.2 lb N per bushel) factor, and adjusted for non-fertilizer N contributions (Sawyer et al., 2006). The rate constant was supported by research demonstrating that optimum yield (yield at the economically optimum N rate) divided by the optimum N rate was about 1.5 kg N hL^{-1} (Fernández et al., 2009). Problems associated with this system included unrealistic yield goals, lack of a general use consensus, and poor relationships of actual optimum N rates and N recommendations (Vanotti and Bundy, 1994). In Michigan, the yield goal approach was used to develop a linear N recommendation equation based on yield potential (Warncke et al., 2009). Using the yield goal approach, over-application of N could result from the assumption of limitless yield with increasing N application.

Illinois soil N test versus the Michigan presidedress soil nitrate test: Assessing the soil's ability to supply N for plant uptake may help synchronize available N with uptake and reduce the potential for NO_3^- leaching (Andraski et al., 2000; Dinnes et al., 2002). Soils where manure has been previously applied may not respond to additional N applications (Scharf et al., 2002). Soils unresponsive to N illustrate the importance of determining first year N availability from animal manure and credit this portion of available N towards N application rates (Oberle and Keeney, 1990). Mulvaney et al. (2006) utilized the Illinois soil N test (ISNT) to quantify corn response to N fertilization in nonresponsive soils in various rotations at a critical test level of 230 mg kg^{-1} amino sugar N. The ISNT measured amino sugar N compounds which may mineralize during the growing season. Fernández et al. (2009) stated when cool soils limited N mineralization into early June, high values of the ISNT did not correspond to reduced fertilizer N needs. In Michigan the presidedress soil nitrate (PSNT) test is used to measure residual NO_3^- from previous season inputs and early-season mineralized organic-N (Warncke et al., 2009). The PSNT involves sampling approximately 6 weeks after planting and indicates the additional amount of fertilizer N to supply and appropriate N credits when tests levels are $\geq$10 and $\leq$ 25 mg NO_3^- kg^{-1} (Dinnes et al., 2002; Fernández et al., 2009). Sidedress N rates with PSNT adjustment have resulted in <8.0 mg L^{-1} flow-weighted NO_3^- concentrations in percolate and 8-10% less total N applied when compared to unadjusted N rates on a Woodbridge fine sandy loam soil (Guillard et al., 1999).

Corn N application timing

Michigan State University does not recommend fall applications of N to corn in Michigan. May rainfall and soil temperature >10°C promoting nitrification can reduce corn grain yield and N uptake when N demand is low (Randall and Vetsch, 2005). A survey in 2005 indicated <5% of total annual corn N was fall applied in MI over 2.2 million acres (Randall and Sawyer, 2008). Sanchez and Blackmer (1988) found 49-64% of fall applied N to be lost from the upper 1.5 m of surface soils, and wet and warm soil conditions during the spring may exacerbate N losses (Vetsch and Randall, 2004). Nitrogen recovery and grain yield were reduced 42 and 20% (year 1), respectively, with fall versus spring applied N in addition to decreased corn grain N concentration from a poorly drained clay-loam soil and did not include a nitrification inhibitor (Vetsch and Randall, 2004). In northern states (i.e., Minnesota) soils can be poorly drained, and spring pre-plant N applications are often utilized in conventionally tilled soils (Vetsch and Randall, 2004).

Failure to supply N to corn at critical stages of growth may lead to yield loss (Binder et al., 2000; Subedi and Ma, 2005; Walsh et al., 2012). Weather, planting date, and N application timing may affect N uptake (Scharf et al., 2002). Traditional sidedress N timing in Michigan is V4 to V6 corn which leaves time for soil incorporation by rainfall and does not require the use of high-clearance equipment. Early studies by Karlen et al. (1988) suggested two patterns of N accumulation: 1) V12-V18, and 2) during grain fill. Recently Bender et al. (2013) observed N uptake by corn is greatest during V10-V14 and at reproductive (R) stages R2-R6 with limited uptake at tasseling (VT)/R1. Utilizing modern corn hybrids Bender et al. (2013) observed that corn had taken up 65% of total N by R1 and total grain N comprised 58% of total N uptake when

N was not limiting. Improved fertility practices should focus on matching available N during these periods of high vegetative uptake.

Applying corn fertilizer N after planting may be an effective strategy to increase NUE and reduce overall N rates (Gehl et al., 2005; Walsh et al., 2012). Utilizing split-N applications, Gehl et al., (2005) concluded yield-goal based N rates could be reduced 40% as compared to a single pre-plant application in Kansas. Split N applications (45 kg pre-plant/45kg V6 or V10) maximized NUE and doubling these N rates maximized grain yield (Walsh et al., 2012). Delayed N applications may avoid exposure to early-season weather events and reduce opportunities for N loss, but growers can risk irreversible yield loss due to N stress (Binder et al., 2000; Scharf et al., 2002). Jokela and Randall (1997) further noted N uptake was reduced from 54 to 48 kg ha^{-1} when N application was delayed until V8 due to extremely dry soil conditions limiting N availability. Maximum grain production will require earlier N applications when corn is N stressed (Binder et al., 2000). A single N application at V6 was found to reduce yield up to 12% when the chlorophyll meter N sufficiency index was below 0.90 (Binder et al., 2000), but a grower missing the opportunity to apply N early could apply N up to silking with a 15% yield reduction (Scharf et al., 2002). Walsh et al. (2012) determined sidedress N applied at V6 allowed for crop recovery when no pre-plant N was supplied but sidedress N beyond V6 did not. When pre-plant N was provided (45 or 90 kg ha^{-1}) no yield differences were observed among sidedress timings of V6, V10, or tasseling (VT) (Walsh et al., 2012). In-season weather variables should guide sidedress decisions where increased June rainfall can reduce corn N availability (Gagnon and Ziadi, 2010; Kyveryga et al., 2010).

Corn N fertilizer source

Corn response to N fertilizer source can be affected by corn management and weather and may affect urea-N fertilizer sources. Up to 35% of applied N has been lost due to non-incorporated applications of urea and UAN if no rain fell within 6 days of application in no-till corn (Fox and Hoffman, 1981). Volatilization of surface applied urea to wheat were found to exceed 50% when urea was surface applied to Saskatchewan soil types (Aridic, Typic, Vertic, and Udic Haploborolls) (Fowler and Brydon, 1989). On a Kamouraska clay soil (Orthic Humic Gleysol) a split application of calcium ammonium nitrate (CAN) + UAN sidedressed at V6 produced comparable corn yields relative to polymer coated urea (PCU) applied in a single pre-plant incorporated (PPI) application (Gagnon et al., 2012). In the same study, PCU, UAN at V6 sidedress (injected), and urea with a nitrification inhibitor N sources resulted in yields ≥ 0.6 Mg ha^{-1} as compared to urea PPI in wet soils when average June-July soil temperatures were cool (17.6ºC). In cool and humid climates UAN has increased mean apparent N recovery 44.4% as compared to CAN and aqua ammonia (AA) (Gagnon and Ziadi, 2010). Increased apparent N recovery of UAN corresponded to a lower optimum N rate of 100 kg ha^{-1} required to maximize grain yield relative to 124 and 128 kg N ha^{-1} observed for CAN and AA, respectively (Gagnon and Zaidi, 2010).

Application of enhanced efficiency fertilizer (EEF) N sources can be used to extend N release for periods of corn N uptake and achieve comparable recovery efficiency (48-56%) to UAN sidedress V6 (Gagnon et al., 2012). Observing leaf chlorophyll values during grain fill, Hatfield and Parkin (2014) demonstrated the effectiveness of EEF sources (i.e., PCU, stabilized urea with urease and nitrification inhibitors, and UAN with or without a combination urease and nitrification inhibitor). In their study delayed leaf senescence resulted in a greener canopy and corresponded to improved grain yield despite variable weather among study years. Economic analysis revealed PCU and UAN increased net return \$95 to \$263 ha^{-1} in wet years relative to urea but in a warm and dry season UAN returned \$154 ha^{-1} as compared to PCU \$-46 ha^{-1} (Gagnon et al., 2012). Michigan studies on Capac loam soils attained mean corn yields of 9.7 Mg ha^{-1} (NUE = 0.61 Mg grain kg N^{-1}) when PCU was blended with urea (75:25) and broadcast + incorporated 2 to 4 weeks before planting as compared to yields of 8.3 Mg ha^{-1} (NUE = 0.46 Mg grain kg N^{-1}) achieved with a similar application of 100% urea (Steinke and Chomas, 2014). When 102 mm less rainfall was avoided with at-plant N applications, a 25:75 blend PCU + urea was sufficient to increase corn yield 2.7 Mg ha^{-1} relative to pre-plant urea-N applications demonstrating the potential of PCU fertilizer to reduce N loss for surface N applications (Steinke and Chomas, 2014).

Corn starter N fertilizer placement

Collectively, fertilizers placed with or near the seed at planting are generally referred to as starter fertilizer. Starter fertilizer may utilize either single element or complete NPK mixtures even in soils testing high in P and K (Niehues et al., 2004; Rehm et al., 2009). Starter fertilizer increased corn yield in Wisconsin 0.27 Mg ha^{-1} and decreased grain moisture at harvest 0.5% regardless of soil test phosphorus (STP) (Bundy and Andraski, 1999). Starter fertilizer has increased yield in Iowa 1.1-2.4% and early growth (V5-6 whole plant dry weight and N uptake) 20-32% but studies noted early season corn growth and nutrient uptake associated with starter N do not always correspond to yield improvements (R=0.44) or STP (Bermudez and Mallarino 2002; 2004; Kaiser et al., 2005). Plant biomass (V6-V8) has been increased more in irrigated corn (30%) at 5 sites as compared to rainfed corn (10%) at 3 of 5 sites with starter fertilizer application although grain yield was only increased 0.86 Mg ha^{-1} at irrigated sites (Wortman et al., 2006).

In-furrow: Michigan growers sometimes place fertilizer with corn seed at planting to improve early corn growth. In-furrow nutrient placements (sometimes referred to as "pop-up" or “seed-placed” starter) can provide immediate nutrient access to young corn roots (Niehues et al., 2004). In-furrow placements have been more sensitive to fertilizer rate than sub-surface band placements (Niehues et al., 2004). Salt rates ($N+K_2O$) in excess of 11.2 kg ha^{-1} could delay seedling emergence until precipitation occurs and result in uneven corn stands (Raun et al., 1986). Reported salt indices for N sources utilized by corn growers are 20.0, 63.0, and 74.4 for ammonium polyphosphate (10-34-0), UAN 28%, and urea, respectively (Kaiser and Rubin, 2013). Niehues et al. (2004) found 56 kg N ha^{-1} placed in-furrow reduced corn populations 12,000 and 21,000 plants ha^{-1} in two notably dry years on a silt loam textured soil. When April + May rainfall increased 100%, little effect on corn stand was observed at the 45 and 56 kg N + K_2O rates illustrating the potential dilution of reduced N rates at planting (Niehues et al., 2004). Niehues et al. (2004) concluded the N component was responsible for reduced seedling emergence in contrast to Rehm and Lamb (2009) who recommended the sum of $N+K_2O$ should guide considerations due to salt damage. Common in-furrow corn fertilizers include 10-34-0, 7-21-7, and 9-18-9. At equal P_2O_5 rates (0, 14, 28, and 56 kg ha^{-1}), 10-34-0 and 7-21-7 have resulted in less corn stand reduction (>93%) as compared to 9-18-9 (41 to 66%) (Gerwing et al., 1996). To prevent seedling injury Michigan recommendations for in-furrow applications to corn are $\leq$5.6 kg ha^{-1} N + K_2O with seed where CEC is < 7 cmol(+) kg^{-1}, and $\leq$ 9 kg ha^{-1} $N+K_2O$ where CEC is $\geq$ 8.

Subsurface band: Subsurface placement can vary relative to seed placement with bands 5cm below and 5cm laterally ("2x2" or "sub-surface [SS]band") as compared to within the seed furrow "pop-up" or "in-furrow" (Niehues et al., 2004). In-furrow and SSband placements have been utilized at corn planting in Michigan to encourage early season plant growth and nutrient uptake where cool and moist soil conditions associated with early planting dates can affect corn ontogeny (Bollero et al., 1996). Rates of SSband N up to 134 kg N ha^{-1} did not affect plant population while 34 kg N ha^{-1} optimized yield (5.4 to 8.3 Mg ha^{-1}) on silt loam textured soils in Kansas. Increased yield consistency has been observed with the use of SSband placement as compared to in-furrow placements (Ritchie et al., 1996).

Corn Response to Starter N

Predicting corn response to starter fertilizer application has been inconsistent (Randall and Hoeft, 1988). A review of placement methods by Randall and Hoeft (1988) concluded that efficacy of starter fertilizer placement was dependent on crop, soil texture, tillage system, and climatic conditions. Magnitude of corn yield response to starter fertilizer has been more likely when STP is <16 mg kg^{-1} suggesting response to a P component (Bermudez and Mallarino, 2002; 2004; Kaiser et al., 2005; Wortman et al., 2006). Grid-point soil sampling confirmed spatially-variable yield response to starter NPK to portions of fields with low STP (15 mg kg^{-1}) deemed otherwise unresponsive (P=0.16) (Bermudez and Mallarino, 2004). Starter fertilizer has also increased corn yield where soil STP ≥24 and attributed to an N or P component of the product (Bermudez and Mallarino, 2002; 2004; Vetsch and Randall, 2002). For example corn yield response to starter fertilizer was increased (0.20 to 0.67 Mg ha^{-1}) in fields where STP was low (<16 mg P kg^{-1}, Bray-P_1) (Bermudez and Mallarino, 2002). In soils with high STP (21 to 30 mg kg^{-1}) corn yield response to in-furrow placement (4.5 to 9.1 kg N ha^{-1}) was less (0.08 to 0.19 Mg ha^{-1}) compared to a SSband placement (16.3 to 27.2 kg N ha^{-1}) (0.17 to 0.47 Mg ha^{-1}) (Bermudez and Mallarino, 2002). Bundy and Andraski (1999) determined an arithmetic combination of relative hybrid corn maturity date (increasing maturity date) + plant date (increasing plant date) (PDRM) increased the profitable yield response 34% where soil K levels were below excessively high (EH; <140ppm) and 17% where soils were above EH. Bundy and Andraski (1999) attributed increased yield from SSband placement relative to a no SSband control to increased yield potential realization due to early season growth stimulation. Positive economical response to the SSband placement was most likely when PDRM > 235 and soil K levels are < 140 mg kg^{-1} (Bundy and Andraski, 1999).

Tillage has affected corn response to starter fertilizer. Spring (beginning Julian day 100) soil temperatures and gravimetric water content have been increased 3 to 5° and reduced 20 to 40 g kg^{-1}, respectively, in conventional till (CT) (fall chisel 20 cm + spring disk [2x] 10 cm) as compared to reduced (fall) or no-till (NT) corn on a silt loam soil in Wisconsin (Wolkowski, 2000). The authors reported yield increased 0.5 Mg ha^{-1} with SSband starter as compared to a no starter control across tillage systems despite excessively high STP (48 mg kg^{-1}) and STK (167 mg kg^{-1}) but observed no yield increase when SSband was used in a CT system (Wolkowski, 2000). On a silt loam soil in Minnesota corn emergence and yield were reduced 2.7 d and 7%, respectively, in NT continuous corn (CC) as compared to CT (Vetsch and Randall, 2000). An SSband starter N-P-K increased corn height >5 cm observed 35 d after emergence and yield 0.5 Mg ha^{-1} as compared to a no starter control in a corn-soybean (CS) and CC rotation despite high

STP and high STK (≥24) and ≥130 mg kg^{-1}, respectively) (Vetsch and Randall, 2000). Deep placement of starter fertilizer (15 cm) was not an effective placement as compared to SSband for increasing yield in the north (Minnesota and Wisconsin) (Vetsch and Randall, 2000; Wolkowski, 2000). In Iowa corn response to liquid starter placement (SSband or in-furrow N-P-K) was similar across CS tillage systems (NT or spring tillage) in reference to a no starter control when P and K were broadcast 1 to 4 mo. before tillage (Bermudez and Mallarino, 2004). The authors further noted soil test levels (P, K, pH, and OM) and soil series were not useful predictors of a yield increase to starter fertilizer (Bermudez and Mallarino, 2004).

Soil texture (clay loam and silty clay loam as compared to loamy fine sand) has affected corn yield response to N rate (13.1 as compared to 6.6 kg ha^{-1}) using 10-15-0 (placed either in-furrow, 1.25, or 1.90 cm away from seed) (Rehm and Lamb, 2009). Grain yield reductions of 6.9 and 14.3% corresponded to reduced emergence of 8.6 and 31.9%, respectively, when 10-15-0 was applied at 13.1 kg N ha^{-1} on a loamy fine sand textured soil (Rehm and Lamb, 2009). Grain yield was not increased relative to a control (without starter) regardless of starter grade, rate, or placement in contrast to increased V4-5 whole plant weight and P uptake on clay loam and silty clay loam textured soils (Rehm and Lamb, 2009). Stand loss was noted on sandy soils with in-furrow placement of 93.5 L ha^{-1} 10-15-0 but equivalent rates of 4-4-8 N-P-K and 63.6 L ha-1 3-8-15 N-P-K did not (Rehm and Lamb, 2009). In fine-textured soils placement of all grades did not impact emergence demonstrating the importance of soil texture when considering in-furrow sources and rates (Rehm and Lamb, 2009).

Corn Nutrition Status

Chlorophyll meter

Determining corn N status may be a useful tool to correct in-season N deficiencies and improve N management (Hawkins et al., 2007; Varvel et al., 1997). The Konica Minolta SPAD 502 chlorophyll meter (CM) has been used to determine chlorophyll content of plants relative to corn receiving non-limiting rates of N (rel. chlorophyll). Light passing through a leaf is captured by another sensor below the leaf and the filtered wavelengths is a relative indication of chlorophyll content per leaf unit area (Richardson et al., 2002). Studies have determined chlorophyll contents obtained in corn leaves 1 week before silking (a + b=total) were correlated to tissue N content (r^2=0.97) and a predictor of N sufficiency (Asghari and Hanson, 1984; Lohry, 1989). Leaf tissue N has been correlated to CM readings while differences in greenness associated with hybrids emphasize the importance of reference strips (Rorie et al., 2011; Schepers et al., 1992; Varvel et al., 1997). Readings have been used to detect N deficiencies in irrigated corn to initiate additional N applications as well as N sufficiency where normalized readings were determined in regards to a high N reference strip to estimate excessive N fertilization (Blackmer and Schepers, 1995; Piekielek et al., 1995). Nitrogen sufficiency indices (i.e., sufficiency index) can be used to monitor crop N status and are determined using readings taken for a crop divided by the reference or reading taken from the crop where N was not limiting. Varvel et al. (1997) used the CM to separate N deficient and sufficient corn beginning at V8 using a sufficiency index of 95% and used this to make 30 kg N ha^{-1} applications beginning at V8 corn to obtain maximum yields. Corn N supply was deemed insufficient when V8 corn fell below a sufficiency index of 90% and full yield was not achieved with further N additions (Varvel et al., 1997). At the late milk stage Piekielek et al. (1995) observed a corn N sufficiency index of 0.93. Samborski et al. (2009)

provided a comprehensive review of methods and strategies which included normalization of CM data used to establish relationships between N rates and CM data and indicated the need for accurate reference strips.

NDVI

Normalized difference vegetation index (NDVI) is an indicator of green biomass, indicates plant vigor, and has been used as a tool to assess in-season corn N status and adjust N rates (Tucker, 1979; Teal et al., 2006; Samborski et al., 2009). Deriving N rates utilizing NDVI requires correlation of NDVI and yield to determine yield potential at a particular corn growth stage (Teal et al., 2006). Using the GreenSeeker sensor, Teal et al. (2006) observed significant relationships between yield and NDVI and noted V8 corn resulted in the strongest correlation (R^2=0.77). In-season measurements at V9 could not determine treatment differences due to canopy closure (Teal et al., 2006). Normalizing NDVI with growing degree days (GDD_{50}) increased the timeframe for assessment plus N application where NDVI sensed at 800 to 1000 GDD's was correlated with yield (R^2=0.76), which encompassed V7-9 corn (Teal et al., 2006).

Basal cornstalk nitrate test

The Basal Cornstalk Nitrate Test (CSNT) is a late-season assessment of deficient/sufficient/excess corn N supply for the growing season taken 1-3 weeks after black layer formation on 80% of kernels (Fox et al., 2001). The CSNT indicates N supply to the corn plant enabling growers to assess optimization of N management practices. In Michigan, 'low' (<700 mg NO_3^- kg^{-1}), 'optimal' (700 to 2000 mg NO_3^- kg^{-1}) and 'excessive' (>2000 mg NO_3^- kg^{-1}) CSNT levels are used as criteria (Silva, 2011). Brouder et al. (2000) determined a critical cornstalk NO_3-N level of 1.67 g kg^{-1} separated sufficient from excessive N categories while at 2900 mg kg^{-1} fertilizer efficiency approached zero. Values of CSNT have been positively correlated to residual soil nitrate illustrating the test's ability to detect luxury consumption (Forrestal et al., 2012; Fox et al., 2001). Precipitation can impact results of the CSNT. Less than 2000 mg NO_3^- kg^{-1} (excessive) did not always correspond to low residual soil NO_3-N values where drought reduced corn production (Forrestal et al., 2012). Likewise, excessive rainfall in June has been found to reduce N availability which decreased the likelihood of obtaining 'excessive' CSNT values (Kyveryga et al., 2010).

Cover Crops and Soil N Dynamics

High N demands of crop systems coupled with soil N mobility present a niche were the use of living ground covers can be used to alter N cycling. Cover crops (CC) have been found useful to reduce soil erosion, increase OM, reduce soil compaction, and provide suppression of weeds (Kaspar and Singer, 2011). Winter annual CCs have been used to reduce the risk of N loss in the non-cash crop growing season of Michigan corn rotations, but spring kill date can impact synchrony of N mineralization and corn N needs (Crandall et al., 2005; McSwiney et al., 2010). In Michigan crop rotations winter wheat (soft red or soft white) (*Triticum aestivum* L.) sometimes precedes corn, and is often harvested in July leaving approximately a 90 d period of fallow soils prior to the first fall freeze (-2.2°C) that CCs may be established (National Oceanic and Atmospheric Administration [NOAA], http://www.weather.gov/dtx/firstFallfreeze; verified 02 Aug. 2017). In one Michigan study, winter wheat and cereal rye CCs preceding a corn cash crop were used to "tighten" the N cycle where early-season N availability preceding the corn cash crop was immobilized until N uptake by the corn in August (McSwiney et al., 2010). In the same study, no differences in corn yields or total N uptake were observed between CC and no CC plots. On an irrigated loamy sand Andraski et al. (2005) increased corn grain yield (1.4 Mg ha^{-1}) utilizing oat, winter triticale, and winter rye CCs, reduced risk of N loss on sandy soils, and reduced mean EONR 32 kg N ha^{-1} as opposed to winter fallow.

Cover crop kill date and incorporation method preceding a corn cash crop may affect in-season corn N availability (McSwiney et al., 2010). Delaying CC kill date can decrease the amount of available N for early corn N uptake (Crandall et al., 2005). A cereal rye CC killed 7 d before corn planting in May in Illinois resulted in reduced corn biomass, N uptake, and yield (Crandall et al., 2005). Grassy CCs similar to cereal rye may need to be killed no sooner than 14 d preceding a corn cash crop to avoid yield reductions and indicate corn N strategies that include the use of delayed N applications may have to be implemented to account for reduced N availability due to CC (Crandall et al., 2005). Cover crop residue decomposition and N mineralization and immobilization are dependent on the carbon (C) to nitrogen ratio (C:N) and lignin concentrations (Wagger et al., 1998; Jahanzad et al., 2016). Growers may need to consider CC biomass quality and quantity when deliberating between fertilizer N strategies to synchronize corn N demand and N availability.

Radish cover crops

Radish (*Raphanus sativus* L.) is a non-leguminous *Brassicaceae* CC utilized as a N scavenger in the non-growing season to sequester available soil NO_3-N (Mutch and Thelen, 2003; Warncke et al., 2009). Radishes are a large-taproot annual that grow quickly in late summer and autumn and have been observed to reduce soil compaction and suppress winter annual weeds (Williams and

Weil, 2004; Clark, 2007; Lawley et al., 2011). Radish winterkills when air temperatures drop below -4 °C eliminating the need for chemical termination (Thomas et al., 2017). Corn grain yields following an autumn radish cover crop have varied showing both no effect (Vyn et al., 2000; Gieske et al., 2016a; 2016b) and increased yield (Dapaah and Vyn, 1998; Vyn et al., 1999; O'Reilly et al., 2012) compared to no cover crop. Decreased C:N associated with a radish cover (14 to 31:1) relative to a grassy cover (15 to 38:1) (e.g., oats, triticale, or ryegrass) may help explain the 43 and 24% greater soil NO_3-N concentrations with a radish cover crop at mid-May corn planting and mid-June N sidedress application in Ontario, respectively (Vyn et al., 1999; Andraski and Bundy, 2005).

Oat cover crops

Oat (*Avena sativa* L.) is a non-leguminous cover crop also used to sequester residual soil N and prevent wind and water erosion (Warncke et al., 2009). Similar to radish, oats are an annual cover crop and that is subject to winterkill (Johnson et al., 1998; Snapp et al., 2005). Unlike radish, oat has a dense, fibrous root system characteristic of cereal cover crops (Thorup-Kristensen, 2001). Increased lignin and cellulose may reduce decomposition rates and dry matter loss in relation to a radish CC (Jahanzad et al., 2016). The C:N ratio of oat may exceed 30:1 which can immobilize soil N and reduce availability for corn uptake (Adraski and Bundy, 2005; Dean and Weil, 2009). Corn yield following oat cover crops have been generally unaffected or decreased in rainfed studies (Johnson et al., 1998; Vyn et al., 2000; O'Reilly et al., 2012). However, Andraski and Bundy (2005) suggested corn yield gains due to oat cover crops in an irrigated study were an effect of rotation and not N contributions as evidenced by similar May soil NO_3-N contents and reduced EONR (32 ± 8 kg N ha^{-1}). Variable corn yield response to antecedent CCs suggest corn N fertilizer strategies may have to account for N availability affected by CC decomposition rates.

Plant, Soil, and Microbial Community Interactions

The soil is a living system composed of biological, chemical, and physical fractions (Doran and Zeiss, 2000). Plants, soils, and soil microbes function simultaneously to influence plant health and productivity (Chaparro et al., 2012). Plant-microbe interactions can enhance plant growth through ecosystem services such as nutrient cycling, plant pathogen control (biocontrol agents), and phytohormone production (e.g. auxin) (Nihorimbere et al., 2011). Soil microorganisms mediate transformations of N P, K, S, Fe, Zn, Cu, and Mn to soluble forms while plants can stimulate microbes through rhizodeposition of C-rich root exudates and residues, root morphology, and genotype (Berg and Smalla, 2009; Lambers et al., 2009; Aira et al., 2010; Prashar et al., 2014; Coyne and Mikkelsen, 2015). Greater than 95% of soil N is in organic form and requires microbial conversion for plant uptake (Havlin et al., 2014). Microflora can also impact soil structure. Plant roots and fungal hyphae can "enmesh" larger aggregates, while secretions from roots, fungal hyphae, bacteria, and fauna (i.e. earthworms) are involved in stabilizing smaller aggregates (Oades, 1993). In a barley rhizosphere, Cytophaga-like bacteria produced exopolysaccharides to decompose soil OM and contributed to soil aggregation (Johansen and Binnerup, 2002). A recent study conducted at four locations across Michigan determined the interaction of location, plant species, and soil attributes explained more variation (27%) in structure of bacterial communities than when each factor was considered alone (3.5%, 2.1%, and 0.7%, respectively) ($P \leq 0.15$) and illustrated the complex relationship between bacterial populations and their environment (da C. Jesus et al., 2010).

Increased soil bacterial diversity (e.g. functional, taxonomic) may enhance functional resiliency and increase overall microbial community stability against stress (i.e., environmental variation or disturbance) (Eisenhauer et al., 2012; Awasthi et al., 2014). The removal of crop residues, tillage, and reduced cropping system diversity may affect C substrate quality and reduce soil bacterial community diversity and abundance (Ceja-Navarro et al., 2010; Figuerola et al., 2015; Sun et al., 2016). Nitrogen fertilization strategies may select for distinct microbial communities or reduce community diversity while CCs may provide opportunities to increase cropping system diversity and affect microbial communities that can benefit cash crop health (Berg and Smalla, 2009; Li et al., 2016; Soman et al., 2017).

Effect of Plant Diversity on Soil Microbial Communities

Plant species diversity can influence the structure and function of microbial communities (Berg and Smalla, 2009). In greenhouse studies, introduction of new plant species to soils which had been in corn monoculture over 50 years resulted in increased microbial community richness, diversity, and new microbial species within eight weeks (Maul and Drinkwater, 2010). Addition of hairy vetch (*Vicia villosa*) and cereal rye (*Secale cereal*) to a tomato production system increased the absolute microbial biomass but increased available C from the cover crops reduced Gram-positive bacteria (Buyer et al., 2010). In the same study the microbial biomass under hairy vetch was greater than that of bare soil in May and illustrated the effect of root exudation and turnover (Buyer et al., 2010). Using phospholipid fatty acid (PLFA) analysis Zak et al. (2003) observed increased plant diversity altered heterotrophic microbial community composition, and increased the supply of soil N (Zak et al., 2003). Some plant species such as Velvetleaf (*Abutilon* *theophrasti*), Common Lambsquarter (*Chenopodium album*), Redroot Pigweed (*Amaranthus retroflexus*), and Green Foxtail (*Setaria viridis*) can be unwanted in corn systems, and when growth is left uncontrolled in the spring can have a deleterious effect on the abundance of arbuscular mycorrhizal fungi (AMF) (Wortman et al., 2013). Plant roots can also serve as a habitat to soil microbes in addition to plant anchorage and nutrient acquisition. Root surface area has been found as a good predictor of microbial diversity and community structure using terminal restriction fragment length polymorphism targeting the 16S rRNA gene (Maul and Drinkwater, 2010).

Influence of Soil Properties on Microbial Community Composition

Soil physiochemical factors such as pH can influence microbial community composition and function. Using PCR-DGGE Wakelin et al. (2008) observed decreasing catabolism of C-substrates with declining pH levels between fine and coarse-textured soils. Plants utilizing NH_4^+ release H^+ in the process, which can decrease the pH of the rhizosphere (Abbott and Murphey, 2003). Nutrient management practices that influence pH may therefore impact microbial dynamics. In contrast, Buyer et al. (2010) did not find soil pH to be a significant factor in microbial community composition in bulk soil. A difference of 1.1 and 0.6 pH units in different years explained only 2.7% and 1.5% of microbial community composition variance in bulk soil (Buyer et al., 2010). Soil nutrient levels may also affect soil microbial community composition. Redundancy analysis has indicated that soil pH, P, K, Ca, NH_4-N, and OM concentrations have correlated to soil bacterial community structure in soybean, canola, sunflower, and switchgrass rhizosphere soils at four Michigan locations (da C. Jesus et al., 2010).

Rhizosphere Soil

The rhizosphere is an area of soil in the vicinity and influence of plant roots consisting of the endorhizosphere, rhizoplane, and ectorhizosphere and extends 1-2 mm the root surface (Prashar et al., 2014). Bulk soil is the volume not influenced by plant roots. Plant roots are able to congregate microbial communities through the exudation of rhizodeposits, growth, respiration, and nutrient exchange making the rhizosphere a "hot spot" of microbial activity in relation to the surrounding bulk soil (Mahmood et al., 2005; Prashar et al., 2014). Rhizodeposits are carbon-(C) rich allowing microbes to utilize as a source of energy or be inhibited by them (Mahmood et al., 2005; Neumann and Römheld, 2007). Rhizodeposits include sloughed root cells and lysates, border cells, and root exudates in the form of sugars, amino acids and amines, aliphatic-, aromatic-, and fatty acids, sterols, enzymes, miscellaneous vitamins, and plant growth regulators (Neumann and Römheld, 2007). As opposed to bulk soil the rhizosphere may contain bacteria, actinomycetes, and fungi in 2-20, 5-10, and 10-20 proportions, respectively (Morgan et al., 2005). Actinomycetes are a member of the Actinobacteria phylum and contain subgroups of *Streptomyces* associated with crop disease suppressiveness (Wiggins and Kinkel, 2005; Mendes et al., 2011). Microbial activity may be up to 50 times higher in rhizosphere soil as compared to the bulk soil (Prasher et al., 2014). A corn rhizosphere soil was found to contain 3.6x more microbial biomass and 1.2x higher bacterial population density relative to surrounding bulk soil demonstrating higher C-substrate availability (Mahmood et al., 2005). Rhizosphere bacterial phyla found most abundant in a canola, corn, soybean, sunflower rotation were Acidobacteria, Proteobacteria, Actinobacteria, and Verrucomicrobia (da C. Jesus et al., 2010). Bacterial population density was found to be higher on corn and wheat roots relative to the corresponding rhizosphere soil and lowest in the bulk soil which suggests similar soil samples contaminated with unequal amounts of root biomass may have contrasting amounts of bacterial populations (Mahmood et al., 2005).

Plant Growth Promoting Rhizobacteria

Bacteria beneficial to plant growth and productivity have been termed Plant Growth Promoting Rhizobacteria (PGPR) and can colonize plant root surfaces in the rhizosphere, rhizoplane, and within plant tissue (endophytes) (Tilak et al., 2005). Colonization of PGPRs can be symbiotic or non-symbiotic (includes free-living, associative, or endophytic) and both groups are capable of N fixation (Tilak et al., 2005). Symbiotic nitrogen fixing PGPRs include cyanobacteria of the genera Rhizobium, Bradyrhizobium, Azorhizobium, Allorhizobium, Sinorhizobium, and Mesorhizobium (Hayat et al., 2010). Free-living nitrogen fixing PGPRs have included bacterial species of Azospirillum, Enterobacter, Klebsiella, and Pseudomonas (Hayat et al., 2010). Plant growth promotion by symbiotic and non-symbiotic PGPRs can be direct through the fixation of atmospheric N, phosphorus solubilization, and production of plant growth horomones such as auxins, cytokinins, gibberellins, ethylene, and abscisic acid (Tilak et al., 2005). Indirectly, PGPRs enhance plant growth by siderophore production for Fe uptake and provide protection from deleterious phytopathogenic organisms (Hayat et al., 2010). In vitro studies have found *Pseudomonas* spp. produced more hydrogen cyanide, a phytopathogen inhibitor, than *Bacillus* spp. in addition to an increase in siderophore production of 2.87 mm 5 day-1 (Jarak et al., 2012). Symbiotic PGPR reside inside plant cells and produce nodules often in association with leguminous plants although non-legumes (i.e., other crops in rotation) may benefit from fixed N (Hayat et al., 2010).

Non-leguminous plants such as corn, wheat, rice, sugarcane, and cotton can benefit from PGPR effects of non-symbiotic diazatrophs through increased vegetative growth and yield (Kennedy et al., 2004). For example corn dry weight was increased 49.4-81.8% ($P<0.05$) in the presence of fertilizer N after 6 weeks of growth in greenhouse studies using seed inoculated with *H. seropedicae* in non-sterilized soils as compared to an un-inoculated control. However, when N was withheld dry weight increased an average 15.6%, illustrating the potential of diazotrophic bacteria to increase NUE for biomass production in corn (Riggs et al., 2001). Field studies in Africa utilizing corn seed inoculated with a combination of PGPR's including *Azotobacter chroococcum*, *Pseudomonas* spp., and *Bacillus* spp. increased V3-6 corn dry weight 61.3% and yield >1000 kg ha^{-1} as compared to a non-inoculated control and concluded that co-inoculation increased dry matter production over a single inoculation of each PGPR (Jarak et al., 2012).

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CHAPTER 2

CORN GROWTH AND YIELD RESPONSES TO PRE-PLANT AND IN-SEASON NITROGEN COMBINATIONS

Abstract

In-season nitrogen (N) applications offer flexibility to synchronize availability with corn (Zea mays L.) uptake but deliberating between early (V4) and late sidedress [SD] (V11) require further validation due to increased climate variability. A six site-year study investigated N-placement, timing, and source combinations on corn yield, profitability, and growth. Three strategies were investigated involving pre-plant incorporated (PPI), in-furrow [IF] (7.8 kg N ha-1), or sub-surface banded N fertilizer below the furrow [5x5] (44.8 kg N ha-1). Treatment combinations within the IF and 5x5 starter N strategies included sidedress (SD) at V4, V11, or 50/50 (split) V4 and V11. Pre-plant incorporated (PPI) strategies involved 100% urea, 25/75 mix of urea with polymer-coated urea, and poultry manure applied at 2.2 Mg ha-1 plus SD N V11. With the exception of a poultry manure application in wet soils, there was no yield benefit to late season N (i.e., V11) application with similar profitability trends. The IF strategy required reduced N rates at planting and sometimes reduced yield potential when SD was delayed from V4 to V11, unlike the 5x5 strategy. Splitting N application (i.e., multi-pass) increased yield 4.4 to 16.1% compared with a one-pass PPI strategy in 4 of 6 site years. Canopy NDVI at V6 indicated success of SD time may depend on the ability of N strategies to supply N until SD time in order to maintain yield potential. Risk of reduced yield potential late (i.e., V11) was greater than the risk of reduced yield potential early (i.e., V4) suggesting the V11 timing should only be utilized as a rescue application when growers miss a V4 timing to obtain yields ≥ 11.4 Mg ha-1.

Introduction

In the Northern Corn Belt spring preplant N applications are considered a best-management practice in conventionally tilled, poorly drained, medium- to fine-textured soils (Vetsch and Randall, 2004). Corn yield potential is affected by interacting agronomic practices [e.g., fertility management], cultivars, and the environment (Evans and Fischer, 1999). However, recent studies project more heat waves (5°C above climatic normal), increasing air temperatures (1.5 to 2.0°C) over the next 30 years, changing early spring frost dates (1 wk) in the northern hemisphere, and increasing precipitation frequency in winter and spring months with more intense events [> 50.8 mm in 48 hours] (Schwartz et al., 2006; Tebaldi et al., 2006; Hayhoe et al., 2007; Karl et al., 2009). Warming spring temperatures are likely to move corn planting dates earlier than currently recommended [1 and 7 May] (Lauer et al., 1999). Weather variability may increase risk of early applied N loss (immobilization, leaching, denitrification, and clay fixation) and suggests N management strategies should be revisited in response to climate change (Scharf et al., 2002; Scavia et al., 2014; Dove and Chapra, 2015). Optimizing fertility management includes adjusting strategies to account for the right placement, time, source, and rate (e.g., '4R') of nitrogen (N) adapted to site or region-specific environments since corn response to N is mediated by soil texture and weather (Snyder et al., 2009; Tremblay et al., 2012; Norton, 2014; Venterea and Coulter, 2015). Since rapid corn N uptake does not occur until V10, early planting dates combined with spring weather volatility suggest delayed N applications (up to V10) would mitigate the time lapse between N availability and uptake but further research is needed to refine current grower practices (Bender et al., 2013).

One N management strategy used by growers are pre-plant incorporated (PPI) applications. The PPI strategy represents a one-pass system where 100% of the N inputs are applied up to planting time and incorporated. Other one-pass systems include pre-emergent applications (PRE) applied 100% at-plant or within 1 to 3-d after planting (e.g., weed 'n' feed) using urea or urea ammonium nitrate (UAN), and spring pre-plant N applications. In MI, the majority of corn acreage is located on calcareous soils with a soil pH > 7.2 which can potentially increase NH_3 volatilization losses, potential for immobilization, and reduce the efficiency of surface applied urea containing N sources (Vetsch and Randall, 2000; Rawluk et al., 2001; Havlin et al., 2014). In MI studies, urea blended with polymer coated urea (PCU) (75/25 percent PCU/urea blend ratio) have improved corn yields up to 1.38 Mg ha^{-1} relative to 100% urea PPI (2-4 weeks) when Apr. and May rainfall were above avg (Franzen, 2017). In the same studies a PCU/urea blend (75/25) extended N activity in dry soils which increased corn grain yield 1.07 Mg ha^{-1} relative to a V4-6 urea surface banded sidedress (SD) application. Despite the recommended use of spring preplant N applications in the Northern Corn Belt, early weather can be volatile with above-average rainfall in April, May, and June, increasing the risk of N loss conditions (Vetsch and Randall, 2004). Single spring pre-plant N applications have reduced yield 0.39 Mg ha^{-1} relative to split applications in Minnesota and 2.45 Mg ha^{-1} relative to a single at-plant application in MI (Randall et al., 2003; Franzen, 2017). Reduced efficiency of early applied N has prompted some MI growers to utilize a multi-pass system to improve N recovery but limited MI data utilizing the PPI strategy warrant further research including in-season N applications (Warncke et al., 2009).

In an attempt to improve synchrony between N applications and plant N uptake, grower interest in late N (e.g., post-V11) applications has increased. In-furrow (IF) nutrient placement (i.e., "popup" or “seed-placed” starter) is one strategy to provide immediate nutrient access to emerging corn roots as fertilizer is placed with the seed at planting (Niehues et al., 2004). Due to less planter equipment, the IF placement is popular in the Northern Corn Belt (Kaiser et al., 2016). However to prevent yield reductions due to seedling injury or delayed emergence, reduced fertilizer rates and non-urea containing forms of N are used to avoid excessive salt concentrations or ammonia toxicity (Raun et al., 1986; Laboski et al., 2008; Rehm and Lamb, 2009). Michigan IF fertilizer recommendations to corn include ≤ 5.6 kg N+ K2O ha^{-1} where CEC is < 7 cmol(+) kg^{-1} and ≤ 9.0 kg N+K_2O ha^{-1} where CEC is ≥ 8 cmol(+) kg^{-1} (Steinke, 2013). In-furrow starter fertilizer has increased early season plant height and kernel mass, while decreasing days to silking and grain moisture (Kaiser et al., 2016). Despite increased early season plant growth IF applications have not corresponded well to grain yield (r = 0.44) (Bermudez and Mallarino, 2002; 2004). In-furrow starters often contain phosphorus (P) and may contain an N, P, and K combination. In soils testing low in phosphorus (STP) (<16 mg P kg^{-1}, Bray-1 test) positive grain yields using IF starter fertilizer were attributed to a P-component as opposed to the N-component when positive yield responses were noted in high STP soils (>23 mg kg^{-1}) in Iowa (Bermudez and Mallarino, 2002, 2004). In corn production regions with compressed growing seasons and thus shorter maturity length hybrids (i.e., MI), data on fertilizer strategies that provide minimal early-season N in favor of later applications are minimal and require further investigation.

To encourage early season plant growth and nutrient uptake, sub-surface banded N applications 5 cm below and 5 cm laterally (5x5) relative to the seed furrow is another option for at-plant N applications. The 5x5 placement requires installation of an extra planting coulter that may reduce planting speeds and can be more affected by soil moisture than IF due to bringing more soil to the surface. However, the 5x5 placement can utilize multiple N sources, and allow growers more flexibility for N rate selection relative to reduced N rates required by IF placements (Niehues et al., 2004). Corn V6 plant biomass and grain yield have increased more consistently with no-till 5x5-placed starter relative to IF placements in Illinois while 5x5 placement (N-P) increased yield 0.82 Mg ha^{-1} relative to a no 5x5 control in six no-tillage strip trials in Missouri (Ritchie et al., 1996; Scharf, 1999). In a Wisconsin study 5x5 placed starter fertilizer increased yields up to 0.7 Mg ha^{-1} relative to a no starter control when combined across multiple tillage regimes (Wolkowski, 2000). When compared to the IF placement positive yield responses to the 5x5 placement have been attributed to corn stand maintenance and increased 5x5 N rates [16.3 to 27.2 kg N ha^{-1}] (Lamond and Gordon, 2001; Bermudez and Mallarino, 2002). Corn response to 5x5 starter may also depend upon the interaction between growing season length and hybrid maturity class. On Missouri silt-loam soils increased early corn growth (6-weeks after planting) and reduced days to silking were influenced by hybrid maturity class, tillage, and 5x5 starter (N-P) application but yield was not affected (Cromley et al., 2003, 2006). Bundy and Andraski (1999) observed that a positive yield and economic response to 5x5 starter was linearly related (R^2=0.51) to the sum of hybrid relative maturity (RM) and planting date in Julian days (PD) and most likely when RM+PD > 235. Corn responded 33% and 53% of the time to starter fertilizers with relative maturity classes < and > 100 days, respectively, with a mean yield increase of 251 kg ha^{-1} relative to a no 5x5 control (Bundy and Andraski, 1999). When using the 5x5 strategy in a split-application approach (i.e., 5x5 + SD) few data exist to validate deliberation among SD times and require further investigation for use in the northern corn belt.

Growers who choose to split apply often utilize SD applications up until the V6 growth stage but interest in delayed (i.e., post-V6) N applications has increased as a strategy to better synchronize N availability and uptake while reducing N loss (Binder et al., 2000; Scharf et al., 2002). Corn N uptake does not begin to accelerate until V6 to V8 (Bender et al., 2013). However weather, planting date, and fertilizer application strategy can affect the N uptake ability of a maturing corn plant and the potential for plant N stress prior to SD time resulting in unrealized yield potential (Bundy and Andraski, 1999; Scharf et al., 2002). When compared to at-plant N applications, N applied at V8 was found to increase N recovery 11% at N rates between 75 - 150 kg N ha^{-1} (Jokela and Randall, 1997). In Missouri, Scharf et al. (2002) did not observe a yield loss when N was delayed until V11, but noted a 0-3% reduction with later applied N (i.e., V12 to V16). However delayed N applications may have less effect on plant growth and development in the Midwestern U.S. Corn Belt as opposed to northern production regions. Midwest states tend to have protracted growing seasons as compared to northern production regions, where fewer suboptimal growing days due to a compressed growing season and use of shorter relative maturity class corn hybrids may influence N synchrony (Scharf et al., 2002). Nitrogen stress due to late N application emphasizes the importance of satisfying early-season corn N requirements, and the need to identify corn response to delayed sidedress N applications. The objective of this study was to investigate the effects of early- and late-season sidedress N applications in combination with other grower N strategies on early corn growth, corn grain yield, and profitability.

Materials and Methods

Field trials were conducted from 2014-2016 at the Saginaw Valley Research and Extension Center (SVREC) (43°23'58.2"N, -83°41'52.7994"W) near Richville, MI on a Tappan-Londo loam soil (fine-loamy, mixed, active, calcareous, mesic Typic Epiaquolls) and at the South Campus Research Farm (SCRF) (42°40'24.24"N, -84°29'13.1994"W) in Lansing, MI on a Capac loam soil (fine-loamy, mixed, active, mesic Aquic Glossudalf). Fields were autumn chisel plowed following soybean [Glycine max (L.) Merr.] harvest, and followed with spring tillage using a soil finisher to a 10-cm depth. Soil samples were collected to a depth of 20 cm prior to fertilizer application, air-dried, and ground to pass through a 2-mm sieve. Soil characteristics at SVREC included 7.6-7.7 pH (1:1 soil/water) (Peters et al., 2015), 19-24 mg kg-1 P (Bray-P1) (Frank et al., 2015), 138-164 mg kg-1 K (ammonium acetate method) (Warncke and Brown, 2015), and 27-28 g kg-1 soil organic matter (loss-on-ignition) (Combs and Nathan, 2015). Soil characteristics at SCRF included 6.5-6.8 pH, 25-47 mg kg-1 P, 91-114 mg kg-1 K, and 28-34 g kg-1 soil OM. Broadcast P and K fertilizer were pre-plant incorporated (10-cm depth) prior to planting as monoammonium phosphate (MAP) (11-52-0 N-P-K) and muriate of potash (MOP) (0-0-62) based on soil tests. Weed control at SVREC consisted of S-metolachlor [Acetamide, 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)-,(S)] and glyphosate [N-(phosphonomethyl)glycine] (6 June 2014, 28 May 2015 [glyphosate only], and 9 June 2016) followed by a second application of glyphosate (20 June 2014 and 22 June 2015). Weed control at SCRF consisted of S-metolachlor and glyphosate (10 June 2014), acetochlor [2-chloro-N-ethoxymethyl-N-(2-ethyl-6-methylphenyl) acetamide] and glyphosate (defined previously) (2 June 2015 and 10 June 2016) followed by a second application of glyphosate (26 June 2014, 26 June 2015, and 27 June 2016). Environmental data were collected throughout the year using the

Michigan Automated Weather Network (http://www.agweather.geo.msu.edu/mawn/, Michigan State University, East Lansing, Michigan; verified 24 Jul. 2017) from an on-site weather station.

Ten treatments (including a non-fertilized control) were arranged in a randomized complete block with four replications. Plot width was 4.5 m by 12.1 m in length. Treatments were equalized to a total N rate based on the site-specific maximum return to nitrogen rate (MRTN) (202 and 157 kg N ha-1 for SVREC and SCRF, respectively) (Sawyer et al., 2006). Treatments were grouped into three strategies including 1) broadcast pre-plant incorporated (PPI) N, 2) in-furrow (IF) starter N (7.8 kg N ha-1 ammonium polyphosphate [10-34-0]), and 3) starter N sub-surface banded 5 cm beside and 5 cm below the furrow (5x5) (44.8 kg N ha-1 urea ammonium nitrate [UAN; 28-0-0]). Combinations within the IF and 5x5 N strategies included sidedress (SD) at V4, V11, or a 50/50 split at V4 and V11. Pre-plant incorporated N strategies included 100% urea (46-0-0), 25/75 mix of urea with polymer-coated urea (PCU) (44N-0P-0K, Agrium Inc., Calgary, Alberta, Canada), and dried poultry litter (PL) (4-3-2) applied at 2.2 Mg ha-1 plus SD N V11. Corn V4 N was UAN coulter injected 5 cm deep and 38 cm to the side of each row (Ritchie et al., 1997). Corn V11 N was UAN mixed with a urease inhibitor (CO(NH2)2 + n-(n-butyl) thiophosphoric triamide] (Koch Agronomic Services, LLC, Wichita, KS) to prevent N volatilization and banded 10 – 15 cm to the side of each row. A non-limiting N reference (280 kg N ha-1) was included to normalize canopy sensor data. An untreated control which did not receive N fertilizer was also included. A Monosem planter (Monosem Inc., Kansas City, Kansas) equipped with Yetter floating planter-unit mounted row cleaners (Yetter Manufacturing, Colchester, IL) and liquid fertilizer applicators was used to apply IF and 5x5 starter. At all sites

Dekalb DKC48-12 RIB (98 d relative maturity) (mid-season hybrid) (Monsanto Co., St. Louis, MO) was planted in 76-cm rows at 84,510 seeds ha-1 (Table 1).

Corn plant density (V3) was determined as number of corn plants in both harvest rows (9.3 m2 total area) expressed as plants per ha-1. Canopy normalized difference vegetation index (NDVI) [V6 and V11] was collected using a GreenSeeker® Model 505 handheld red-band optical sensor (Trimble Agriculture Div., Westminster, CO). Nitrogen status using the ear leaf (R1 and R4) was determined with a Minolta SPAD 502 chlorophyll meter (CM) (Konica Minolta, Tokyo, Japan) and normalized to the non-limiting N reference per replicate (Shapiro et al., 2013).

The center two rows of each plot were harvested with a research plot combine to determine grain yield, moisture, and test weight. Yield data were reported at 155 g kg-1 moisture.
Treatment profitability was calculated as net return = gross return from yield – input costs (Table 2).

Data were subject to analysis of variance using the GLIMMIX procedure in SAS assuming fixed effects of site, year, and treatment, and random block effects (SAS Institute, 2011). The UNIVARIATE procedure was used to test for normality of residuals ($P \leq 0.05$). Levene's test was used to investigate homogeneity of error variances using squared- and absolute-values of residuals ($P \leq 0.05$). The LINES option of the slice statement was used to separate lsmeans when ANOVA indicated a significant interaction ($P \leq 0.10$). Multiple degree of freedom (df) contrasts were constructed using the mean of three treatment combinations within each N strategy (e.g., the 5x5 strategy included a mean of three sidedress treatments which received 5x5 starter fertilizer). Pearson product-moment correlations were used to investigate the relationship of canopy sensor data and weather with grain yield ($P \leq 0.05$) using the CORR procedure.

Results and Discussion

Growing conditions

Total growing season (Apr. – Sept.) precipitation differed by -9, 20 and 1% and 4, -19, and -26% from the 30-yr mean during 2014-2016 at Lansing and Richville, respectively (Table 3). May and June 2015 Lansing precipitation was 24 and 103 mm greater than the 30-yr mean, but Richville 2014-2016 May and June precipitation was deficient by 6-44 mm and 20-52 mm, respectively. August precipitation was 2 – 79 mm above the 30-yr mean across all site years. Mean April air temperatures were below the 30-yr mean across all years and locations while May and June air temperatures were within 0.3 – 1.6 ºC at each location. In July 2014 air temperatures were 3.1 – 3.3 ºC below the 30-yr mean at each location. August and September mean air temperatures were within 0.5 – 2.2 ºC of the 30-yr mean across all site years.

Corn plant density

Means were combined across treatments within each strategy (i.e., IF, PPI, and 5x5) as no SD N applications occurred prior to V3 stand counts. In 2014 the IF strategy reduced plant density up to 2.6% relative to other strategies including the untreated control (Table 4). At the Lansing location dry soil conditions (i.e., 8.9 mm rainfall over 14 d following planting) may have limited diffusion of fertilizer into the soil solution while at the Richville location cool soil temperatures (4.4 °C cooler than Lansing with 5 d of consecutive minimums near 7.2 °C) may have decreased root growth. Either scenario may have increased the amount of time germinating corn roots were exposed to the fertilizer band and resulted in injury (Laboski et al., 2008). Stand reductions were not observed in other years. Plant density was evaluated as a covariate in the statistical analysis of grain yield, found not significant ($P \leq 0.05$), and was not included in grain yield analysis.

Grain yield

Pearson correlations indicated mean grain yields of treatments receiving N fertilizer increased in association with mean minimum June air temperatures ($r = 0.94$; $P=0.005$) and mean maximum July air temperatures ($r = -0.85$; $P=0.030$). Other studies have reported the influence of air temperature on post-silking dry matter production and growth rate which affect grain yield (Zhou et al., 2016). Weather correlation to yield and treatments receiving N fertilization but not the untreated control emphasizes the impact in-season weather variability may have on N strategy and corn growth.

Grain yield was influenced by the interaction of year, site, and treatment and a significant grain yield response to treatment was observed in 4 of 6 site years (Table 5 and 6). Treatment means were presented separately for each site year. Mean grain yields (i.e., treatments receiving N, 2014-2016) were 11.8 – 14.7 and 11.1 – 15.0 Mg ha-1 at the SCRF and SVRF locations, respectively, with the greatest yields occurring in 2014 at each location. In the two non-significant site years (i.e., SVRF 2015 and SCRF 2016), deficit spring rainfall (i.e., Apr.-June) resulted in dry soils leading to water-stressed corn, reduced mobility of urea-N, and no response to N placement and timing combinations (Venterea and Coulter, 2015; Maharian et al., 2016). For the four significant site years, treatments are discussed by near normal to deficit rainfall (i.e., 3 site years) and above normal rainfall (i.e., 1 site year).

When Apr. – June rainfall was near normal (i.e., SCRF and SVRF 2014) to deficit (i.e., SVRF 2016) with near normal air temperatures, no yields gains occurred with a V11 SD timing (Table 6). Precipitation timing at SVRF in 2016 coincided with SD applications and provided ≥ 6.9 mm cumulative rainfall within 3 d of each SD N application assisting N movement to corn roots and indicated that yield gains with SD still depend upon rainfall. Within the IF strategy, delaying N application from V4 to V11 resulted in similar or 7.6 – 10.1% yield reductions. No statistical differences were observed using the 5x5 strategy with a V4 or V11 SD timing, indicating a more consistent yield response across variable weather conditions and concurs with previous findings (Ritchie et al., 1996). A lack of differences indicated the additional 37 kg N ha-1 provided by the 5x5 strategy was sufficient for attaining similar yields with V4 or V11 SD relative to the IF strategy and emphasizes the importance of considering starter N strategies for unconstrained corn growth when deliberating between early and late SD timings. Relative to a split (50/50) application, SD delayed until V11 resulted in similar or 5.6 – 6.9% yield reductions across both the IF and 5x5 strategies. Yield reductions indicate that starter N (8 to 45 kg N ha-1) was likely exhausted and reduced yield potential due to deficit N supplies by V11 SD. Scharf et al. (2002) suggested that a protracted growing season (e.g., Michigan) may reduce the duration of corn N uptake and may explain full yield potential realization with a V11 SD application in longer growing season climates (e.g., Missouri). Relative to a V4 timing, full SD at V11 was too late to increase yield in near normal to deficit Apr. – Jun. rainfall conditions. Split SD N (50/50) applications did not provide additional yield gains compared to full V4 SD. Reduced yield potential suggests the V11 timing should be utilized as a rescue application only to maintain yields ≥ 11.4 Mg ha-1 when growers miss a V4 timing.

Under the same near normal to deficit rainfall conditions discussed above, pre-plant incorporated PL followed by V11 SD produced similar yields as IF and V4 SD, indicating yield potential was not increased with a slowly mineralizable N source (60.5 kg N ha-1 first year mineralizable-N) (Table 6). Poultry litter was able to extend N activity relative to other early applied N treatments (i.e., IF or 5x5 strategies) and resulted in similar or increased yields (6.6 to 9.7%) when full SD was delayed until V11. When compared to a urea or PCU and urea blends applied PPI, a PL plus V11 SD application achieved similar or increased yields (8.1 to 19.8%), respectively, across site years while the PCU did not provide yield gains as compared to urea alone. The above average June rainfall (32 mm) at the SCRF (2014) and near-normal to deficit May and June rainfall at SVRF (2014, 2016) may have reduced N loss opportunities and provided no benefit to using PCU.

Above normal May – June rainfall (127 mm excess) occurred at SCRF in 2015 resulting in wet soils. In this scenario, pre-plant incorporated PL followed by V11 SD increased yield 8.6 to 13.9% relative to IF or 5x5 starter and V4 SD (Table 6). Moist soil conditions but a lack of heavy rainfall events in May (< 21 mm) may have prevented some degree of early PPI N loss and hindered the effectiveness of PCU. Yield results with PCU were in contrast to Gagnon et al. (2012) where corn yield gains (0.8 to 1.6 Mg ha-1) due to pre-plant incorporated PCU fertilizer in Canada were observed relative to urea fertilizer in a wet spring similar to the current study. However average air temperatures in Lansing, MI were 5 to 6 °C warmer than the Canadian study which may have increased the rate of N release (Franzen, 2017). Although delaying the majority of N application until V11 may have reduced the opportunity for N losses in wet years, this practice did not increase the opportunity for yield gains in other years.

Multiple degree of freedom (df) contrast statements were constructed to compare means across strategies (e.g., PPI strategy is a mean of urea, PCU and urea, and PL and V11 SD treatments) as well as one pass (i.e., urea and PCU with urea) and multi-pass N (i.e., treatments with SD N) application systems. In 5 of 6 site years the IF and 5x5 strategies achieved similar yields (Table 7). Relative to the PPI strategy, in dry years (i.e., SVRF 2015 and 2016; SCRF 2016) the 5x5 strategy increased yield 5.0 to 9.2% and illustrated the difficulty associated with PPI N uptake in dry soils. Similar yields were obtained with the IF and 5x5 strategy except at the SCRF (2016) where the 5x5 strategy increased yields 5.8%. However, no differences among strategies were observed when May – June rainfall was above normal to excessive (i.e., SCRF 2014 and 2015). Multi-pass N application systems are a university recommended best management practice in MI to improve N recovery (Warncke et al., 2009). In 4 of 6 sites years a multi-pass system increased yields 4.4 to 16.1% relative to a one-pass system. In variable weather conditions yield gains in multi-pass systems suggests improved synchrony of N application with corn uptake. Increased starter N rates with the 5x5 strategy may offer more consistent yield response from increased N supply at V6 as opposed to low N rates applied IF.

Profitability

When total treatment costs were subtracted from treatment gross returns there was a treatment x site x year interaction (Tables 2, 5). Treatment means are presented separately by site and year (Table 7). Across years profitability was correlated with grain yield ($r \geq 0.82$; $P < 0.01$) and total treatment cost ($r \leq -0.29$; $P \geq 0.01$). A negative correlation with total treatment costs suggests that growers should consider N and application costs in addition to yields when maximizing profitability.

Full SD N applied at V11 did not increase profitability (Table 7). When cumulative Apr. – June rainfall was near normal to deficit, SD delayed from V4 to V11 using 8 kg N ha-1 with the IF strategy resulted in similar or reduced profitability (192 to 195 $ ha-1) (3 of 6 site years). A similar reduction in profit was observed (99 $ ha-1) (1 of 6 site years) with the 5x5 strategy under reduced N loss conditions (i.e., SVRF 2014). A reduced frequency of profit loss indicated the 45 kg N per ha-1 applied at planting stabilized profit variability among SD timings. Increased N rates at planting with the 5x5 strategy may allow greater flexibility to achieve similar profitability when deliberating between a V4 and V11 SD application. Despite similar yields split-applied SD (50/50) resulted in similar or reduced (152 $ ha-1) profitability relative to IF or 5x5 starter plus full V4 SD and suggested no profit benefit to a second SD application. Over two years of optimal growth conditions (i.e., SCRF and SVRF, 2014) a full V11 SD application reduced profitability relative to split-applied SD with starter IF (95 to 117 $ ha-1) or 5x5 (100 to 105 $ ha-1). Although similar profits were often achieved with IF and 5x5 plus V4 SD, growers using a V11 SD rescue application were more likely to maintain profitability if preceded by increased N rates in the 5x5 strategy.

Under the same near normal to deficit Apr. – June rainfall conditions discussed above, a PL and V11 SD application resulted in similar or reduced profitability (154 to 252 $ ha-1) relative to PPI urea or PCU and urea (Table 7). Likewise, PCU and urea resulted in similar or reduced profitability (98 $ ha-1) relative to a urea PPI application. Reduced profitability and increased PCU N costs ($0.42 to 0.54 kg-1 N) relative to urea N emphasizes the risk associated with enhanced efficiency N when N loss conditions are not present (i.e., SVRF 2014). Profitability with a single V11 SD application was most affected in dry years (deficit Apr. – June rainfall) (i.e., SVRF 2015 and 2016; SCRF 2016) and emphasized the importance of considering N costs when deliberating N strategies. For instance, despite similar yields V11 SD N applications that utilized PL sometimes reduced profitability compared to an IF strategy (129 $ ha-1) and 5x5 strategy (145 to 278 $ ha-1). At the same time a 5x5 strategy and V11 SD increased profitability (200 to 228 $ ha-1) relative to the IF strategy and illustrates the increased capacity of an additional 37 kg N ha-1 at planting in a 5x5 strategy to increase profits as compared to 8 kg N ha-1 in the IF strategy. In dry years a PL with V11 SD reduced profitability relative to a V4 SD with IF (190 to 245 $ ha-1) or 5x5 strategy (186 to 346 $ ha-1) with fewer apparent differences during near normal rainfall conditions. The PL-N source cost up to 5.7x more than urea- and UAN-N sources, which were both reduced 26 to 38% after 2014 and contributed to the reduced PL profitability in dry years. Despite greater yields in a wet year, similar profits with PL and V11 SD compared to IF or 5x5 starter with V4 SD indicated N savings through improved synchrony of N application and uptake was not sufficient to offset the increased cost of the PL treatment. A lack of profitability gains with a V11 SD suggested post-V4 SD increased risks of reduced profitability as a result of cost and reduced yield.

Profitability between IF, PPI, and 5x5 strategies corresponded to yield data as indicated by multiple df contrasts. When Apr. – June rainfall was near normal to excessive, strategies often resulted in similar profitability (Table 7). However under deficit Apr. – June rainfall conditions, PPI profitability was reduced relative to the 5x5 (155 to 220 $ ha-1) and IF strategies (88 to 177 $ ha-1) and illustrates the difficulty associated with PPI N uptake, grain production, and subsequent profitability in dry soils. In two instances an IF strategy reduced profitability (60 to 140 $ ha-1) as compared to the 5x5 strategy. In 4 of 6 site years no differences were observed between one and multi-pass N application systems indicating weather variability impacted profitability less than yield. In two contrasting years reduced N loss opportunities increased profitability of a one pass system (122 $ ha-1) while apparently reduced PPI N uptake in dry soils reduced profitability (201 $ ha-1) relative to the multi-pass system. Growers often perceive yield loss as a larger risk than profit loss. One pass systems increased the frequency of yield loss while a 5x5 strategy achieved similar or increased profitability relative to other strategies. Results suggest a multi-pass system with a 5x5 strategy may reduce risk of profit loss where weather variability influences yield response.

Plant characteristics

NDVI: Pearson correlations indicated significant relationships between V6 NDVI and yield across years at the Lansing ($r=0.42$; $P<.01$) and Richville ($r=0.76$; $P<.01$) locations, and similar to an r value of 0.46 used to predict grain yield with V8 NDVI in another study (Liu and Wiatrak, 2011). In the current study, positive relationships suggest corn yield increases in relation to NDVI and emphasizes the importance of N management strategies to sufficiently supply N to maintain yield potential until SD time. Active canopy sensing at V6 corn indicated a significant year x treatment and location x treatment interaction and data are presented separately for year and location (Tables 5, 8). NDVI is an indicator of green biomass and has been used to compare plant growth response and N management (Tucker, 1979). When cumulative Apr. –

Jun. rainfall was deficit at both sites (i.e., 2016) or near normal to deficit across years (i.e., SVRF) treatment comparisons were not significant and suggests that deficit rainfall reduces corn growth response to N strategies. No significant differences between SD timings within the IF or 5x5 strategies indicated that corn NDVI had not yet responded to V4 SD N applied 4 to 11-d prior to V6 measurements. In optimal growing conditions with reduced N loss opportunities (i.e., 2014), NDVI increased in corn receiving a full N rate at planting as urea (9.2 to 11.7%) or PCU (6.3 to 8.8%) relative to starter N plus V4 or V11 SD. Multiple df contrast statements showed that NDVI increased with PPI or IF N strategies relative to a 5x5 N placement and suggested increased positional N availability when soils were not dry. Compared to corn receiving no N (i.e., untreated), the IF, 5x5, and PPI N increased corn NDVI (7.8 to 19.2%). Increased NDVI in corn receiving N at planting suggested native soil N supplies were insufficient to achieve similar growth in unfertilized corn. Corn requires nearly 15% of the total N uptake by V6 which may be supplied by soil N mineralization (Bender et al., 2013). Although corn yield potential is set prior to V8, N deficiencies 10 – 42 days after emergence have decreased stem elongation, leaf area, and net photosynthetic rate resulting in less dry matter accumulation and unrealized yield potential emphasizing the importance of strategies to supply sufficient N early in the growing season when photosynthetic rates are high (i.e., μmol CO2/m2/s) (Varvel et al., 1997; Binder et al., 2000; Zhao et al., 2003; Yu et al., 2016).

Active canopy sensing at V11 was not influenced by site or year and data are presented by treatment (Tables 5, 9). When full SD rates were delayed from V4 to V11 the IF strategy reduced NDVI 6.6% as compared to 1.8% with the 5x5 strategy. The larger NDVI reduction with the IF strategy indicated that the 8 kg N ha-1 was not sufficient to supply the required N for both green biomass and chlorophyll production and suggested less potential to capture photosynthetically active radiation (PAR) (Zhao et al., 2003). However the PL application which had not yet received V11 SD increased NDVI (3.9 to 6.6%) relative to the IF and 5x5 strategies across all site years and suggested increased potential to maintain yield until the V11 SD timing. All strategies increased V11 NDVI relative to unfertilized corn. Increased NDVI in corn receiving PPI N (5.1%) relative to IF N but not 5x5 N suggests N rates > 8 kg N ha-1 were required to improve NDVI but then may affect stand density via saltation.

Chlorophyll content: Chlorophyll meter indices normalized to a non-limiting N control (280 kg N ha-1) at R1 were used to indicate corn N status. Values were influenced by the interaction of year, site, and treatment and data are presented by treatment for each site year (Tables 5, 10). Except for a wet year (i.e., SCRF 2015), relative CM indices indicated reduced plant chlorophyll when SD was delayed from V4 to V11 within both the IF (2.0 to 22%) and 5x5 (4.1 to 9.2%) strategies. Decreased chlorophyll reduced photosynthesis for corn grain production (Hatfield and Parkin, 2014; Yu et al., 2016). Reduced CM values when delaying SD from V4 to V11 indicated less N was sequestered and assimilated into canopy tissues for photosynthesis and corresponded to yield reductions. When May – June rainfall was below normal (i.e., SVRF 2014 – 2016; SCRF 2016), PL with V11 SD often reduced CM values relative to IF or 5x5 starter and V4 SD (5 to 10%) and other PPI's (4.3 to 6.3%) likely due to reduced moisture required for mineralization. In the same years, PL and IF strategies receiving V11 SD resulted in CM values below criterion previously used to trigger N application in MI (≤ 0.96) and indicated N stress (Elwadie et al., 2005). However, studies have indicated that 35% of total corn N uptake occurs post-silking in modern hybrids so despite CM values below a 0.96 threshold, statistically higher grain yields in all years with PL applications suggested that a slowly mineralizable N source may continue to provide sufficient N for uptake and assimilation during corn reproductive stages (Bender et al., 2013). Across treatment strategies and one- versus multi-pass systems, multiple df contrasts indicated CM values were generally ≥ 0.96, with few biologically significant differences.

Relative CM indices at R4 were not affected by year or location and treatment means are presented across factors (Tables 5, 11). Studies by Hatfield and Parkin (2014) suggest corn N strategies which increased green leaf area (i.e., stay-green) at the onset of grain-fill enhanced the ability of the plant to capture and convert PAR into greater yield. When a split or full V4 SD was delayed until V11, CM values decreased (6.1 to 8.8%) regardless of IF or 5x5 strategy and corresponded to similar reductions at R1. Despite similar or reduced greenness with the PL and V11 SD relative to the IF and 5x5 starter and V4 or split SD N treatments, similar greenness at R4 (and R1) was not required to attain similar yield. Rather a CM threshold (0.96) corresponded more to reduced yield associated with V11 SD (i.e., IF or 5x5) and urea or PCU/urea applied PPI which indicated N deficiency at R4. A PL application increased greenness between R1 and R4 (numerically) likely due to post-silking N mineralization and uptake. The IF or 5x5 strategies increased CM values (3.2 to 4.3%) in regard to a PPI strategy while a multi-pass system increased relative CM content (4.3%) as compared to a one-pass system. Despite few differences at R1, reduced greenness at R4 with PPI strategies applied in a one pass system suggested less N was sequestered post-silking and reduced opportunities for increased dry matter accumulation (i.e., yield).

Conclusions

When Apr. – June rainfall was near normal to deficit, full SD delayed from V4 to V11 did not result in yield gains. Reduced N rates required by the IF strategy resulted in similar or reduced yields 7.6 – 10.1% and profitability 192 – 195 \$ ha-1 when SD was delayed from V4 to V11. The 5x5 strategy resulted in no yield differences and reduced profitability (99 \$ ha-1) in only 1 of 6 site years when SD was delayed from V4 to V11 which indicated that increased N rates afforded by the 5x5 strategy may stabilize profit and yield variability. Regardless of 5x5 or IF strategy, similar or reduced yields 5.6 – 6.9% with delayed SD (V11) relative to a split (50/50) SD indicated that starter N (8 to 45 kg N ha-1) may have been exhausted due to deficit N supplies, but reduced profitability 152 \$ ha-1 due to the split SD indicate no benefit to a second SD application. Poultry litter did not increase yield potential relative to other strategies (i.e., IF or 5x5 plus V4 SD), but when full SD was delayed until V11 increased yields 6.9 – 9.7% due to PL were offset by the increased N cost and reduced profitability 129 to 278 \$ ha-1. Substituting urea with PCU did not affect yields in relation to 100% urea but reduced profitability 98 \$ ha-1 and emphasized the risk of enhanced efficiency N use when N loss conditions are not present. In wet soils (above normal May – June rainfall) PL followed by V11 SD increased yield 8.6 to 13.9% relative to IF or 5x5 strategies and V4 SD. Relative to one-pass systems yield gains 4.4 – 16.1% of multi-pass systems in 4 of 6 site years suggest improved synchrony N application with corn N uptake. Delaying the majority of N application until V11 may have reduced the opportunity for N losses in wet years, this practice did not increase the opportunity for yield gains in other years. Since SD N applications are often dependent on seasonal weather patterns growers must leverage risk when deliberating between early and late SD. The ability of N strategies to sufficiently supply N to corn until SD remain critical in order to maintain yield potential. Canopy sensory data suggest the IF, PPI, and 5x5 strategies can increase plant vigor at V6 and V11 relative to an unfertilized strategy, but reduced N rates required by the IF strategy may reduce the capacity to maintain yield potential when full SD is delayed from V4 to V11. Weather forecast models can vary in their predictive accuracy and reduced yield potential and economic response to V11 SD N suggest less risk is associated with the V4 timing. However, growers missing the V4 application window can utilize the V11 timing as a rescue application to achieve yields ≥ 11.2 Mg ha-1. Growers utilizing a rescue application are more likely to maintain yield and profitability if preceded by increased N rates in the 5x5 strategy.

As models predict warmer temperatures, the use of relative maturity class corn hybrids greater than 98 d during a lengthened growing season may create greater opportunities for late season N applications to have a significant impact on grain yield in northern climates. Future studies that include similar treatments and multiple corn hybrids replicated across Corn Belt regimes may provide evidence to substantiate this idea as well as additional data and tools to address N management in a changing 21st century climate.

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APPENDICES

APPENDIX A

CHAPTER 2 TABLES

Table 2.01. Corn planting date, V4, and V11 sidedress N application dates for SCRF (Lansing, MI) and SVRF (Richville, MI), 2014-2016.

	---------------SCRF---------------			---------------SVRF---------------		
	Planting	V4	V11	Planting	V4	V11
2014	19 May	09 June	07 July	08 May	04 June	30 June
2015	01 May	02 June	29 June	28 April	28 May	25 June
2016	18 May	06 June	05 July	09 May	03 June	29 June

Table 2.02. Prices received and variable input costs utilized used for profitability analysis, 2014-2016.

Years		2014	2015	2016
	Returns	--------US \$ Mg^{-1}-------		
Prices Received†	Corn	138.18	142.91	131.88
	Costs	--------US \$ kg^{-1}--------		
Fertilizer‡	Urea-N	1.20	1.01	0.74
	28% urea ammonium nitrate (UAN)	1.26	1.14	0.81
	10-34-0	5.68	7.19	6.17
	Polymer coated urea (PCU)	1.63	1.54	1.23
	Poultry Litter (PL)	3.78	4.23	4.23
		--------US \$ L^{-1}--------		
Nitrogen stabilizer§	Urease Inhibitor	19.01	19.01	19.01
		--------US \$ ha^{-1}--------		
Application¶	5x5 starter applicator	5.24	5.12	4.99
	Urea broadcast application	17.27	14.65	15.59
	Urea incorporation	15.96	17.54	21.62
	UAN injection application (V4)	27.63	31.88	30.32
	UAN high-clearance application (V11)	19.00	25.03	22.76
	Grain hauling (farm to mkt or elv.)	0.32	0.32	0.32

†Fall grain prices for each year (USDA – NASS 2014; 2015; 2016).

‡Spring urea and UAN prices for each year (USDA – AMS 2014; 2015; 2016); PL, 10-34-0, and PCU prices obtained from local vendors.

§Urease inhibitor price obtained from local vendors and applied at label rates.

¶Application costs obtained from Michigan State University Extension custom machine and work rate estimates for each year (http://msue.anr.msu.edu/topic/farm_management/firm_publication_archive, Michigan State University, East Lansing, Michigan; verified 23 May 2017).

Table 2.03. Growing season (April – September) and 30-yr mean temperature† (°C) and precipitation (mm) data for SCRF (Lansing, MI) and SVRF (Richville, MI), 2014 – 2016.

Site	Year	Apr.	May	Jun.	Jul.	Aug.	Sept.	Total
					°C			
SCRF	2014	8.0	14.4	20.0	18.8	20.2	15.4	--
	2015	8.2	16.3	19.2	20.9	20.3	19.1	--
	2016	7.5	14.8	20.3	23.0	22.9	17.4	--
	30-yr	8.7	14.7	20.0	22.1	21.3	16.9	--
SVRF	2014	7.4	14.3	20.2	19.0	19.7	15.5	--
	2015	7.4	15.7	18.5	20.7	20.0	18.6	--
	2016	5.6	14.8	19.7	22.6	22.4	18.2	--
	30-yr‡	7.7	14.2	19.7	22.1	20.9	16.8	--
					mm			
SCRF	2014	22	83	123	61	86	85	460
	2015	23	109	192	61	123	95	604
	2016	75	52	18	96	163	106	510
	30-yr	73	85	89	83	84	92	505
SVRF	2014	101	78	70	106	99	77	531
	2015	50	73	68	56	100	67	413
	2016	33	40	38	88	131	52	382
	30-yr‡	81	84	90	79	82	98	513

†Air temperature and precipitation data were collected from Michigan Automated Weather Network (http://www.agweather.geo.msu.edu/mawn/) and determined as a mean of the average monthly high and low.
‡30-yr mean source for air temperature and precipitation data: NOAA (https://www.ncdc.noaa.gov/cdo-web/datatools/normals).

Table 2.04. Impact of N strategy on V3 corn plant density prior to in-season sidedress applicaitons using multiple degree of freedom contrasts†, across sites, 2014 - 2016.

N strategy	2014		2015		2016	
			plants ha^{-1}			
In-furrow starter (IF)‡	79,115	b#	81,806	a	80,729	a
Pre-plant, incorporated (PPI)§	81,268	a	81,268	a	80,729	a
5x5 starter¶	80,729	a	80,729	a	81,806	a
Untreated control	81,268	a	80,191	a	78,038	b
P > F	<0.001		0.612		0.042	

†Contrasts consisted of 3-treatment means which utilized each strategy.
‡Corn received 8 kg N ha^{-1} with the IF strategy.
§Corn received 2.2 Mg ha^{-1} poultry litter [61 kg N ha^{-1} first-year mineralizable N] or 157 – 202 kg N ha^{-1} PPI.
¶Corn received 45 kg N ha^{-1} with the 5x5 strategy.
#Treatment means within each column followed by the same letter are not significantly different at $P \leq 0.10$.

Table 2.05. Analysis of variance for V3 stand count, V6 and V11 NDVI, R1 and R4 rel. chlorophyll meter (SPAD), and grain yield in Lansing, and Richville, MI, 2014 - 2016.

Observation	Source of variation (P > F)						
	Location (L)	Treatment (T)	Year (Y)	L x T	L x Y	T x Y	Y x L x T
NDVI V6	<0.001	0.026	<0.001	<0.001	<0.001	0.037	0.158
NDVI V11	0.088	0.007	0.001	0.122	0.074	0.870	0.240
Rel. SPAD R1	0.921	<0.001	0.090	<0.001	0.143	<0.001	<0.001
Rel. SPAD R4	0.644	<0.001	0.165	0.479	0.944	0.165	0.313
Grain yield	0.957	<0.001	<0.001	0.786	0.113	0.013	0.002
Net return	0.1723	<0.001	<0.001	0.721	0.165	0.010	0.001

Table 2.06. Corn grain yield† as affected by in-furrow (IF), pre-plant incorporated (PPI), and 5x5 N strategies in combination with sidedress timings, at SCRF (Lansing, MI) and SVRF (Richville, MI)‡ 2014 - 2016.

N strategy	2014				2015				2016			
	SCRF		SVRF		SCRF		SVRF		SCRF		SVRF	
					Mg ha^{-1}							
IF + V4	14.5	ab§	14.0	bc	12.2	bc	12.7	a	12.2	a	13.8	a
IF + V11	13.4	d	13.6	c	12.7	bc	11.4	a	11.8	a	12.4	b
IF + V4/V11	14.4	abc	14.5	ab	13.2	ab	12.4	a	12.0	a	12.9	ab
PPI: urea	13.6	cd	15.0	a	11.9	c	11.2	a	11.8	a	11.2	c
PPI: PCU/urea	13.3	d	14.7	ab	12.0	c	11.5	a	12.4	a	11.1	c
PPI: PL + V11	14.7	a	14.5	ab	13.9	a	12.0	a	12.1	a	13.3	ab
5x5 + V4	13.9	bcd	14.4	abc	12.8	bc	11.9	a	13.1	a	13.0	ab
5x5 + V11	13.4	d	13.7	c	13.1	ab	12.6	a	12.4	a	12.8	b
5x5 + V4/V11	14.2	abc	14.6	ab	12.7	bc	12.3	a	12.7	a	13.3	ab
P > F	0.010		0.049		0.046		0.133		0.349		<0.001	
Untreated¶	7.2		6.0		5.9		7.2		8.5		5.8	
Multiple *df* contrasts												
IF strategy#	14.1	a	14.0	b	12.7	a	12.1	a	12.0	b	13.0	a
PPI strategy	13.9	a	14.7	a	12.6	a	11.5	b	12.1	b	11.9	b
5x5 strategy	13.8	a	14.2	b	12.9	a	12.3	a	12.7	a	13.0	a
P > F	0.633		0.019		0.712		0.065		0.066		0.004	
One pass††	13.5	b	14.9	a	11.9	b	11.3	b	12.1	a	11.2	b
Multi-pass	14.1	a	14.2	b	12.9	a	12.2	a	12.3	a	13.0	a
P > F	0.035		0.006		0.001		0.011		0.487		<0.001	

†Grain yield at 155 g kg^{-1} moisture.
‡Total maximum return to N rate used at Lansing and Richville locations was 157 and 202 kg N ha^{-1}, respectively, across years.
§Treatment means within each column followed by the same letter are not significantly different at $P \leq 0.10$.
¶Untreated control not included in statistical analysis.
#Contrasts consisted of 3-treatment means that utilized each strategy.
††One pass system multiple degree of freedom contrast was mean of urea and PCU and urea treatments. Multi-pass system was a mean of all other treatments combined.

Table 2.07. Corn profitability as affected N strategy at SCRF (Lansing, MI) and SVRF (Richville, MI)[†] 2014 - 2016.

N strategy	2014				2015				2016			
	SCRF		SVRF		SCRF		SVRF		SCRF		SVRF	
						Net Returns[‡] ($ per ha)						
IF + V4	1663	a[§]	1544	bcd	1412	a	1451	ab	1349	bcd	1521	a
IF + V11	1501	d	1475	d	1470	a	1256	de	1288	d	1329	cde
IF + V4/V11	1618	abc	1570	bc	1514	a	1372	abcd	1300	cd	1369	bcd
PPI: urea	1593	abcd	1719	a	1450	a	1321	bcde	1349	bcd	1244	ef
PPI: PCU/urea	1499	d	1621	b	1395	a	1288	cde	1386	abcd	1167	f
PPI: PL + V11	1558	bcd	1467	d	1498	a	1206	e	1159	e	1276	def
5x5 + V4	1620	abc	1623	b	1550	a	1393	abcd	1505	a	1463	ab
5x5 + V11	1539	cd	1524	cd	1586	a	1484	a	1418	abc	1421	abc
5x5 + V4/V11	1639	ab	1629	ab	1495	a	1416	abc	1435	ab	1464	ab
P > F	0.064		0.001		0.376		0.025		0.002		<0.001	
Untreated[¶]	959		798		813		990		1080		741	
					Multiple *df* contrasts							
IF strategy[#]	1595	a	1532	b	1466	a	1360	a	1313	b	1406	a
PPI strategy	1550	a	1602	a	1448	a	1272	b	1298	b	1229	b
5x5 strategy	1599	a	1592	a	1544	a	1431	a	1453	a	1449	a
P > F	0.338		0.062		0.145		0.005		0.002		<0.001	
One pass[#]	1546	a	1670	a	1422	a	1305	a	1367	a	1205	b
Multi-pass	1591	a	1548	b	1504	a	1368	a	1351	a	1406	a
P > F	0.218		<0.001		0.109		0.152		0.683		<0.001	

†Total maximum return to N rate used at Lansing and Richville locations was 157 and 202 kg N ha^{-1}, respectively, in all years.

‡Net returns calculated as gross return [yield x corn price] minus total costs [N + N protectant + application cost] ha^{-1} per respective year.

§Treatment means within each column followed by the same letter are not significantly different at $P \leq 0.10$.

¶Untreated control not included in statistical analysis.

#Contrasts consisted of 3-treatment means that utilized each strategy.

††One pass system multiple degree of freedom contrast was mean of urea and PCU and urea treatments. Multi-pass system was a mean of all other treatments combined.

Table 2.08. Mean V6 NDVI readings as affected by N strategy across locations at SCRF (Lansing, MI) and SVRF (Richville, MI) 2014 – 2016.

Treatment combinations	Years 2014		2015		2016		Location SCRF		SVRF	
					NDVI					
IF + V4	0.4620	cd‡	0.3974	ab	0.3296	a	0.4678	a	0.3248	a
IF + V11†	0.4696	cd	0.3965	ab	0.3182	a	0.4647	a	0.3248	a
IF + V4/V11	0.4768	bcd	0.4087	a	0.3187	a	0.4701	a	0.3327	a
PPI: urea	0.5132	a	0.3703	bcd	0.3085	a	0.4358	bc	0.3589	a
PPI: PCU/urea	0.4998	ab	0.3799	bc	0.3233	a	0.4488	ab	0.3531	a
PPI: PL + V11	0.4886	abc	0.3909	ab	0.3176	a	0.4573	ab	0.3408	a
5x5 + V4	0.4700	cd	0.3560	cd	0.3162	a	0.4197	cd	0.3418	a
5x5 + V11	0.4595	d	0.3502	d	0.3072	a	0.4071	d	0.3376	a
5x5 + V4/V11	0.4860	abcd	0.3599	cd	0.3083	a	0.4242	cd	0.3454	a
P > F	0.021		0.002		0.935		<0.001		0.168	
Untreated§	0.4263		0.3350		0.3133		0.4174		0.2977	
					Multiple *df* contrasts					
IF starter only¶	0.4696	b	0.3965	a	0.3182	a	0.4647	a	0.3248	b
PPI strategy#	0.5005	a	0.3804	a	0.3164	a	0.4473	a	0.3509	a
5x5 starter only	0.4595	b	0.3502	b	0.3072	a	0.4071	b	0.3376	ab
Untreated control	0.4263	c	0.3350	b	0.3061	a	0.4174	b	0.2943	c
P > F	<0.001		<0.001		0.856		0.001		<0.001	

†Not all treatments at V6 have received total seasonal N application (i.e., V11 N applications).
‡Treatment means within each column followed by the same letter are not significantly different at $P \leq 0.10$.
§Untreated not included in analysis of pairwise comparisons.
¶The IF and 5x5 strategies indicated as 'starter' as these contrasts only contained those treatments not receiving SD N to enable comparison with PPI N and the untreated control.
#'strategy' indicates a mean of all three treatments utilizing the PPI strategy.

Table 2.09. Mean V11 NDVI readings as affected by N strategy across locations and years† in Lansing and Richville, MI, 2015 - 2016.

N strategy	NDVI	
IF + V4	0.7309	a§
IF + V11‡	0.6823	d
IF + V4/V11	0.7223	ab
PPI: urea	0.7071	bc
PPI: PCU/urea	0.7157	abc
PPI: PL + V11	0.7274	ab
5x5 + V4	0.7132	abc
5x5 + V11	0.7003	cd
5x5 + V4/V11	0.7190	abc
P > F	0.050	
Untreated¶	0.6526	
Multiple *df* contrasts		
IF starter only#	0.6823	b
PPI strategy††	0.7168	a
5x5 starter only	0.7003	ab
Untreated control	0.6526	c
P > F	<0.001	

†Data were not collected in 2014.
‡Not all treatments at V11 have received total seasonal N application (i.e., V11 N applications).
§Treatment means followed by the same letter are not significantly different at $P \leq 0.10$.
¶Untreated not included in analysis of pairwise comparisons.
#The IF and 5x5 strategies indicated as 'starter' as these contrasts only contained those treatments not receiving SD N to enable comparison with PPI N and the untreated control.
††'strategy' indicates a mean of all three treatments utilizing the PPI strategy.

Table 2.10. Mean R1 relative† chlorophyll (CM) meter measurements as affected by N strategy at SCRF (Lansing, MI) and SVRF (Richville, MI), MI‡, 2014 - 2016.

N strategy	2014				2015				2016			
	SCRF		SVRF		SCRF		SVRF		SCRF		SVRF	
	Relative CM index											
IF + V4	0.99	a§	0.97	cd	0.95	bc	1.00	a	1.00	a	1.04	a
IF + V11	0.97	bcd	0.91	e	0.94	c	0.78	e	0.92	d	1.00	bc
IF + V4/V11	0.99	abc	1.02	ab	0.99	a	0.97	ab	1.00	a	1.01	abc
PPI: urea	0.99	abc	1.00	abc	0.98	ab	0.96	bc	0.98	abc	0.99	c
PPI: PCU/urea	0.96	d	1.00	bc	0.95	bc	0.94	c	1.00	a	1.00	bc
PPI: PL + V11	0.99	ab	0.95	d	0.99	a	0.90	d	0.95	c	0.95	d
5x5 + V4	0.97	cd	1.03	a	0.99	a	0.98	ab	1.01	a	1.02	ab
5x5 + V11	0.93	e	0.97	d	0.98	a	0.89	d	0.96	bc	0.99	bc
5x5 + V4/V11	0.97	abcd	1.01	abc	0.99	a	0.97	abc	0.99	ab	1.03	a
$P > F$	0.052		<0.001		0.019		<0.001		<0.001		<0.001	
Untreated¶	0.74		0.71		0.72		0.74		0.84		0.73	
Multiple *df* contrasts												
IF strategy#	0.98	a	0.97	b	0.96	a	0.92	b	0.97	a	1.02	a
PPI strategy	0.98	a	0.98	ab	0.97	a	0.93	ab	0.98	a	0.98	b
5x5 strategy	0.96	b	1.00	a	0.99	a	0.95	a	0.98	a	1.01	a
$P > F$	0.013		0.074		0.119		0.078		0.523		<0.001	
One pass††	0.97	a	1.00	a	0.96	a	0.95	a	0.99	a	0.99	a
Multi-pass	0.97	a	0.98	a	0.97	a	0.93	b	0.97	a	1.00	a
$P > F$	0.818		0.124		0.410		0.074		0.129		0.135	

†Readings normalized to non-limiting N control (280 kg N ha^{-1}).
‡Total maximum return to N rate used at Lansing and Richville locations was 157 and 202 kg N ha^{-1}, respectively, in all years.
§Treatment means within each column followed by the same letter are not significantly different at $P \leq 0.10$.
¶Untreated control not included in statistical analysis.
#Contrasts consisted of 3-treatment means that utilized each strategy.
††One pass system multiple degree of freedom contrast was mean of urea and PCU and urea treatments. Multi-pass system was a mean of all other treatments combined.

Table 2.11. Mean R4 relative† chlorophyll (CM) meter measurements as affected by N strategy across locations‡ and years§, 2015-2016.

Treatment combinations	Rel. CM index	
IF + V4	0.99	ab¶
IF + V11	0.93	cd
IF + V4/V11	0.99	ab
PPI: urea	0.93	d
PPI: PCU/urea	0.95	cd
PPI: PL + V11	0.96	bc
5x5 + V4	1.02	a
5x5 + V11	0.94	cd
5x5 + V4/V11	0.99	ab
P > F	<0.001	
Untreated#	0.57	
Multiple *df* contrasts		
IF strategy††	0.97	a
PPI strategy	0.94	b
5x5 strategy	0.98	a
P > F	0.01	
One pass‡‡	0.94	b
Mutli-pass	0.98	a
P > F	0.001	

†Readings normalized to non-limiting N control (280 kg N ha^{-1}).
‡Total maximum return to N rate used at Lansing and Richville locations was 157 and 202 kg N ha^{-1}, respectively, in all years.
§Data not collected in 2014.
¶Treatment means followed by the same letter are not significantly different at $P \leq 0.10$.
#Untreated control not included in statistical analysis.
††Contrasts consisted of 3-treatment means that utilized each strategy.
‡‡One pass system multiple degree of freedom contrast was mean of urea and PCU and urea treatments. Multi-pass system was a mean of all other treatments combined.

APPENDIX B

CHAPTER 2 DATA COLLECTED AND NOT USED FOR PUBLICATION

Table B.01. Total vegetative N uptake† at physiological maturity as affected by in-furrow (IF), pre-plant incorporated (PPI), and 5x5 N strategies in combination with sidedress timings, at SCRF (Lansing, MI) and SVRF (Richville, MI)‡ 2014 - 2016.

N strategy	2014				2015				2016			
	SCRF		SVRF		SCRF		SVRF		SCRF		SVRF	
					kg ha^{-1}							
IF + V4	61.5	ab§	38.1	bc	33.3	a	46.3	a	42.5	a	37.7	a
IF + V11	53.7	bc	40.4	bc	51.7	a	26.7	e	39.9	a	39.6	a
IF + V4/V11	66.4	a	54.7	a	48.0	a	38.2	abc	44.2	a	33.7	a
PPI: urea	52.9	bc	46.3	ab	42.5	a	36.4	bcd	42.8	a	40.6	a
PPI: PCU/urea	48.3	c	46.2	b	42.3	a	33.4	cde	49.2	a	39.4	a
PPI: PL + V11	65.7	a	39.1	bc	45.7	a	32.9	cde	38.9	a	41.4	a
5x5 + V4	48.8	c	46.9	ab	40.4	a	43.1	ab	45.9	a	36.3	a
5x5 + V11	50.2	c	35.6	c	42.6	a	28.3	de	39.7	a	34.1	a
5x5 + V4/V11	51.8	bc	41.8	bc	36.6	a	35.6	bcd	41.9	a	38.3	a
P > F	0.0172		0.0181		0.1718		0.0051		0.8244		0.8105	
Untreated¶	22.6		19.2		17.5		15.1		22.2		17.1	
Multiple *df* Contrasts#												
IF strategy	60.6	a	44.3	a	44.3	a	37.1	a	42.2	a	37.0	a
PPI strategy	55.7	ab	43.9	a	43.5	a	34.2	a	43.6	a	40.5	a
5x5 strategy	50.3	b	41.1	a	39.9	a	35.6	a	42.5	a	36.1	a
P > F	0.0308		0.5201		0.5380		0.6978		0.8973		0.2415	
PPI: urea	53.1	a	46.3	a	42.5	a	36.4	a	42.8	a	40.6	a
PPI: PCU/urea	48.3	a	46.2	a	42.3	a	33.4	a	49.2	a	39.4	a
Split-N	56.9	a	42.2	a	42.6	a	35.9	a	41.9	a	37.2	a
P > F	0.1758		0.3533		0.9978		0.8351		0.2175		0.5536	

†Determined as total vegetative biomass multiplied by percent total N.
‡Total maximum return to N rate used at Lansing and Richville locations was 157 and 202 kg N ha^{-1}, respectively, across years.
§Treatment means within each column followed by the same letter are not significantly different at $P \leq 0.10$.
¶Untreated control not included in statistical analysis.
#Contrasts consisted of 3-treatment means that utilized each strategy.

Table B.02. Soil residual nitrate† at corn harvest as affected by in-furrow (IF), pre-plant incorporated (PPI), and 5x5 N strategies in combination with sidedress timings, at SCRF (Lansing, MI) and SVRF (Richville, MI)‡ 2014 - 2016.

N strategy	2014				2015				2016			
	SCRF		SVRF		SCRF		SVRF		SCRF		SVRF	
					kg N ha^{-1}							
IF + V4	13.6	a§	14.0	cd	11.3	a	20.3	a	19.8	a	10.7	a
IF + V11	26.6	a	41.2	a	13.5	a	11.1	bcde	11.8	a	9.2	a
IF + V4/V11	15.1	a	27.9	a	13.8	a	19.1	ab	12.2	a	10.1	a
PPI: urea	16.7	a	21.0	bc	12.8	a	8.6	cde	9.6	a	10.3	a
PPI: PCU/urea	15.1	a	27.0	b	13.6	a	8.0	e	13.5	a	12.5	a
PPI: PL + V11	25.5	a	18.6	bc	11.5	a	14.3	bc	10.5	a	8.6	a
5x5 + V4	13.8	a	11.5	d	12.4	a	18.7	a	12.1	a	7.8	a
5x5 + V11	15.2	a	20.9	bc	11.7	a	12.1	bcd	8.5	a	8.8	a
5x5 + V4/V11	16.7	a	16.2	bcd	13.6	a	8.1	de	13.2	a	8.8	a
P > F	0.2792		0.0003		0.9905		0.0002		0.1860		0.6226	
Untreated¶	13.6		9.1		9.2		7.4		8.3		8.6	
					Multiple *df* Contrasts#							
IF strategy	18.4	a	27.7	a	12.9	a	16.8	a	14.6	a	10.0	a
PPI strategy	19.1	a	22.9	ab	12.6	a	10.3	b	11.2	a	10.5	a
5x5 strategy	15.2	a	16.2	b	12.6	a	13.0	ab	11.3	a	8.4	a
P > F	0.6019		0.0757		0.9629		0.0395		0.1080		0.1363	
PPI: urea	16.7	a	22.4	a	12.8	a	8.6	b	9.6	a	10.3	ab
PPI: PCU/urea	15.1	a	27.8	a	13.6	a	8.0	b	13.5	a	12.5	a
Split-N	18.1	a	21.5	a	12.5	a	14.8	a	12.6	a	9.1	b
P > F	0.8909		0.6860		0.8391		0.0243		0.2987		0.0597	

†Determined at the 0 – 30 centimeter depth.
‡Total maximum return to N rate used at Lansing and Richville locations was 157 and 202 kg N ha^{-1}, respectively, across years.
§Treatment means within each column followed by the same letter are not significantly different at $P \leq 0.10$.
¶Untreated control not included in statistical analysis.
#Contrasts consisted of 3-treatment means that utilized each strategy.

Table B.03. Corn stalk nitrate† at corn physiological maturity as affected by in-furrow (IF), pre-plant incorporated (PPI), and 5x5 N strategies in combination with sidedress timings, at SCRF (Lansing, MI) and SVRF (Richville, MI)‡ 2014 - 2016.

N strategy	2014				2015				2016			
	SCRF		SVRF		SCRF		SVRF		SCRF		SVRF	
	mg NO_3-N kg^{-1}											
IF + V4	447	a§	359	abc	445	a	1579	a	748	a	84	c
IF + V11	268	a	195	bc	138	a	91	c	121	bc	326	b
IF + V4/V11	467	a	226	abc	426	a	592	b	211	abc	53	cd
PPI: urea	99	a	220	abc	432	a	636	b	347	ab	934	a
PPI: PCU/urea	217	a	504	a	482	a	867	b	544	a	1238	a
PPI: PL + V11	516	a	391	ab	529	a	82	c	49	c	62	c
5x5 + V4	179	a	213	c	233	a	2227	a	625	a	35	cd
5x5 + V11	240	a	156	c	366	a	325	b	145	bc	40	cd
5x5 + V4/V11	548	a	357	ab	655	a	744	b	267	abc	7	d
Pr>F	0.4327		0.0886		0.8351		<.0001		0.0439		<.0001	
Untreated¶	1.3		0.0		1.9		3.4		1.1		0.3	
Multiple *df* Contrasts#												
IF strategy	393	a	264	a	336	a	754	b	359	a	155	b
PPI strategy	281	a	372	a	481	a	528	b	313	a	744	a
5x5 strategy	322	a	248	a	418	a	1099	a	351	a	27	c
Pr>F	0.3364		0.1936		0.6187		0.0046		0.6493		<.0001	
PPI: urea	109	b	220	a	432	a	636	a	347	a	934	a
PPI: PCU/urea	217	ab	504	a	482	a	867	a	544	a	1238	a
Split-N	380	a	275	a	399	a	806	a	311	a	87	b
Pr>F	0.0631		0.2207		0.9262		0.7033		0.2046		<.0001	

†Determined using 20 centimeter stalk segments collected 15 centimeters above the soil surface.
‡Total maximum return to N rate used at Lansing and Richville locations was 157 and 202 kg N ha^{-1}, respectively, across years.
§Treatment means within each column followed by the same letter are not significantly different at $P \leq 0.10$.
¶Untreated control not included in statistical analysis.
#Contrasts consisted of 3-treatment means that utilized each strategy.

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CHAPTER 3

NITROGEN CYCLING, CORN GROWTH AND GRAIN YIELD RESPONSE TO NON-LEGUMINOUS COVER CROPS AND FERTILIZER

Abstract

Cover crops (CC) seeded prior to a corn (Zea mays L.) cash crop may influence nitrogen (N) availability but little validation has occurred when combined with corn sidedress (SD) N applications. Field studies were conducted in 2015 and 2016 to evaluate the effects of a Daikon radish [Raphanus sativus (L). var. The Buster], Forage oat [Avena sativa (L.) var. Magnum], or no CC established following wheat (Triticum aestivum L.) on soil NO3-N levels, corn growth, N uptake and grain yield. Nitrogen management strategies included 179 kg N ha-1 applied as urea pre-plant incorporated (PPI), poultry litter (PL) PPI (61 kg N ha-1) plus SD N V11, starter N (52 kg N ha-1) applied 5 cm beside and 5 cm below the furrow (5x5) followed by SD at V4, V11, or V4 plus V11, and a zero N control. Cover crops reduced autumn soil NO3-N levels 78 to 84% compared with the no cover control but did not increase N availability in corn. When 5x5 starter was followed by full SD at V11 radish and oats reduced yield 3.2 – 3.9% and profitability 11.8 – 13.2% from the no CC control and indicated CCs reduced the effectiveness of 5x5 starter to maintain yield potential until SD time. In the zero N strategy, CCs reduced grain yield 11.8 – 14.2% and increased yield response to N 63 – 79%, suggesting CCs reduced N availability. In years where excessive rainfall increases N loss opportunities CCs may exacerbate soil NO3-N reduction and increase grain yield reductions. Regardless of CC, growers may reduce risk of yield and profitability loss when full SD at V4 follows 5x5 starter N.

Introduction

Degradation of Great Lakes water quality may require further improvement upon corn nitrogen (N) management strategies to simultaneously increase nitrogen use efficiency (NUE) and reduce opportunities for N loss (Dove and Chapra, 2015; USEPA, 2017). Michigan, located in the the northern corn belt and the central basin of the Great Lakes, is characterized by a temperate, humid growing season. Winter wheat (Triticum aestivum L.) often precedes corn in Michigan crop rotations and is harvested in July leaving approx. a 90 d fallow period prior to autumn soil freezing (-2.2°C) (National Oceanic and Atmospheric Administration [NOAA], http://www.weather.gov/dtx/firstFallfreeze; verified 02 Aug. 2017).

Climate change has prolonged the period when autumn soil temperatures remain above 50 degrees F which may extend organic matter mineralization and increase soil NO3-N availability (Schwartz et al., 2006; USEPA, 2016; Schultze et al., 2016). In fallow soils, residual soil NO3-N combined with residue mineralization and no crop N uptake can increase the potential for N loss (Weinert et al., 2002; Derby et al., 2009). To assimilate available N (i.e., post-harvest autumn residual N, inorganic N from soil organic matter (SOM) mineralization) and ameliorate N loss in humid climates, cover crops (CC) are sometimes established in the non-growing season (Shipley et al., 1992; Macdonald et al., 2005; Tonitto et al., 2006; McSwiney et al., 2010). Cover crop biomass production and ensuing N assimilation may improve N cycling and use efficiency (NUE) in maize, maintain and support corn yield, and improve profitability (Decker et al., 1994; McSwiney et al., 2010; O'Reilly et al., 2012). However, corn yields following non-leguminous CCs may be affected by the rate of residue decay and resulting N availability (Dapaah and Vyn, 1998; Vyn et al., 2000; O’Reilly et al., 2012; Gieske et al., 2016a). Corn yield gains and profitability may depend upon the synchrony of CC N availability and N fertilizer timing.

Radish (Raphanus sativus L.) is a non-leguminous Brassicaceae CC utilized as a nutrient scavenger in the non-growing season to sequester nutrients including available soil NO_3-N (Mutch and Thelen, 2003; Warncke et al., 2009). Radishes are a large-taproot annual that grow quickly in late summer and autumn and reduce soil compaction and suppress winter annual weeds (Williams and Weil, 2004; Clark, 2007; Lawley et al., 2011). Radish winterkills when air temperatures drop below -4 °C eliminating the need for chemical termination (Thomas et al., 2017). Autumn radish biomass production in Michigan has ranged from 2.9 to 6.1 Mg ha^{-1} (herbage and roots) after 2 to 3 mo. of growth while N uptake was 10 to 164 kg ha^{-1} and reduced soil inorganic N 24 to 42% compared to no CC (Hill et al., 2016). However, dry autumn soils may limit plant growth and reduce N uptake (Gieske et al., 2016b). Corn grain yields following an autumn radish CC have varied showing both no effect (Vyn et al., 2000; Gieske et al., 2016a; 2016b) and increased yield (Dapaah and Vyn, 1998; Vyn et al., 1999; O'Reilly et al., 2012) compared to no CC. Variable corn yield response may be due to effects of biomass production, chemical composition, and residue decomposition on subsequent N availability. Cover crop residue decomposition and N mineralization and immobilization are dependent on the carbon (C) to nitrogen ratio (C:N) and lignin concentrations (Wagger et al., 1998; Jahanzad et al., 2016). Decreased C:N associated with a radish cover (14 to 31:1) relative to a grassy cover (15 to 38:1) (e.g., oats, triticale, or ryegrass) may help explain the 43 and 24% greater soil NO_3-N concentrations following a radish CC at mid-May corn planting and mid-June N sidedress application in Ontario, respectively (Vyn et al., 1999; Andraski and Bundy, 2005). However when compared to a no CC control, radishes may increase the risk of N loss (i.e., leaching or denitrification) during freeze-thaw cycles in the non cash-crop growing season (Dean and Weil, 2009; Thomas et al., 2017) and may not increase N availability to a subsequent corn crop (Vyn et al., 2000; Gieske et al., 2016a). Adjusting N fertilizer strategies may be needed to synchronize radish N availability with corn N uptake and improve grain yield.

Oat (Avena sativa L.) is a non-leguminous CC also used to sequester residual soil N and prevent wind and water erosion (Warncke et al., 2009). Similar to radish, oats are an annual CC subject to winterkill (Johnson et al., 1998; Snapp et al., 2005). Unlike radish, oat has a dense, fibrous root system characteristic of cereal CCs (Thorup-Kristensen, 2001). Cereal CC residues often contain more lignin and cellulose that may slow decomposition rates and extend N release when compared to radish (Jahanzad et al., 2016). Slower decay of cereal residue may affect synchrony N availability with corn uptake (Wagger, 1989). The C:N ratio of oat may exceed 30:1 which can immobilize soil N and reduce availability for uptake (Adraski and Bundy, 2005; Dean and Weil, 2009). Corn yield following oat CCs have been generally decreased or not affected in non-irrigated studies (Johnson et al., 1998; Vyn et al., 2000; O'Reilly et al., 2012). However, Andraski and Bundy (2005) suggested corn yield gains due to oat CCs in an irrigated study were an effect of rotation and not N contributions as evidenced by similar May soil NO3-N contents. Vyn et al. (2000) suggested oat CCs may reduce corn N availability as indicated by greater corn yield response with added fertilizer N. Reduced corn yield following an oat CC corresponded to reduced soil NO3-N concentrations at corn planting (36%) and at mid-June sidedress (20%) (Vyn et al., 2000). Cereal CCs have also increased gaseous N2O emissions 76% relative to no CC during the non-growing season and may help explain reduced N availability to the subsequent corn crop (Thomas et al., 2017).

Michigan corn growers utilize multiple strategies to optimize corn N management. Some growers prefer one-pass application systems where 100% of the N inputs are applied prior to or within 1 – 3 d after spring planting but this can reduce corn yield fine-textured soils (Franzen, 2017). Volatile spring weather has prompted grower adoption of multi-pass N application systems for improved N recovery (Vetsch and Randall, 2004; Warncke et al., 2009). Multi-pass N application systems may involve starter N (up to 67 kg N ha-1) applied in a sub-surface band placed 5 cm below and 5 cm laterally (5x5) relative to the seed furrow followed by sidedress (SD) N at V4 corn. Recent studies identified maximum corn N uptake between V10 and V14 and suggested delayed in-season N SD applications may improve synchrony between N availability and uptake while reducing losses from early applied N (Binder et al., 2000; Scharf et al., 2002; Bender et al., 2013). However V6 N deficiency has reduced corn yield potential and highlighted the importance of N management strategies that maintain yield potential until SD time (Binder et al., 2000). Although radish and oat CCs can tighten the N cycle by sequestering autumn soil N, additional research is needed to investigate the impact of CCs on corn response to N management. The objective of this study was to evaluate the effect of N placement and timing on corn growth and grain yield in response to a preceding daikon radish or forage oat cover crop.

Materials and Methods

Site characteristics and cultural practices

A two-year field study was conducted (2014 – 2016) at the Michigan State University South Campus Research Farm in Lansing, MI on a Capac loam (fine-loamy, mixed, active, mesic Aquic Glossudalf). Soil samples (20-cm depth) were collected prior to August CC establishment and May corn planting, air-dried, and ground to pass through a 2-mm sieve. Soil characteristics

5.9-6.2 pH (1:1 soil/water) (Peters et al., 2015), 27-51 mg kg-1 P (Bray-P1) (Frank et al., 2015), 105-163 mg kg-1 K (ammonium acetate method) (Warncke and Brown, 2015), and 2.4-3.4 g kg-1 soil organic matter (loss-on-ignition) (Combs and Nathan, 2015). Fields were chisel plowed after July wheat [Triticum aestivum L.) harvest and disk harrowed (10-cm depth) prior to CC establishment. Calcitic lime was spring-applied at 2.2 Mg ha-1 prior to corn planting to a target pH of 6.5. Prior to corn planting fields received disk tillage (10-cm depth) followed by a soil finisher (10-cm depth). Broadcast P and K fertilizer were pre-plant incorporated (PPI) (10-cm depth) as triple super phosphate (0-45-0 N-P-K) and muriate of potash (0-0-62) based on soil test. Corn weed control consisted of acetochlor [2-chloro-N-ethoxymethyl-N-(2-ethyl-6-methylphenyl) acetamide] and glyphosate [N-(phosphonomethyl) glycine] followed by a second application of glyphosate 17-24 d later. Environmental data were collected during the growing season using the Michigan Automated Weather Network (http://www.agweather.geo.msu.edu/mawn/, Michigan State University, East Lansing, Michigan; verified 24 Jul. 2017) from an on-site weather station.

Experimental design and treatment application

Eighteen treatments were arranged in a split-plot, randomized complete block design with four replications. The main plot factor was CC while the subplot factor was N strategy. Three CC treatments included a no cover control, 'The Buster' daikon radish, or 'Magnum' forage oat (Weaver Seed of Oregon, Crabtree, OR) and measured 55 m by 12 m in length. The radish cover was drill-planted at 11.2 kg ha-1 and forage oat cover at 28.0 kg ha-1 in August preceding the ensuing corn crop using a Gandy orbit-Air Seeder coupled with John Deere double disk openers in 19-cm rows (Table 1). The no CC treatment received a single, glyphosate application in the autumn to remain free of vegetative ground cover. To ensure winterkill, CCs were terminated with glyphosate in November (79-83 days of growth).

Subplots consisted of six N placement by N timing strategies applied to corn plots measuring 4.6 m by 12.2 m in length. Nitrogen strategies included a zero N control, pre-plant incorporated (PPI) urea (46-0-0), dried poultry litter (4-3-2) applied PPI at 2.2 Mg ha-1 plus sidedress (SD) N at V11 corn, and starter N subsurface banded 5 cm beside and 5 cm below the seed furrow (5x5) (45 kg N ha-1 urea ammonium nitrate [UAN; 28-0-0]) followed by SD N at V4, V11, or split (50/50) V4 and V11. Corn V4 N was UAN coulter injected 5 cm deep and 38 cm to the side of each row while V11 SD was UAN mixed with a urease inhibitor (CO(NH2)2 + n-(n-butyl) thiophosphoric triamide] (Koch Agronomic Services, LLC, Wichita, KS) to prevent N volatilization and banded 10 – 15 cm to the side of each row. Treatment total N rates were equalized to the maximum return to nitrogen rate (179 kg N ha-1) (Steinke, 2015). Corn seeding and starter N application utilized a Monosem planter (Monosem Inc., Kansas City, Kansas) equipped with Yetter floating planter-unit mounted row cleaners (Yetter Manufacturing, Colchester, IL) and liquid fertilizer applicators. Corn was seeded in 0.76 m rows at 84,510 seeds ha-1 using Dekalb DKC48-12 RIB (98 d relative maturity) (Monsanto Co., St. Louis, MO) (Table 1).

Data collection and statistical analysis

Soil inorganic nitrogen: Ten (0-30 cm) soil samples were collected from each replication at CC establishment, from whole plots at CC biomass collection, and at corn planting (Table 1). After corn planting two (0-30 cm) soil samples were collected monthly from May – October from subplots of the zero N strategy. Samples were analyzed for nitrate-N (cadmium reduction) (Huffman and Barbarick, 1981).

CC measurements: Cover crop measurements included biomass production and total tissue N content at the time of termination (Table 1). Within each whole plot replicate radish (shoots and roots) and oat (shoots only) cover samples were collected within 5 d of termination in the autumn and again in mid-March (oats only) using three 0.25 m2 quadrats. At sampling, radish roots were excavated and washed with water. Fresh weights of cover subsamples were recorded and tissues dried at 60°C and ground using a 1-mm mesh screen. Nitrogen uptake was calculated as the product of percent N and biomass (dry basis).

Corn measurements: Active canopy sensing was conducted to determine normalized difference vegetation index (NDVI) at V6 and V10 using a GreenSeeker® Model 505 handheld red-band optical sensor (Trimble Agriculture Div., Westminster, CO) (Table 1). Chlorophyll content was assessed at R1 to indicate N status using a Minolta SPAD 502 chlorophyll meter (CM) (Konica Minolta, Tokyo, Japan) (Scharf et al., 2006). Corn plant height was measured at V6 and R2 as an average of 20 randomly selected plants plot-1. Measurements were taken from the soil surface to the top of the newest fully developed leaf (height of leaf curve apex) with visible collar (V6) or to the top of the tassel (R2) (Yin and McClure, 2013).

The center two rows of each plot were harvested with a Massey Ferguson 8XP research plot combine (Kincaid Equipment Manufacturing, Haven, KS) to determine grain yield, moisture, and test weight. Yield data were reported at 155 g kg-1 moisture. Treatment profitability was calculated as net return = gross return from yield – input costs. Grain prices and input costs were determined each year using spring N prices (USDA – AMS 2015; 2016), autumn grain prices (USDA – NASS 2015; 2016), and application costs (http://msue.anr.msu.edu/topic/farm_management/firm_publication_archive, Michigan State University, East Lansing, Michigan; verified 23 May 2017) while CC and manure prices were obtained from local vendors.

Data were analyzed in SAS (Ver. 9.4, SAS Institute, 2011) using the GLIMMIX procedure. Corn measurements assumed fixed effects of year, cover, and N management, and random effects of block and block x cover (SAS Institute, 2011). Cover crop influence on soil inorganic N data were analyzed at the whole plot level, assumed block as a random effect, and were compared at each sample time and not over time. Normality and homoscedasticity assumptions were validated with the UNIVARIATE procedure and Levene's test, respectively, using residuals ($P \leq 0.05$). The LINES option of the slice statement was used to separate treatment means when ANOVA indicated a significant interaction ($P \leq 0.10$). Pearson's correlation coefficients were used to investigate linear relationships between monthly soil NO3-N data and R1 SPAD with grain yield ($P \leq 0.05$) using the CORR procedure.

Results and Discussion

Weather

Following cover crop (CC) seeding, mean Aug. – Nov. 2014 air temperatures were below the 30-yr mean but were above the 30-yr mean from Sept. – Nov. 2015 (Table 2). Cover crops received 27 – 35 mm rainfall within 1 week of planting both years followed by near to above normal rainfall in August and September. Following May 2015 corn planting, mean June – Aug. air temperatures were 0.8 – 1.2 °C below normal while Sept. – Oct. was 0.8 – 2.2 °C above normal. Precipitation was 28, 116, and 46% above 30-yr means in May, June, and Aug. 2015, respectively, which may have increased N loss due to denitrification in saturated soils. Following May 2016 corn planting, mean monthly air temperatures were 0.1 – 1.6 °C above normal May through September. In contrast with 2015, 39 and 80% below normal precipitation in May and June, respectively, produced dry soil conditions while 15 – 94% above normal precipitation from July – September produced wet soils later into the growing season.

Autumn CC biomass and nitrogen uptake, and soil NO3-N

Data were combined across years due to no interaction between CC and year for dry matter production (above-ground only for oats), N uptake, and November soil NO3-N measurements ($P \leq 0.10$) (Table 3). Cover crop biomass production represented 74 – 81 d of growth each year. Following autumn termination, mean radish dry matter production (i.e., above- and belowground biomass) was 68% greater than aboveground oat CC biomass which increased total N uptake 56% relative to the oat CC. Radish CCs reached anthesis each year while oats did not and may explain greater radish CC biomass observed (O'Reilly et al., 2012). In contrast, radish and oat biomass (herbage only) or N uptake did not differ in Indiana research (Vyn et. al 2000), suggesting that the growth of non-leguminous CCs was limited by low soil NO3-N (4.4 to 8.3 mg kg-1) concentrations following wheat harvest. Soil NO3-N concentrations at CC planting in the current study were 8.7 to 12.7 mg kg-1 and supports the greater radish and oat biomass production and N uptake values observed. At autumn termination, soil NO3-N concentrations (0 – 30 cm) were reduced 78 to 84% relative to no cover crop, when averaged across CCs. Despite reduced biomass and N uptake compared to radish, oats reduced soil NO3-N levels an additional 25% suggesting that a substantial amount of N remained in the oat roots which were not collected. Both CCs immobilized soil NO3-N compared to no cover which suggests opportunities for tightening of the N cycle and reduced post-harvest N losses (Vyn et al., 2000; Weinert et al., 2002; O'Reilly et al., 2012).

Spring CC biomass and nitrogen uptake, and monthly soil NO3-N

Radish residues decomposed prior to March biomass sampling (Dean and Weil, 2009; Hill et al., 2016). Insufficient radish residues suggested decomposition was rapid in Michigan and that radish N may have been subject to denitrification, leaching, or volatilization N loss mechanisms during the residue degradation process. Approximately 50% of oat residue remained in March (2.9 Mg ha-1, 21% CV) with 38% of the autumn total N uptake remaining immobilized in the biomass (45.9 kg N ha-1, 18% CV). Growers may need to consider different N management strategies to synchronize CC N availability with subsequent crop N uptake.

In June data indicated a significant CC x year interaction on soil NO3-N levels likely due to contrasting precipitation and therefore data are presented by year (Table 4). In wet soils (i.e., 2015), radish and oat CCs reduced June soil NO3-N (28 – 32%) relative to the no cover while in dry soils (i.e., 2016) a 19% reduction was observed with oats only. It is unclear whether dry soils in May and June (i.e., 2016) slowed CC biomass decomposition and reduced radish CC N immobilization or if dry soils reduced opportunities for N loss and offset N immobilization in the radish CC. In both years soil NO3-N reductions indicate N immobilization due to CC decomposition may have contributed to reduced N availability (Ketterings et al., 2015). June soil

NO3-N levels were the most correlated with corn grain yields relative to other months ($r=0.73$; $P <0.001$) and corroborated previous findings (Vyn et al., 1999). Although rapid corn N uptake occurs V10 to V14 corn yield potential can be affected at V6 (Binder et al., 2000; Bender et al., 2013). Reduced N availability at V6 has resulted in unrealized yield potential (Binder et al., 2000). In the current study, reduced June nitrate levels in the no cover zero N control suggest N availability may not synchronize well with N needed for early corn growth.

Except for June, year did not interact with CC response and data were combined across years (Table 4). Soil NO3-N levels (Mar., May, Jul., Aug., and Sept.) were not affected by planting a cover crop, suggesting that radish and oat CCs did not increase NO3-N availability during the ensuing corn growing season (Vyn et al., 2000; O'Reilly et al., 2012; Gieske et al., 2016a). Reduced CC residue for biomass sampling in March indicate winter decomposition and may help explain no increase in CC soil NO3-N availability to the ensuing corn cash crop. Between November and March a net loss of 2.9 mg kg-1 soil NO3-N occurred in the no CC control while an increase of 2.3 and 2.5 mg kg-1 soil NO3-N due to oat and radish CCs, respectively, was observed and suggested residue mineralization contributed to the N pool. Soils in Lansing, Michigan, may not freeze during the winter due to location among the Great Lakes. Studies indicate CC decomposition during freeze-thaw cycles in the winter can supply labile C and N and stimulate N2O emissions (Thomas et al., 2017). Oats reduced soil NO3-N levels 23, 29, and 12% in May, June (2016), and Sept., respectively, compared to radish indicating less N availability to corn. Studies suggest soil NO3-N levels due to oat CC were likely influenced by a slower rate of residue decomposition due to increased lignin concentrations and recalcitrant N contained in root residues (Malpassi et al., 2000; Jahanzad et al., 2016). Soil NO3-N data suggest radish and oat cover crops did not provide an N credit to the ensuing corn crop and growers should not decrease N rates (Andraski and Bundy, 2005; O'Reilly et al., 2012).

Corn response to CCs and N strategies

Grain yield: A cover x N strategy interaction (P=0.071) indicated that N strategy may need to be adjusted for specific CC treatments (Table 5). Year was not a significant factor thus data were combined across years and presented for cover by N strategy (Table 6). Within N strategies, radish and oat CCs did not improve and sometimes reduced yield relative to the no CC control and suggested that N availability was not synchronized with corn N uptake. Few differences between those strategies receiving N compared to the zero N strategy indicated that the N rate used in the study (179 kg N ha-1) was sufficient to offset N immobilization from the cover crops (O'Reilly et al., 2012). Cover crop effects on grain yield were most apparent for the zero N strategy (P=0.025) as radish and oat reduced grain yield 12 and 14%, respectively, compared to the no CC control. Vyn et al. (2000) observed 15 – 22% grain yield reductions from an oat CC compared to a no cover control receiving zero N fertilizer. Cover crop biomass production has explained some variability in subsequent grain yield in previous studies (r= -0.24; P=0.05) (Hill et al., 2016). In the current study aboveground radish biomass accounted for 58% (i.e., 5.8 Mg ha-1) of total production compared to 0.89 – 1.27 Mg ha-1 observed by Vyn et al. (2000) implying that total cover crop biomass production limits grain yield. Growers sometimes apply N to CCs seeded prior to a corn cash crop to maximize growth. Results of the current study suggest increased CC growth may reduce N to a corn cash crop and N applied to encourage CC growth may reduce corn yield gains. Reduced soil NO3-N levels at June soil sampling time (previously mentioned) suggest that CCs may have limited N availability during rapid N uptake and reduced yield potential of the zero N strategy. When mean grain yield of plots not receiving N was subtracted from mean grain yield of plots receiving N, radish and oat CCs increased yield response to N fertilizer by 63 – 79%. Reduced yields in non-fertilized radish and oat and increased yield response to N suggest N availability to corn was reduced where CCs were present. Earlier termination of actively growing cover crops or later corn planting dates may lessen the negative effects of CCs on grain yield, but these practices may not be in agreement with conservation efforts if the goal of non-leguminous cover crops is to produce maximum autumn growth for reduced soil NO3-N availability.

Corn yield was reduced 3 – 5% in the nitrogen PPI treatment in oats as compared to the no cover or radish treatments. When 52 kg N ha-1 was applied at planting with full SD delayed until V11, radish and oat CCs reduced yield 3 – 4% compared to the no cover control. However no differences amongst CCs were observed in V11 SD followed PL or when strategies included V4 SD, indicating that CCs reduced longevity of the 5x5 placement required to supply N until V11 SD time. Success of delayed SD N (i.e., V11) may require a sufficient N supply to meet corn demands prior to SD timing to maintain yield potential (Binder et al., 2000). In Michigan 5x5 starter placement and SD N is a recommended two-pass fertility strategy to reduce risk of early N loss. Corn preceded by radish and oat cover crops may require increased starter N rates (> 52 kg N ha-1) or slowly-available N sources (e.g., PL) when using delayed N applications (i.e., V11).

Across N strategies, N fertilizer increased grain yield ≥ 2.2 Mg ha-1 and confirmed corn N response within each CC treatment. Within the no CC treatment no differences among N strategies indicated no benefit to split-applied N although SD N is a recommended corn N strategy in Michigan to increase N recovery (Warncke et al., 2009). In the radish CC, yield was reduced 4.5% in the 5x5 starter with V11 SD reduced yield 4.5% from the PL and indicated that a slowly mineralizable N source may help maintain yield potential under variable weather conditions. Other than PPI N reducing yield 3.9% relative to the 3 pass system (i.e., 5x5 and split SD), few differences were observed within the oat cover crop. Nitrogen applied PPI following oats may have been partially immobilized by decaying oat residue explaining the greater yield decrease from this treatment. No yield difference between strategies within the no cover treatment yet differences following radish and oat suggests that CCs can impact N application method. The 5x5 plus V4 SD and PL plus V11 SD strategies consistently resulted in the greatest yields among CC treatments, but few differences when no CC was present.

Profitability: A CC x N strategy interaction occurred when total treatment costs were subtracted from treatment gross returns (Table 5). Data were combined across years due to no interaction with year. Across years net return was better correlated with grain yield ($r = 0.85$; $P < 0.01$) than treatment application costs ($r = -0.13$; $P = 0.11$) or total N costs ($r = 0.20$; $P = 0.01$). The significant correlation indicated that profitability was more dependent upon grain yield and suggests when N rate is held constant, strategies that maximize grain yield maximize profitability.

Within N strategies, radish and oat CCs did not increase corn profitability compared to the no cover treatment (Table 7). With the exception for PL in radish, net returns were reduced across strategies receiving N fertilizer with radish ($129 to 214 ha-1) and oat ($163 to 275 ha-1) relative to the no cover. Within the PPI N strategy, oats reduced net return relative to radish by $97 – 106 ha-1. Seed costs of oats averaged $40 ha-1 greater than radish and contributed to net return reductions. Net return differences were most pronounced within the unfertilized strategy where CC reduced return $347 - $427 ha-1. In intensively managed cropping systems inputs may be applied with little data to support agronomic decisions (Orlowski et al., 2016). Radish and oat CCs were not able to improve profitability of N strategies. Future studies should incorporate leguminous CC with N strategies to determine if N availability in corn and if yield and profitability increase. The PL and V11 SD strategy resulted in the lowest net return regardless of CC treatment. While yield consistencies with PL and V11 SD strategy did not correspond to profit consistencies within CC treatments, reduced net returns likely resulted from the increased costs associated with PL. The PL N source cost 333 and 384% more per kg N than urea and 28% UAN-N sources, respectively, while the treatment increased costs over other N fertilizer strategies ($178 to 232 ha-1). Growers with access to inexpensive manure sources similar to that used in this study may increase their potential to impact profitability. The urea-N and 5x5 starter plus V4 SD strategies consistently resulted in the highest net returns although sometimes results were similar to other strategies within each CC. The urea PPI strategy was the least expensive among N fertilizer strategies and reduced cost ($20 ha-1) compared to the 5x5 and V4 SD strategy. However reduced yields associated with the oats and urea PPI strategy may hinder adoption by growers and favor the 5x5 plus V4 SD as yield loss is perceived as a larger risk than N savings (Rutan and Steinke, 2017).

Normalized difference vegetation index

Active canopy sensing at V6 indicated both CC and N strategy affected corn normalized difference vegetation index (NDVI) and results were combined across years for N strategy

(Tables 5, 8). The PL (prior to SD) strategy increased NDVI 8.2% relative to zero N and was similar to other strategies that had received N (i.e., urea PPI or 5x5 and V4 SD). Red-NDVI is sensitive to green biomass and an indicator of plant vigor (Tucker, 1979). Despite contrasting soil moistures at sensing time each year (i.e., 0.23 and 0.09 cm3cm-3 in 2015 and 2016, respectively) increased V6 NDVI with PL may indicate that either mineralization was sufficient to increase plant vigor or trace amounts of other elements (i.e., 0.42, 0.57, 7.2% S, Mg, and Ca, respectively, and 30, 103, 592, 350, and 256 mg kg-1 B, Cu, Fe, Mn, and Zn, respectively) were sufficient to increase NDVI and the capacity to maintain yield potential until SD time.

The radish CC differentially affected corn V6 NDVI response each year (P=0.01) (Table 9). In 2015 radish and oat CCs increased NDVI 22 – 26% relative to the no cover while in 2016 the oat cover increased NDVI 23 – 25% relative to the radish and no CC control. Plant heights at V7 were similar to V6 NDVI measurements across years in that radish and oat increased height 17 – 29% (P=0.01) relative to no cover in 2015 while oat increased height 17 – 22% (P=0.01) in 2016 (data not shown). Increased corn growth with both CCs may have resulted from a rotation effect rather than N contributions (Andraski and Bundy, 2005). In the 2016 dry spring, phosphorus deficiency was observed on V4 corn preceded by radish and the no CC control but not in oats. Phosphorus availability and uptake requires soil moisture for diffusion and indicate oat residues may have increased soil moisture thus limiting P deficiencies. Previous Michigan studies observed increased soil volumetric water content at planting due to previous radish (29%) and cereal rye (79%) residues (Hill et al., 2016). In the current study however, increased early-season corn growth following CCs did not translate into increased yield.

Chlorophyll content (SPAD)

A significant CC x N strategy interaction affected SPAD chlorophyll meter measurements across years (Table 5). Chlorophyll meter (SPAD) values observed at R1 were well correlated with grain yield ($r=0.89$; $P<0.01$) and suggested N status at tasseling impacts grain fill. Within N strategies the effect of CCs on R1 SPAD values were most pronounced in the zero N strategy where radish reduced SPAD values 4% from the no CC control while oats reduced values 6 and 10% from the radish and no CC treatments, respectively (Table 10). Reduced SPAD values in the zero N strategy suggested 1) both CCs augmented R1 N stress and oats reduced N availability to a greater extent compared with radish and 2) demonstrate the ability of N fertilizer to mask the effect of CCs on soil N fertility as suggested by Duiker and Curran (2005). The application of N fertilizer decreased the immobilization of N from radish and oat residues further diminishing any negative effects on SPAD values. Relative to radish, the oat CC reduced SPAD values 3.2% with PPI N applications which corresponded to reduced yield and indicated that the PPI strategy was not able to supply sufficient N to overcome reduced soil NO3-N levels and limited R1 yield potential. Full SD delayed until V11 following oats reduced SPAD values 2.7 – 3.5% relative to the no cover. In order to maintain yield potential, success of the late SD timing (i.e., V11) may depend upon sufficient N supply up until SD. When a 5x5 starter and V11 SD strategy were utilized reduced SPAD (oats) values and yield (radish and oats) relative to the no cover control suggested CCs reduced the ability of 5x5 starter to supply N sufficient to maintain yield potential up to V11 SD. However, when PL was utilized as an N source a lack of yield differences suggested a slowly available N source may have continued to provide post-tasseling N to overcome R1 N stress observed in the oat treatment. The addition of N fertilizer increased SPAD values indicating response to N. When SD included a V4 timing, SPAD values were consistently > 55.0 regardless of CC. Greater SPAD values suggest opportunities to reduce N stress regardless of CC selection and N stress up to R1 caused by CC could be overcome by N fertilizer applications.

Conclusions

Radish and oat covers were non-leguminous species effective at sequestering residual soil NO3-N following a wheat cash crop. While radish covers had greater biomass production as measured by root and shoot growth, oats were more effective at reducing autumn soil NO3-N levels. Although cover crops reduced soil NO3-N in the autumn it cannot be assumed N assimilated will be protected from loss or available to a following corn cash crop. Increased early corn growth due to covers did not correspond to soil N availability and did not translate into improved corn yields or profitability. In the Michigan corn growing season June soil NO3-N levels were correlated to grain yield. However cover crops often reduced soil NO3-N in June and increased yield response to N fertility which suggested reduced N availability to a corn cash crop. Corn yields were unaffected by covers when V4 SD followed 5x5 starter N (52 kg N ha-1) but reduced when full SD at V11 followed 5x5 starter or when urea-N followed an oat cover. Despite few yield differences observed among N strategies within cover crop species reduced N availability suggest years of increased N loss opportunities may exacerbate soil NO3-N reductions due to cover crops and increase grain yield reductions. Results of the current study suggests single species cover crops in winter wheat – corn rotations may be less appropriate as tools for corn N fertility management but more appropriate as tools for soil conservation and water quality management. Contrasting precipitation observed each spring illustrated the impact of climate variability on soil moisture and growing season conditions. Regardless of cover crop use and weather variability growers are likely to increase opportunities for consistent yield and profitability gains when N strategies include the use of 5x5 starter N and full SD early (i.e., V4) as well as PL and V11 SD if the manure source is not cost prohibitive.

Two site-years of data which included wet and dry soils suggest further studies are needed to improve corn N fertility management when CCs are planted. Varying termination dates, seeding rates, and tillage methods may improve synchrony of CC N availability with corn N uptake.

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APPENDIX

Table 3.01. Dates of field operations and observations in 2014 – 2015 and 2015 – 2016, Lansing, MI.

Operation	2014 – 2015	2015 – 2016
	———chronological order———	
Tillage	08 Aug	29 Jul
Pre-cover soil sample	14 Aug	17 Aug
Cover planting	14 Aug	17 Aug
Fall soil sampling	03 Nov	30 Oct
Cover termination	05 Nov	04 Nov
March soil sampling	20 Mar	18 Mar
Lime application	23 Mar	05 Apr
Spring tillage	15 Apr	17 May
Corn planting and soil sampling	01 May	17 May
V4 sidedress	02 Jun	06 Jun
June soil sampling	03 Jun	14 Jun
V6 observations	10 Jun	15 Jun
V10 observations	28 Jun	01 Jul
V11 sidedress	29 Jun	05 Jul
July soil sampling	01 Jul	15 Jul
R1 observations	20 Jul	22-Jul
R2 observations	29 Jul	05 Aug
August soil sampling	03 Aug	15 Aug
September soil sampling	05 Sep	20 Sep
October soil sampling	02 Oct	11 Oct

Table 3.02. Mean monthly and 30-yr precipitation and temperature data for Lansing, MI, 2014 – 2016.

	precipitation†				air temperature‡			
Month	2014	2015	2016	30 yr	2014	2015	2016	30 yr
	---mm---				---°C---			
Jan.	na§	15	22	46	na	-6.9	-3.5	-4.6
Feb.	na	1	18	38	na	-12.3	-1.8	-3.2
Mar.	na	13	101	45	na	-0.1	4.9	1.9
Apr.	na	23	75	73	na	8.2	7.5	8.7
May	na	109	52	85	na	16.3	14.8	14.7
June	na	192	18	89	na	19.2	20.3	20.0
July	na	61	96	83	na	20.9	23.0	22.1
Aug.	86	123	163	84	20.2	20.3	22.9	21.3
Sept.	85	95	106	92	15.4	19.1	17.4	16.9
Oct.	57	48	82	70	9.2	11.2	9.2	10.4
Nov.	37	31	na	71	0.8	6.9	na	4.6
Dec	35	59	na	42	-0.5	4.0	na	-1.9

†Precipitation and air temperature data were collected from Michigan Automated Weather Network (http://www.agweather.geo.msu.edu/mawn/).

‡30-yr mean sources for precipitation and air temperatures: NOAA (https://www.ncdc.noaa.gov/cdo-web/datatools/normals; verified 17 Aug. 2017).

§Data not applicable. Monthly data start with August as cover crop planting began the trial period each year.

Table 3.03. Main effects of cover crop on dry matter biomass production, total N uptake, and soil residual NO_3-N concentrations following autumn growth, Lansing, MI, 2014 – 2015.

Cover crop	Dry matter		Total N uptake‡		soil NO_3-N	
	Mg ha^{-1}		kg ha^{-1}		mg kg^{-1}	
No cover¶	---		---		6.88§	a
Radish	10.0	a#	189	a	1.50	b
Oats	5.9	b	121	b	1.13	c
CV (%)	34		37		98	
Source of variation ($P > F$)						
Cover crop (C)	<.001		0.007		<.001	
Year (Y)	0.013		0.011		0.564	
C x Y	0.152		0.275		0.128	

†Cover crop residues were sampled on 03 Nov 2014 and 30 Oct 2015.
‡Total N uptake relative to aboveground biomass only for oat covers.
§Soil NO_3-N data transformed to meet normality assumption using root function. Untransformed means are presented. CV represents untransformed data.
¶Radish biomass was collected above and belowground in contrast to oats which were aboveground only.
#Means followed by same letter in a column are not significant different at alpha=0.10.

Table 3.04. Impact of preceding cover crops not receiving N fertilizer on ensuing monthly† soil test nitrate (NO_3-N) (0 – 30 cm) concentrations and linear correlation to grain yield (*r*-value), Lansing, MI, 2015 – 2016.

Cover crop	Mar.		May		June		July		Aug.‡		Sept.	
					mg kg^{-1}							
No Cover	3.94	a§	6.56	ab	14.92	.¶	4.93	a	2.24	a	1.71	ab
Radish	3.95	a	7.81	a	12.90	.	5.38	a	1.80	a	1.73	a
Oats	3.42	a	6.01	b	10.81	.	4.08	a	0.77	a	1.52	b
CV (%)	33		29		32		76		153		23	
r-value	0.55**		0.50**		0.73***		0.02		0.18		0.61**	
					$P > F$							
Cover crop (C)	0.317		0.088		0.015		0.680		0.359		0.095	
Year (Y)	0.004		0.002		0.001		0.001		0.010		0.036	
C x Y	0.219		0.881		0.040		0.905		0.642		0.853	

June C x Y interaction	2015		2016	
No Cover	19.37	a§	10.48	a
Radish	13.97	b	11.84	a
Oats	13.18	b	8.44	b
CV (%)	26		21	
$P > F$	0.035		0.042	

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.
†March samples were collected prior to corn plot establishment.
‡August data were transformed using root functions. CV's represent untransformed data.
§Means followed by same letter in a column are not significant different at alpha=0.10.
¶No means separation due to significant cover x year interaction. Data presented separately.

Table 3.05. ANOVA of cover crop, N strategy, and year fixed effects and their interactions on corn V6 NDVI, R1 SPAD, R2 height, grain yield, and net return in Lansing, MI, 2015 – 2016.

Fixed effects	V6 NDVI	R1 SPAD	R2 height	Grain yield	Net return
			Source of variation (*P* > *F*)		
Cover crop (C)	0.001	0.006	0.352	0.035	<.001
N strategy (N)	0.014	<.001	<.001	<.001	<.001
Year (Y)	0.060	<.001	<.001	0.002	<.001
C x N	0.367	0.038	0.061	0.071	0.060
C x Y	0.077	0.124	0.150	0.198	0.188
N x Y	0.672	<.001	0.015	0.129	0.187
C x N x Y	0.282	0.728	0.521	0.628	0.553

Table 3.06. Cover crop and N strategy interaction on corn grain yield (155 g kg^{-1} moisture) and impact of cover crop on response to N, Lansing, MI†, 2015 – 2016.

N strategy	Cover crop: No Cover		Radish		Oat		*P > F*
			Mg ha^{-1}				
Zero N	12.7	b‡A§	11.2	cB	10.9	cB	0.025
PPI	15.3	aA	15.1	abA	14.6	bB	0.077
PL¶ + V11 SD	14.9	aA	15.5	aA	15.0	abA	0.280
5x5 starter# + V4 SD	15.0	aA	15.1	abA	14.8	abA	0.774
5x5 starter + V11 SD	15.4	aA	14.8	bB	14.9	abB	0.061
5x5 starter + V4/V11 SD	15.1	aA	15.1	abA	15.2	aA	0.903
P > F	<.001		<.001		<.001		--
Response to N††	2.4	B	3.9	A	4.3	A	0.034

†Strategies requiring N fertilizer received total maximum return to N rate of 179 kg N ha^{-1} in all years.
‡Means within each column followed by same lower case letters are not significantly different at alpha ≤ 0.10.
§means within each row followed by same upper case letters are not significantly different at alpha ≤0.10.
¶PL = poultry litter applied at 2.2 Mg ha^{-1} PPI
#5x5 starter was applied 5 cm beside and 5 cm below the corn seed furrow at planting (45 kg N ha^{-1})
††Response to N determined as mean yield from plots receiving N fertility minus yield from Zero N plots within each respective cover.

Table 3.07. Interaction of cover crop and N strategy on corn profitability, Lansing, MI[†], 2015 – 2016.

N strategy	Cover: No Cover		Cover: Radish		Cover: Oat		*P > F*
			US$ ha^{-1}				
Zero N	1690	b[‡]A[§]	1343	dB	1263	cB	<.001
PPI	1834	aA	1665	aB	1559	aC	<.001
PL[¶] + V11 SD	1555	cA	1489	cA	1392	bB	0.014
5x5 starter[#] + V4 SD	1773	abA	1644	abB	1574	aB	0.001
5x5 starter + V11 SD	1816	aA	1602	bB	1577	aB	<.001
5x5 starter + V4/V11 SD	1756	bA	1613	abB	1586	aB	0.001
P > F	<.001		<.001		<.001		--

†Strategies requiring N fertilizer received total maximum return to N rate of 179 kg N ha^{-1} in all years.
‡Means within each column followed by same lower case letters are not significantly different at alpha ≤ 0.10.
§means within each row followed by same upper case letters are not significantly different at alpha ≤0.10.
¶PL = poultry litter applied at 2.2 Mg ha^{-1} PPI
#5x5 starter was applied 5 cm beside and 5 cm below the corn seed furrow at planting (45 kg N ha^{-1}).

Table 3.08. Main effect of N strategy† on corn V6 NDVI in Lansing, MI, combined across 2015 – 2016.

N Strategy	V6 NDVI	
Zero N	0.3741	bcd‡
PPI	0.3918	ab
PL§ + V11 SD	0.4048	a
5x5 starter¶ + V4 SD	0.3841	abc
5x5 starter + V11 SD	0.3625	cd
5x5 starter + V4/V11 SD	0.3605	d
P > F	0.014	

†All strategies requiring N fertilizer received total maximum return to N rate of 179 kg N ha^{-1} in all years.
‡Means followed by same lower case letters are not significantly different at alpha=0.10.
§PL = poultry litter applied at 2.2 Mg ha^{-1} PPI
¶5x5 starter was applied 5 cm beside and 5 cm below the corn seed furrow at planting (45 kg N ha^{-1}).

Table 3.09. Corn V6 NDVI measurements as affected by previous cover crop, Lansing, MI, 2015 – 2016.

	———NDVI———			
Cover crop	2015		2016	
No Cover	0.3547	b†	0.3254	b
Radish	0.4311	a	0.3198	b
Oats	0.4457	a	0.4010	a
P > F	0.007		0.006	

†Means followed by same lower case letters are not significantly different at alpha=0.10.

Table 3.10. Interaction of cover crop and N strategy on SPAD R1 chlorophyll meter readings, Lansing, MI†, 2015 – 2016.

	Cover crop						
N strategy	No Cover		Radish		Oats		*P > F*
			SPAD index				
Zero N	49.8	d‡A§	48.0	bB	44.9	dC	<.001
PPI	54.7	bcAB	56.1	aA	54.3	bB	0.066
PL¶ + V11 SD	54.5	cA	55.0	aA	52.6	cB	0.009
5x5 starter# + V4 SD	56.1	aA	55.9	aA	55.5	aA	0.721
5x5 starter + V11 SD	55.8	abA	55.2	aAB	54.3	bB	0.091
5x5 starter + V4/V11 SD	56.3	aA	56.2	aA	55.2	abA	0.255
P > F	<.001		< .001		< .001		

†Strategies requiring N fertilizer received total maximum return to N rate of 179 kg N ha^{-1} in all years.
‡Means within each column followed by same lower case letters are not significantly different at alpha ≤ 0.10.
§Means within each row followed by same upper case letters are not significantly different at alpha ≤0.10.
¶PL = poultry litter applied at 2.2 Mg ha^{-1} PPI
#5x5 starter was applied 5 cm beside and 5 cm below the corn seed furrow at planting (45 kg N ha^{-1}).

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CHAPTER 4

INFLUENCE OF COVER CROPS AND NITROGEN MANAGEMENT ON THE SOIL MICROBIOME TO OPTIMIZE CORN PRODUCTION

Abstract

Nitrogen fertilizer management may affect soil bacterial community composition in a corn (Zea mays L.) rotation but could be altered when following cover crops. Field studies were conducted in 2014 – 2015 (year 1) and 2015 – 2016 (year 2) to evaluate the effects of Daikon radish [Raphanus sativus (L). var. The Buster] and Forage oat [Avena sativa (L.) var. Magnum] on soil respiration, soil bacterial community diversity, richness, and evenness in response to N management strategies. Strategies consisted of 179 kg N ha-1 applied as urea pre-plant incorporated (PPI), poultry litter (PL) PPI (2.2 Mg ha-1) plus sidedress (SD) N V11, starter N (45 kg N ha-1) applied 5 cm beside and 5 cm below the furrow (5x5) followed by SD at V4, V11, or V4 plus V11, and a zero N control. Cover crop autumn biomass production, C and N uptake, soil base cations, and corn yield were also measured. Forage radish increased autumn biomass production 39 and 120% in years 1 and 2, respectively, compared with oats, but 46% less C uptake by oats in year two resulted in a C:N ratio of 17:1 and 23:1 for oat and radish CC, respectively. Relative to an oat or no CC control radishes reduced soil bacterial diversity during active CC growth, but no differences were observed at corn seeding and R1. Nitrogen strategies affected soil bacterial diversity, richness, or evenness at 4 of 6 observations, compared with only 1 observation with CCs. Grain yields were correlated to R1 soil bacterial diversity in both years regardless of N fertilizer application ($r=-0.21$ to -0.63; $P \leq 0.08$) and indicate soil bacterial communities affected corn grain production. The inverse relationship of yield and R1 diversity

suggested the relative abundance of soil bacterial community membership rather than diversity may provide a better indication of yield.

Introduction

The soil is a living ecosystem and functions to sustain healthy plants, animals, and the environment (Doran and Zeiss, 2000). As a living component of soils the microbial biomass provides agricultural services which includes recycling of organic materials and functions as a labile source of carbon (C), N, phosphorus (P), and sulfur (S) (Brookes, 2001). Soil bacteria comprise one of the largest components of the microbial biomass and have direct impact on plant fitness as pathogens or beneficial mutualists, and indirectly as decomposers, or through suppression of deleterious pathogens (Nihorimbere et al., 2011). While soil bacteria include pathogens, beneficials such as genera Streptomyces, Bacillus, and Pseudomonas produce antibiotics antagonistic to other plant pathogens (Rosenzweig, 2014). Plant growth promoting bacteria associated with rhizosphere soil (PGPR) colonize plant roots and stimulate plant growth through N fixation, phytohormone production (e.g., auxin), or reduce the incidence of disease (Naseem and Bano, 2014; Rosenzweig, 2014, Vurukonda et al., 2016). Factors affecting soil bacterial communities may have impact on crop production. Multiple groups of soil bacteria may perform similar functions and influence cropping system resilience and stress adaption through functional redundancy (Sharma et al., 2010). Cropping system resilience to moisture stress may improve consistency of Michigan corn grain yields. Since 2010 Michigan corn grain yields have varied >10% despite little variation in hectares planted (USDA, 2017). Recent studies suggest moisture stress as a result of increased climate variability during cash crop seasons may explain corn yield variation (Mishra and Cherkauer, 2010). A 70-yr projection for Michigan agriculture predicts an additional 5 - 15 days per growing season with air temperatures > 35°C combined with increased spring rainfall and more intense summer droughts (USEPA, 2016). Increased understanding of production-related factors that affect soil bacterial communities may provide opportunities to enhance crop resilience to climate change (e.g. moisture stress) and improve grain yield consistency (Song et al., 2015).

Cover crops (CC) have been used to increase plant diversity and influence soil microbial communities (Buyer et al., 2010; Vukicevich et al., 2017). Following Michigan winter wheat harvest, soils may remain fallow for up to a 9 mo. period and provide a niche for CC incorporation. Plants, soils, and soil microbes function simultaneously to influence rhizosphere processes that contribute to plant health and productivity (Chaparro et al., 2012). Plants modulate microbial community composition through root morphology, C-rich root exudates (i.e., rhizodeposition), and litter quality (Berg and Smalla, 2009; Chaparro et al., 2012; Cleveland et al., 2014). Soil microbial communities provide agroecosystem services (i.e., nutrient cycling, carbon transformations, soil structure maintenance, water availability, and regulation of pests and disease) which in-turn influence crop production (Kibblewhite et al., 2008). Tillage, lack of crop rotations, and crop residue removal reduce soil bacterial community diversity (i.e., richness and evenness) (Lupwayi et al., 1998; Ceja-Navarro et al., 2010; Figuerola et al., 2015; Sun et al., 2016). Studies suggest increased bacterial community diversity may influence crop yield and N uptake, enhance functional resiliency, and protect ecosystem services under abiotic disturbance (Eisenhauer et al., 2012; Awasthi et al., 2014; Tautges et al., 2016). Meta-analysis indicated that crop diversity (i.e., rotations) increased microbial richness (i.e. number of species present) (15%) and diversity (3.4%) scores but was unable to conclude whether microbial diversity sustained ecosystem function (e.g., nutrient cycling) under soil disturbance. Utilizing CCs to influence soil bacterial communities for optimal crop production requires further investigation (Venter et al., 2016).

To improve the synchrony between N availability and corn N uptake, Michigan corn growers utilize multiple N strategies including, pre-plant incorporation, at-plant applications, and multi-pass systems with reduced N rates at planting followed by in-season sidedress (SD) application. Contrasting literature results concerning the impact of N fertilizer on soil bacterial diversity and community composition may implicate both N rate and source (Fierer et al., 2012; Geisseler and Scow, 2014; Ikeda et al., 2014; Li et al., 2016). For example, Ikeda et al. (2014) observed increased bacterial species diversity (i.e., α-Proteobacteria and β-Proteobacteria) from urea-formaldehyde N relative to ammonium sulfate, nitrate-N, or urea. Zhao et al. (2014) observed increased bacterial diversity at the phylum level when manure and mineral N fertilizers were utilized as a combined source. However Orr et al. (2012) indicated organic and mineral N sources had less of an impact on soil bacterial communities as compared to changes in soil temperature and cumulative rainfall received 14 d prior to sampling. Recent data from the Morrow plots (fertilizer and crop-rotation study initiated in 1876) determined that manure increased bacterial diversity and lead to greater relative abundance of bacteria beneficial to plant fitness (Soman et al., 2017). Reduced soil microbial diversity due to N fertilizer applications may be due to changes in soil chemistry and not simply a direct osmotic effect (Lupwayi et al., 2012; Zhalnina et al., 2015; Li et al., 2016). Ammonium-based N fertilizers are a source of soil acidity due to H+ release during NH4+ nitrification with varying degrees of acidification depending upon N source (i.e., anhydrous ammonia, urea, etc) (Havlin et al., 2014). Cation uptake by crops also contributes to soil acidity, which in turn could influence bacterial communities. Soil microbial response to N fertilizer may also depend upon organic C inputs which are used by soil bacteria as a food source (i.e., heterotrophs). Meta-analysis indicated that the addition of mineral N fertilizer to promote crop growth and yield increased crop residue returned to the soil and increased both organic C (13%) and the microbial biomass (15%) (Geisseler and Scow, 2014). In fallow soils N fertilization decreased soil bacterial diversity and reduced copiotrophic (i.e., Actinobacteria) bacteria important for C and N cycling. However, when CCs were used this did not occur, indicating CCs were able to protect soil bacterial diversity from deleterious effects due to N fertilization (Verzeaux et al., 2016). Further research is needed to investigate impacts of N fertilization strategies on both soil bacterial communities and crop yield (Nielsen et al., 2014; Zhao et al., 2014).

High throughput next-generation sequencing (NGS) has enabled researchers to study plant and soil microbiome interactions which are predominated by bacteria not cultivable in laboratory environments (Rosenzweig, 2014). Illumina-based sequencing is a cost effective NGS strategy that can produce up to 500 cycles of a paired-end DNA fragment read while the MiSeq platform can generate 8.5 Gbp using paired 250-nucleotide reads (Kozich et al., 2013). Prior to sequencing, conserved primers which target regions of 16S rRNA are combined with template soil DNA and then amplified (Rosenzweig, 2014). The 16S rRNA gene provides phylogenetic bacterial community soil diversity due to its presence in most bacteria, stability over time, and size (1,500 base-pairs) for informatics purposes (Janda and Abbott, 2007). Sequencing of the 16S rRNA gene has revealed a higher prevalence of Agrobacterium spp. in the corn rhizosphere with variations in bacterial richness, diversity, and relative abundance when compared to bulk soil

(Sanguin et al., 2006; Peiffer et al., 2013). Analysis of corn rhizosphere phylogenetic diversity indicated enriched Proteobacteria, Firmicutes, Bacteroidetes, and Cyanobacteria which function to transform labile and recalcitrant nutrient sources (Li et al., 2014). Corn roots exude 5 – 62% of total net belowground C and may alter rhizosphere bacterial community composition, but C quality may depend on growth stage (Amos and Walters, 2006). Prior to tasseling copiotrophic bacteria, which prefer simple amino acids (i.e., r-strategists), predominate until after tasseling when oligotrophic bacteria, which utilize complex carbohydrates (k-strategists) become more abundant. When compared to the bulk soil corn has the capacity to influence and select bacterial communities (Li et al., 2014). Illumina sequencing of corn rhizosphere and bulk soil samples at multiple growth stages using field-derived studies may provide opportunities to assess the impact of cover crops when combined with N management strategies on the microbial community. The objective of this study was to investigate the effects of a radish, oat, and no cover crop in combination with corn N management strategies on corn grain production, spatial and temporal variation in soil CO2 respiration, and soil bacterial community composition.

Materials and Methods

Site characteristics and cultural practices

Two field experiments were conducted on different sites (2014 – 15 and 2015 – 16) at the Michigan State University South Campus Research Farm in Lansing, MI. Each location consisted of a 2-yr cropping rotation of winter wheat/cover crop (CC) followed by corn on a Capac loam soil (fine-loamy, mixed, active, mesic Aquic Glossudalf). Following July wheat harvest, fields were chisel plowed, disk-harrowed, and leveled (10-cm depth) with a soil finisher. Prior to cover crop planting soils were sampled (20-cm depth), air-dried, and ground to pass through a 2-mm sieve. Soil properties included 5.8 – 6.0 pH (1:1 soil/water) (Peters et al., 2015), 24 – 55 mg kg-1 P (Bray-P1) (Frank et al., 2015), 122 – 136 mg kg-1 K (ammonium acetate method) (Warncke and Brown, 2015), and 26 – 34g kg-1 organic matter (loss-on-ignition) (Combs and Nathan, 2015).

To control volunteer wheat the following season, CCs were terminated each autumn with glyphosate [N-(phosphonomethyl) glycine]. To control vegetation encroachment, the no CC treatment received an additional glyphosate application prior to CC termination. Each site year received lime (2.2 Mg ha-1) applied 39 to 42 d prior to corn planting to adjust soil pH to 6.5. Broadcast P and K fertilizer were pre-plant incorporated 1-d prior to corn planting with triple super phosphate (0-45-0 N-P-K) and muriate of potash (0-0-62) based on soil tests. Weeds were controlled with acetochlor [2-chloro-N-ethoxymethyl-N-(2-ethyl-6-methylphenyl) acetamide] and glyphosate followed by a second application of glyphosate 17 – 24 d later. On-site environmental data were collected using the Michigan Automated Weather Network (http://www.agweather.geo.msu.edu/mawn/, Michigan State University, East Lansing, Michigan; verified 04 Oct. 2017).

Experimental design and treatment application

Eighteen treatments were arranged in a randomized complete block design with four replications. The whole plot factor was CC (no cover crop, ‘The Buster™’ daikon forage radish, or ‘Magnum™’ forage oat [Weaver Seed of Oregon, Crabtree, OR]) and measured 54.9 m by 12.2 m in length. Cover crops were drill-planted (radish and forage oats at 11.2 and 28.0 kg ha-1, respectively) 14 Aug. and 17 Aug. in 2014 and 2015, respectively, with a Gandy orbit-Air Seeder coupled with John Deere double disk openers in 19 cm rows.

The subplot factor consisted of six N strategies that measured 4.6 m by 12.2 m in length. Total N rate was equalized to the maximum return to N rate (MRTN) (179 kg N ha-1) (Steinke, 2015). Nitrogen management strategies included PPI N (1-d prior to corn planting) as 100% urea, dried poultry manure (4-3-2-0.42S-0.57Mg-7Ca) (PM) (2.2 Mg ha-1) applied PPI plus V11 sidedress (SD), three starter N (45 kg N ha-1 urea ammonium nitrate [UAN; 28-0-0]) treatments applied 5 cm beside and 5 cm below the furrow (5x5) followed by SD at V4, V11, or V4 and V11, and a zero N control. Corn V4 N was UAN coulter injected 5 cm deep and 38 cm to the side of each row. At V11 SD a urease inhibitor (CO(NH2)2 + n-(n-butyl) thiophosphoric triamide] (Koch Agronomic Services, LLC, Wichita, KS) was mixed with UAN to prevent N volatilization and banded 10 – 15 cm to the side of each row. Corn was seeded in 0.76 m rows at 84,510 seeds ha-1 with Dekalb DKC48-12 RIB (98 d relative maturity) (Monsanto Co., St. Louis, MO).

Data collection and processing

Corn: The center two rows of each plot were used to determine V3 corn density (plants m-1) and harvested for grain yield, moisture, and test weight on 14 Oct. and 18 Oct. 2015 and 2016, respectively, using a Massey Ferguson 8XP research plot combine (Kincaid Equipment Manufacturing, Haven, KS). Final grain yields were corrected to 155 g kg-1 moisture.

Cover crops: Whole radish plants and oat canopies were sampled for biomass production and total tissue N and organic C content within 5 d of autumn termination and again mid-March (oats only) from three 0.25 m2 quadrats. Cover crop tissues were dried at 60°C, ground using a 1-mm mesh screen, and sent for commercial analysis (A&L Great Lakes Laboratories, Inc., Fort Wayne, IN) of total C and N via dry combustion. Total C and N uptake were calculated as the product of tissue concentration and dry biomass.

Soil chemical properties: Soils were sampled for NO_3-N (cadmium reduction) (30 cm depth) (Huffman and Barbarick, 1981), and P, K, Mg, Ca, pH, and CEC (20 cm depth) (previously described). Soils were sampled from each replication at CC establishment, from whole plots during autumn and mid-March (inorganic N only) simultaneous to cover crop biomass sampling, during May corn planting, and from subplots not receiving N fertility June through October (inorganic N only).

Soil basal CO_2 respiration and labile amino-N (SLAN): Soils were sampled (15 cm depth) from each replication and analyzed for CO_2 respiration and SLAN (Solvita®, Mt Vernon, ME) at cover crop establishment, from whole plots at autumn biomass sampling and May corn planting, and during corn tasseling (R1) and harvest (R6) (38 cm from corn row). Observations in the autumn of 2014 and May of 2015 followed a protocol which required soils to be dried, ground, and rewet but was changed for soil samples thereafter to the 'basal' protocol which utilized unground soils at field moisture levels (https://solvita.com/wp-content/uploads/2014/04/Solvita-and-Soil-Structure_Rewetting-2015.pdf, Solvita® & Woods End Laboratories; verified 02 Nov. 2017). For the basal field test, 6 – 10 soil samples were collected and homogenized and a 110 g subsample was used for processing at field soil moisture levels. Soils used for SLAN testing at R1 and R6 were sampled from the zero N strategy only and required soils to be dried.

Soil bacterial phylogenetic diversity: Soils were sampled (10 cm depth) for bacterial DNA analysis at cover crop establishment and termination, corn planting, R1, and R6. Soils at R1 and R6 were sampled from subplots and included bulk soil (i.e., 38 cm from corn row) and rhizosphere soil (i.e., between two corn plants within a row). Soils were air-dried and ground to pass through a 2-mm sieve.

Soil genomic DNA was extracted from 0.25 g with the MO-BIO Power Soil® kit (MO BIO Laboratories, Carlsbad, CA) and stored at -20°C. Polymerase chain reaction (PCR) was used to amplify the V4 hypervariable region (length 250 bp) of the 16S gene using genomic DNA as a template and high fidelity polymerase (Accuprime™ Pfx Supermix, ThermoFisher Scientific, Waltham, MA). Amplicons and libraries were constructed per previously described methods (Kozich et al., 2013). The MiSeq Illumina platform (San Diego, CA) was used to sequence amplicon libraries submitted to the Michigan State University Research and Technology Support Facility for next-generation sequencing (East Lansing, MI). Sequence data were processed through the Michigan State University High Performance Computing Center (East Lansing, MI) and analyzed using a previously described analysis pipeline (Kozich et al., 2013) and protocol (http://www.mothur.org/wiki/MiSeq_SOP; verified 5 Oct. 2017) with the mothur software package (ver. 1.33.2b; Schloss et al., 2009). Operational taxonomic units (OTU's) were based on a 97% sequence identity and classified to the SILVA database. Soil bacterial alpha diversity indicators (i.e., membership of taxa within a single sample) were assessed to describe diversity, richness, and evenness. The inverse Simpson's diversity index (ISD) was used to describe bacterial diversity and represented the number of uniformly abundant OTUs needed to observe the same level of diversity found in the community (Schloss and Handelsman, 2006). Soil bacterial community richness and evenness were assessed using the Chao1 estimator and Shannon index, respectively.

Data analysis

Data were subjected to analysis of variance using the GLIMMIX procedure in SAS (ver. 9.4; SAS Institute, 2011). Whole plot data considered cover crop and year as fixed effects and assumed random block effects. Additionally, subplot data (soil respiration, stand density, and corn yield) included N management strategy as a fixed effect and block x cover as a random effect. Microbial DNA sequencing data were processed separately by year due to computational load (Smith et al., 2016) and included soil sampling time and sampling zone as additional fixed effects in the model. Normality and homoscedasticity assumptions were validated with the UNIVARIATE procedure and Levene's test, respectively, using residuals ($P \leq 0.05$). When ANOVA generated a sig. F value ($P \leq 0.10$) treatment means were separated using the LINES option of the LSmeans statement. The SLICE statement was used to investigate interacting effects ($P \leq 0.10$). The CORR procedure was used to generate Pearson correlation coefficients for investigating relationships between edaphic variables and grain yield each year ($P \leq 0.10$).

Results and Discussion

Environmental conditions

Each year cover crops (CC) received 27 – 35 mm cumulative rainfall within 1-wk after seeding in Aug. that helped to aid in establishment (data not shown). Following CC seeding cumulative rainfall in Aug. and Sept. was 5 mm below the 30-yr mean in 2014 and 42 mm above in 2015 (Table 1). Oct. and Nov. received a cumulative rainfall deficit 47 and 62 mm below the 30-yr in 2014 and 2015, respectively. Air temperatures in the non-cash crop growing season (Aug. – Nov.) were 1.0 – 3.7 °C below the 30-yr mean in 2014 and 0.7 – 2.3 °C above in 2015 (except Aug.).

At corn seeding cumulative May and June rainfall was above normal (128 mm) in 2015 but below normal (103 mm) in 2016 and resulted in contrasting wet and dry soils, respectively. August rainfall was 39 – 79 mm above normal in 2015 – 2016. Cumulative Sept. and Oct. rainfall was 19 and 26 mm below and above the 30-yr mean in 2015 and 2016, respectively. Mean air temperatures (Apr. – Oct.) were -0.5 – 2.1 °C of the 30-year mean in 2015 and -1.3 to 1.6 °C in 2016.

Soil min. and max. moisture and temperature were generally higher in year 2 relative to year 1 in the 14-d prior to each soil sampling time except for soil moistures at R1 and soil temperatures at CC planting. Soil moisture at R1 was less in year 2 (relative to year 1) and likely due to deficit May and June rainfall followed by less precipitation in the 14-d prior to R1 observations in 2016 (Table 2). Soil moisture and soil temperature can shift soil bacterial community composition (i.e., β-diversity) with different phyla taxon responding differently to moisture stress and alter

CO2 efflux in soils under corn management (Ding et al., 2010; Bainard et al., 2016). Yearly variation in soil moisture and temperature at each observation likely influenced soil bacterial community composition each site year.

Studies were conducted on separate fields each year (183-m apart). Despite similar soil series (i.e., Capac loam soil) soil properties varied yearly and within each field. For example prior to CC planting in year 1 standard deviation between replicates for soil organic matter (OM) (g kg-1), pH, K (mg kg-1), and magnesium (Mg) (mg kg-1) was 1.9, 0.15, 21, and 25, respectively. Year 2 standard deviation was 0.22, 29, and 31 for soil pH, K, and Mg, respectively. Soil bacterial diversity (i.e., inverse simpson index) and relative abundance of bacterial phyla can be influenced by soil properties such as OM, pH, K, and Mg and vary spatially within fields (Rosenzweig et al., 2017). Differences in soil edaphic properties (pH, soil moisture, soil temperature, available phosphate) have shifted soil bacterial community composition within a growing season regardless of crop species (Bainard et al., 2016). The current study was conducted for two years on similar sites but variation within fields or between years in soil edaphic properties may have also influenced soil bacterial diversity and relative abundance.

Cover crop biomass production and quality

At autumn CC harvest in 2014 radish biomass was 39% greater compared with oat cover crop (Table 3). Leaf tissue analyses indicated radishes increased total N uptake 63% while oats resulted in an increased C:N ratio. Similar biomass results were observed in 2015 where the radish CC increased production 120% relative to the oat CC. In year 2 (i.e., autumn 2015) oat CC biomass production was reduced 44% relative to a 12% reduction with radishes from year 1 due to crown rust (Puccinia coronata) in the oats. Due to less biomass production C and N uptake was reduced 46 and 32%, respectively, and resulted in a lower oat CC C:N ration relative to the radish. Litter quality (i.e., CC) can influence soil bacterial community composition due to labile and recalcitrant C and N contributions and their relative proportions which provide sources of nutrients and energy (Paterson et al., 2008). Microbial metabolism can become N limited above a 20:1 C:N threshold and immobilize available soil N during CC litter decomposition (Mooshammer et al., 2014). The C:N ratios observed in the current study are near the 20:1 threshold observed in other studies and suggest that both CC's have the potential to affect N cycling. Additionally grass species (i.e., oats) contain more recalcitrant C (i.e., lignin) compared with radishes which has increased the relative abundance of Verrucomicrobia and Actinobacteria and may prolong decomposition and N mineralization (Sagova-Marechova et al., 2011; Jahanzad et al., 2016; Ye et al., 2017).

In March of both years radish residues were nearly decomposed and only oat CC residues were able to be sampled for biomass, and C and N uptake which precluded a formal analysis. In March of 2014 oat CC residues constituted 2429 kg ha-1 biomass, and 40 and 1041 kg ha-1 of N and C, respectively. In 2015, 3415 kg ha-1 oat residues remained and contained 52 and 1319 kg ha-1 of N and C, respectively. Spring C/N ratios of remaining biomass were similar each year (26:1) but higher than observed in the fall indicating a recalcitrant C source for soil bacteria.

Cover crop effects on base cation levels

At autumn CC biomass harvest in 2014, soil test phosphorus (P), potassium (K), magnesium (Mg), and calcium (Ca) levels were 51 – 64, 95 – 105, 155 – 206, and 878 – 1052 mg kg-1, respectively. After 81-d of CC growth oats had significantly increased autumn soil P levels 25% relative to radishes (P=0.0912) while no differences were observed between CC and the no cover control. In contrast, radishes increased autumn soil Ca levels 18 – 20% (P=0.0308) relative to other treatments. A previous Michigan study indicated that CC (i.e., radish) can increase soil test nutrient levels, but was after CC termination and incorporation into the soil (Wang et al., 2008). In the current study CC were actively growing and soils were subject to CC root exudation. Studies suggest graminaceous plants root exudates contain phytosiderophores – iron chelating compounds (e.g., mugineic acids) and may help explain increased P levels due to oat CCs (Sugiura and Nomoto, 1984). Mean soil pH at was 6.1 at cover crop sampling and phytosiderophores could have reduced the potential for Fe- and Al-P binding. Other studies have suggested plant root exudates solubilize FePO4 and AlP04 to release P which may have also contributed to P levels (Otani et al., 1996). Changes in autumn soil Ca and P levels in 2014 illustrate the potential of CC to impact soil properties and indirectly influence soil bacteria communities (Tao et al., 2017). At corn planting in May soil test P, K, Mg, and Ca levels were 48 – 52, 93 – 104, 169 – 173, and 1128 – 1167 but were unaffected by CC's.

At autumn CC biomass harvest in 2015, soil test P, K, Mg, and Ca levels were 20 – 23, 95 – 105, 155 – 206, and 878 – 1052 mg kg-1, respectively, and at corn planting in May were 27 – 30, 164 – 180, 179 – 210, and 990 – 1178 but were unaffected by cover crops.

Cover crop effects on corn grain yield

In 2015, grain yield was affected by N strategy but not by CC despite numerically higher yields in the no cover treatment (Table 4). The zero N strategy reduced corn yields 22 – 24% from strategies receiving N fertilizer. A single urea PPI application reduced grain yield 3% from strategies receiving a single SD application at V11 likely as a result of increased N loss opportunities with early applied N due to above-normal May (29%) and June (116%) rainfall. In 2016 grain yields were affected by CC's and N strategy. Oats reduced corn grain yield 4% relative to other CC treatments while the zero N strategy reduced grain yield 21 – 23% from other N fertilizer strategies. Unlike 2015 the 2016 field season was dry in May and June as a result of below normal rainfall in May (38%) and June (80%) rainfall. Reduced yields due to oat cover crops suggests that under dry soil conditions residue decomposition rates and subsequent N availability may not be in synchrony with corn N uptake. June soil sampling indicated that radish and oat cover crops reduced soil NO3-N levels 28 – 32% from the no cover (P=0.0533) in 2015 and 20 – 29% (P=0.0394) (oats only) in 2016 (data not shown). At June soil sampling each year corn was at growth stage V6 to V8 where N stress has reduced yield potential (Binder et al., 2000). Results suggest that cover crops may impact soil N availability in June thus growers should consider crop N uptake periods when increasing plant diversity with using non-leguminous cover crops too stimulate soil bacterial communities.

Cover crop effects on SLAN content and soil basal CO2 respiration

SLAN: Soil labile amino-N was observed in whole plots under the influence of CC treatments. Pearson correlations indicated SLAN was not affected by soil moisture, temperature, or precipitation experienced 14-d prior to each observation (Table 5). In 2014, the radish CC increased SLAN 15% relative to the no cover control at autumn biomass harvest (Table 6). In 2015 oats increased SLAN 18 and 14% relative to the radish CC and no cover control, respectively. Root exudates provided by actively growing cover crops in the autumn may contain amino acids and contribute to SLAN (Huang et al., 2014). Soil microbes can directly assimilate free amino acids and increased SLAN due to covers may offer opportunity to affect soil bacterial communities (Jan et al., 2009). Despite non-significant correlations between SLAN and soil moisture, precipitation, or temperature, soil minimum and maximum moisture were correlated with soil NO3-N ($r=-0.62$ to -0.64; $P \leq 0.06$) and NH4-N were correlated ($r=-0.78$ to -0.79; $P \leq 0.01$). Significant correlations with soil NO3-N and NH4-N but not SLAN suggest that N contained in SLAN is less affected by climatic factors and a more stable criterion when evaluating CC to impact microbial communities. While observations the following May in 2015 indicated no differences in SLAN, the presence of root and shoot biomass from cover crop residues are a source of proteins for enzymatic amino acid synthesis and microbial assimilation (Jan et al., 2009).

Respiration: Soil respiration was observed in whole plots prior to corn planting and in subplots of the zero-N strategy at R1 and R6. Data will be discussed pertaining to whole plots first (not under the influence of corn) (i.e., autumn and the following May prior to corn planting) for year 1 and year 2, and then subplots for year 1 and year 2. In contrast to Han et al. (2007) Pearson correlations indicated CO2 was not affected by soil moisture, temperature, or precipitation experienced 14-d prior to observations at CC planting, autumn CC harvest, corn planting, corn tasseling, and corn harvest (Table 5). In 2014 CC did not significantly impact soil respiration at CC harvest time in the autumn or at corn planting the following May in 2015 (Table 6). At CC harvest in the fall of 2015, radish and oat covers increased soil respiration 46 – 61%. Increased autumn CO2 respiration due to cover crops are an indication of increased microbial activity as both heterotrophic microbial oxidation of organic C by rhizosphere and bulk soil microbes and CC (autotrophic) root respiration could have contributed to CO_2 efflux (Raich and Mora, 2005).

After corn planting and subplot establishment in 2015 oat and radish cover crops increased soil CO_2 respiration 12 – 15% relative to the no cover control and indicated that CC residues were continuing to decompose between R1 and R6 (Table 7). Nitrogen strategies had no impact on CO_2 respiration, but when values were averaged across CC and N strategies respiration at R1 increased 21% relative to R6. Previous studies have indicated microbial community structure is influenced by corn growth stage with greatest bacterial diversity observed at tasseling and the least at physiological maturity (i.e., R6) (Cavaglieri et al., 2009). Increased CO_2 respiration at R1 may stimulate microbial activity due to the influence of an actively growing corn crop. In 2016 a CC by N strategy interaction was observed ($P=0.0374$) indicating that CO_2 respiration due to N strategy was affected by the previous CC (Table 8). When splitting the interaction by CC, CO_2 respiration was increased 22% in the radish CC when the early-applied SD N rate (i.e., V4) was reduced and the late-applied SD N rate (i.e., V11) was increased. In the same site year radish cover crops resulted in a higher C:N ratio than oats (previously mentioned). Previous studies have indicated that increased CC C:N ratio can have a legacy effect on soil CO_2 respiration in response to secondary low C:N inputs (i.e., starter N plus SD N) and reduce soil CO_2 respiration (Marschner et al., 2015). Deficit rainfall in May and June (i.e., 2016) may have prolonged cover crop decomposition and suggest consideration of CC C:N ratio management (e.g., termination dates) when using CC to stimulate soil microbial activity.

DNA sequence analysis

In year 1 (2014 – 2015) and year 2 (2015 – 2016) 7.1 – 9.2 x 106 valid sequences were obtained after quality filtering and represented a mean of 29,047 and 21,928 sequences per sample in each year, respectively. Despite soil sampling efforts each year rarefaction analysis did not produce a plateauing curve which indicated increased sampling frequency may have increased the number of recovered unique sequences. In spite of rarefaction results sequences were subsampled each year (i.e., 9,500=year 1 and 6,000=year 2) to help rarify data. Sequences covered 91,376 and 49,203 OTUs using 97% identity as the cutoff and suggests previous estimates (2,000 – 5,000 OTUs) are conservative (Schloss and Handelsman, 2006). In year 1 and year 2, 17,020 and 14,930 OTUs were represented by more than 5 reads which was 112 – 142% greater than reported in a previous study which utilized the Roche 454 platform to sequence soils treated with straw and N fertilizer (Pitombo et al., 2016). Sequencing identified 25 and 27 bacterial phyla in years 1 and 2 (24 phyla appearing across both years) and 682 and 655 genera, respectively. The top five phyla shared in each year represented 82 – 87% of the total OTUs identified. However, each year 42 – 44% of phyla and 76 – 78% of genera were unclassifiable and represented the largest group in each taxonomic rank. Collectively, singletons and doubletons (i.e., sequences observed 1x and 2x per sample, respectively) accounted for 73 and 56% of all sequences identified in years 1 and 2, respectively, and indicate rarity was common in the agricultural soils sampled.

Alpha diversity indicators (Inverse Simpson [ISD], Shannon Evenness [Shannon] and Chao1 Richness Estimator [Chao]) before and after corn establishment

Soil bacterial diversity (ISD), richness (Chao), and evenness (Shannon) was observed in whole plots prior to corn planting and in subplots of the zero-N strategy at R1 and R6. Data will be discussed pertaining to whole plots first (not under the influence of corn) for year 1 (2014 – 2015) and year 2 (2015 – 2016), and then subplots for year 1 and year 2.

Before corn establishment: Estimation of OTU diversity using the inverse Simpson index (ISD), which accounts for richness (i.e., the number of species per sample) and evenness (i.e., the relative abundance of species in a sample) of soil bacterial communities indicated an effect of CC in year 1 (i.e., autumn, 2014, and May, 2015) (Table 9). Despite the abundance of singletons (previously mentioned) which can increase richness of samples, the ISD helps control for rarity and gives more weight to abundant species where OTU diversity increases as ISD increases. The radish CC reduced diversity 50 – 52% relative to oats or the no cover control which were similar in the autumn. Members of Brassicacae (i.e., radish) contain quantities of glucosinolates that break down to isothiocyanates which may have biofumigant effects on soil microbial taxa and help explain reduced soil bacterial diversity to an ensuing corn crop observed in other studies (Kirkegaard and Sarwar, 1998; Fernandez et al., 2016; Tao et al., 2017). Results contrast the common assertion that increased plant diversity increases soil biological diversity. Instead radishes may have favored growth of copiotrophic taxa and decreased bacterial richness and evenness in the short term (Fernandez et al., 2016). Despite no sig. differences a trend for reduced OTU richness and evenness was evident which supports the previous hypothesis. In May (2015) radishes reduced diversity 23% relative to the oats while diversity in the no cover control remained similar to CCs which suggested that overwintering radish residues were continuing to affect soil bacterial membership more than the oat CC but to a lesser extent as CC decay progressed.

In year 2 CC did not affect diversity in the autumn (2015) or the following May (2016). Pearson correlations indicated that diversity in the May of corn establishment negatively corresponded to soil NO_3-N levels in both years ($r=-0.71$; $P \leq 0.01$), similar to other studies (Zhao et al., 2014; Li et al., 2016). Differing C:N ratios of radish CC biomass each autumn suggest differences in decomposition rates that could affect substrate availability as residue was broken down by soil microbiota. A 23:1 C:N ratio in year 2 (relative to 17:1 in year 1) may have reduced soil bacterial substrate availability and placed less emphasis on copiotrophic behavior (Koch, 2001; Fernandez et al., 2016). In May radish and oat CCs increased richness 7 – 11% and reduced evenness 0.9 – 1.0% which considering both factors may be an indication of increased number of rare OTUs. Within the two – year timeframe of this study it was unclear if CCs have a consistent effect on soil bacterial diversity, richness, and evenness prior to corn planting and warrants further investigation (Fernandez et al., 2016).

After corn establishment: When soil sample time and zone were included in the statistical model ANOVA of alpha diversity indicators did not indicate significant interactions of CC and N strategy when analyzed within soil sample zone, timing, or both and suggested soil bacterial communities responded similarly to N management strategies despite previous CCs (Table 10). Main effects of CC and N strategy are discussed separately. Soil bacterial diversity, richness, and evenness were not affected by CC while N strategy effects were significant in 4 of 6 observations which suggests soil bacterial communities were affected by N fertilization. Soil sampling zone and timing were significant in 5 of 6 observations which suggest that spatial and temporal variation had more impact on alpha diversity indicators than applied treatments.

In 2015 the PL plus V11 sidedress (SD) strategy increased ISD 7.2 – 9.4% relative to other N fertilizer strategies. The PL plus V11 SD treatment contained a natural organic plus inorganic NPK in addition to C, sulfur (S), Mg, Ca, boron (B), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) provided by PL. Other studies have also observed increased soil bacterial diversity due to organic plus inorganic fertilization which may provide C and N substrates for bacterial function (Zhao et al., 2014). All N sources have the capacity to reduce soil pH which can indirectly due to release of H+ during corn root NO3-N uptake, but urea and UAN-N sources may contribute directly to soil acidity due to 2H+ as a byproduct during decomposition (Havlin et al., 2014). Soil pH can be a determinant of soil bacterial diversity (Zhalnina et al., 2015). Urea and UAN fertilizers utilized in the current study may have created zones of acidity and reduced soil pH and reduced ISD relative to a natural organic N + inorganic N source (i.e., PL and V11 SD) although no differences were observed relative to the control. Richness was not affected within CCs and illustrates the dominance of spatial and temporal variation on richness. Cover crop residues had less influence to soil bacterial communities than N strategies as measured by ISD, chao richness, and Shannon evenness when combined across R1 and R6 sample times but growers may supplement a natural organic N source in their fertilizer program to increase diversity.

In 2015 a CC x soil sample zone x sample timing interaction was observed for diversity (i.e., ISD) (P=0.0091) and richness (P=0.0431) (Table 10). Slicing the ISD interaction indicated no diversity differences in soil sampling zone x timing within the radish CC only and suggests radish residues were able to reduce the spatial and temporal variation of soil bacterial diversity relative to oat and no CC soils (data not shown). Radish and oat CCs increased diversity 11 –

15% relative to no cover in bulk soils at R6 and not R1 and suggests 1) an actively growing cash crop may select for bacterial communities and reduce the influence of CC residues, 2) CC residues affecting diversity at corn maturity indicate recalcitrant residues may still influence soil bacterial diversity 11 months after termination, and 3) reduced influence of a corn rhizosphere after reaching physiological maturity. Studies observing no relationship of plant species richness and bacterial diversity have suggested that rhizosphere bacterial community composition may depend on the composition of bulk soil communities (de Ridder-Duine et al., 2005) or due to the functional redundancy of soil bacterial communities to utilize common root exudates (Zhalnina et al., 2015). Slicing the CC x soil sample zone x sample timing interaction for richness indicated CC did not have an effect on soil bacterial community richness regardless of soil sample timing or zone. Data suggest that future studies investigating CC influence to bacterial diversity in an actively growing corn cash crop may consider actively growing CC during the corn cash crop season due to the potentially interacting effects of plant rhizospheres and surrounding bulk soil bacterial communities.

In 2016 soils were dry following N applications in May and June due to deficit monthly rainfall. Cover crops did not affect ISD, Chao, or Shannon but each were affected by N strategy and suggests N fertilization may have more influence on bacterial OTUs in dry soils than wet (i.e., 2015) (Table 10). Limited rainfall to assimilate N fertilizer into the soil matrix may have increased soil solution osmotic potential, become toxic due to V4 UAN SD, and reduced community diversity 6 – 8% from soils receiving urea or PL PPI or no zero N and evenness 1.5% from the zero N strategy (Li et al., 2016). Strategies including V4 SD were coulter injected which may have preserved fertilizer band prevalence to reduce diversity relative to surface banded V11 SD strategies. Pearson correlations indicated soil moisture was positively associated with bacterial diversity and evenness and negatively with soil inorganic N levels (Table 5). Associations suggest cumulative rainfall near N application timing may influence the effect of N strategy on soil bacterial communities. Similar to 2015, the PL and V11 SD resulted in similar diversity, richness, and evenness as the zero N strategy and suggest that an inorganic + organic N source may promote bacterial community stability across space and time. In both years diversity and evenness were increased at R6 relative to R1. Bulk soil diversity was increased in 2016 relative to the rhizosphere. Previous studies have suggested that environmental heterogeneity (e.g., pH and soil moisture) and field location interact to impact corn rhizosphere soil bacterial diversity (Pieffer et al., 2013). Deficit rainfall in May and June may have limited corn growth as evidenced by reduced yields in 2016 and reduced the influence corn had on bacterial diversity in dry spring soils as bulk soil diversity was similar each year (ISD 342 – 343).

In 2016 a N x Z x T interaction was observed for ISD ($P<0.0001$) and Shannon evenness ($P=0.0159$) (Table 10). Inspection of the interaction for ISD values indicated significant differences at R1 between strategies receiving N fertilizer in the bulk soil, but not the rhizosphere. In contrast Shannon evenness at R1 was affected by N strategies in the rhizosphere but not in the bulk soil. Other studies have illustrated the selection pressure plant rhizospheres can have on soil bacterial communities (Kowalchuk et al., 2002). Despite the influence of N strategies on bulk soil diversity no differences in ISD observed due to strategies receiving N fertilizer within R1 corn rhizosphere soils indicate corn influenced ISD more than N placement x timing. In lieu of ISD values, differences in Shannon evenness values in R1 rhizosphere soils indicate specific communities were influenced by the corn rhizosphere in regard to N fertilizer strategy and suggest that N fertilizer strategy may have influenced corn selection of rhizosphere soil bacterial membership.

Pearson correlations indicated that R1 diversity in the rhizosphere was neg. associated with corn grain yield each year where N fertilizer had been applied (r=-0.21 to -0.44; $P \leq 0.08$) or had not been applied (r=-0.53 to -0.63; $P \leq 0.08$). Negative correlations suggest that relative abundance may explain more variation in grain yield. Reduced diversity were sometimes associated with treatments receiving UAN SD at V4. Evenness was negatively correlated with yield in the bulk soil (r=-0.25 to -0.36; $P \leq 0.05$) which suggests OTU relative abundance was affected and further supports reduced diversity associated with increased grain yield. Studies often do not report associations of crop yield with soil bacterial diversity, or have observed no correlation of plant biomass yield with microbial diversity (Zhalnina et al., 2015) and suggested bacterial abundance as indicators of crop yield (Tautges et al., 2015). While data indicating reduced R1 corn rhizosphere diversity and increased crop yield were consistent in both years reduced grain yield in a dry year (i.e., 2016) suggest future studies investigate variation of this effect across landscapes and climates for improved understanding on cropping system and soil bacterial community resilience to stress.

Conclusions

Two years of data demonstrated that CCs preceding a corn cash crop were able to affect soil labile amino acid N, soil CO2 respiration, soil P and Ca levels, and soil bacterial diversity (i.e., ISD), richness (i.e., chao), and evenness (i.e., Shannon) but varied temporally. After corn cash crop seeding decomposing CC residues continued to affect soil respiration when cumulative May

– June rainfall was excessive (128 mm above normal) while under deficit May – June cumulative rainfall N fertilizer placement x timing treatments preceded by a radish CC affected CO2 respiration. Daikon radish increased autumn biomass production and N uptake relative an oat CC, but less residue remaining in March of the following year suggesting fewer opportunities to affect soil microbial diversity in the ensuing corn cash crop season. During the corn cash crop season CCs had less influence on soil bacterial diversity, richness, and evenness in relation to the effects observed due to N strategies while more instances (5 of 6 site years) of interacting factors of soil sampling time and soil sampling zone (i.e., rhizosphere or bulk soils) affected soil bacterial diversity, richness, and evenness and suggest spatial and temporal variation were a large determinant of bacterial community membership. Nitrogen fertilizer which included a natural organic N source (i.e., poultry litter) increased diversity in relation to strategies that included V4 SD. Full V4 SD reduced diversity 6 – 8% from soils receiving urea, PL, or zero N in dry soils. An actively growing corn cash crop reduced the impact of N strategies on R1 rhizosphere soil bacterial diversity in relation to differences observed in the bulk soil. Grain yield was inversely proportional to soil bacterial diversity at R1 ($r=-0.21$ to -0.63; $P \leq 0.08$) regardless of fertilizer N application and indicates corn tasseling may be a time in corn ontogeny to increase opportunities to affect grain yield but suggests relative abundance of bacterial community membership may explain more variation in grain yield than diversity. However reduced grain yields in dry soils due to an oat CC suggest growers may need to select CC species according to agronomic objectives. Two site years of data with variable May and June rainfall suggest the main driver of soil microbial diversity, richness, and evenness during a corn cash crop season is N fertilizer strategy and not previous CC, but it remains unclear due to spatial and temporal variation between years. Relationship of alpha diversity indicators to corn grain yields and cropping system resilience may depend on multi-year observations of crop rotations where the rotation itself is replicated across site years. A more extensive soil sampling and sequencing effort may be necessary to characterize relationships occurring between bacterial OTUs and corn rhizospheres. Future studies may be enhanced with analysis of microbial functional groups and not taxonomic relatedness.

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APPENDIX

Table 4.01. Monthly precipitation and temperature departures from normal and 30-yr means for Lansing, MI, 2014 – 2016.

	precipitation†				air temperature‡			
Month	2014	2015	2016	30-yr mean	2014	2015	2016	30-yr mean
	difference (mm) from avg.			mm	difference (°C) from avg.			°C
Aug.	+2	+39	+79	84	-1.0	-1.0	+1.6	21.3
Sept.	-7	+3	+14	92	-1.5	+2.1	+0.5	16.9
Oct.	-13	-22	+12	70	-1.3	+0.7	-1.2	10.4
Nov.	-34	-40	-22	71	-3.7	+2.3	+0.1	4.6
Apr.	-51	-50	+2	73	-0.7	-0.5	-1.3	8.7
May	-2	+25	-32	85	-0.3	+1.6	0.0	14.7
June	+34	+103	-71	89	0.0	-0.8	+0.3	20.0
July	-22	-21	+13	83	-3.3	-1.2	+1.0	22.1

†Precipitation and air temperature data were collected from Michigan Automated Weather Network (http://www.agweather.geo.msu.edu/mawn/).
‡30-yr mean sources for precipitation and air temperatures: NOAA (https://www.ncdc.noaa.gov/cdo-web/datatools/normals; verified 17 Aug. 2017).

Table 4.02. Two-year soil moisture (30-cm), precipitation (cm), and soil temperature (5-cm) data for the 14 d period prior to each soil sampling event (cover crop planting [CC plant], autumn cover crop harvest [CC harvest], corn planting [corn plant], corn tasseling [corn R1], and corn harvest [corn R6]) in Lansing, MI, 2014 – 2016.

	CC plant†	CC harvest	Corn plant	Corn R1	Corn R6
2014-15	14-Aug	3-Nov	1-May	20-Jul	9-Oct
Max. soil moist. (cm^3 cm^{-3})	0.110	0.211	0.197	0.273	0.174
Min. soil moist. (cm^3 cm^{-3})	0.110	0.202	0.190	0.257	0.170
Precip. (mm)	17.0	17.8	7.1	46.2	14.0
Max. soil temp. (°C)	24.0	4.0	12.0	27.0	16.5
Min. soil temp. (°C)	18.4	2.5	6.3	16.5	13.2
2015-16	17-Aug	30-Oct	17-May	20-Jul	11-Oct
Max. soil moist. (cm^3 cm^{-3})	0.200	0.232	0.250	0.085	0.237
Min. soil moist. (cm^3 cm^{-3})	0.179	0.224	0.235	0.079	0.222
Precip. (mm)	65.3	32.5	37.3	42.2	80.8
Max. soil temp. (°C)	22.9	11.4	16.5	30.0	19.3
Min. soil temp. (°C)	18.9	8.1	10.5	21.4	14.6

†Cover crop planting and harvest relative to 2014 and 2015, respectively. Corn planting, R1, and R6 relative to 2015 and 2016, respectively.

Table 4.03. Cover crop biomass and quality at autumn cover crop harvest, Lansing, MI, 2014 - 2015.

	Biomass		C uptake		N uptake		C/N	
Sample			2014					
			kg ha^{-1}				mg kg^{-1}	
Radish†	10567	a‡	3866	a	230	a	17:1	b
Oats	7604	b	3149	a	141	b	23:1	a
P > F	0.0667		0.1651		0.0550		0.0724	
			2015					
Radish	9351	a	3298	a	149	a	23:1	a
Oats	4255	b	1765	b	101	b	17:1	b
P > F	0.0030		0.0072		0.0065		0.0508	

†Radish biomass unable to be sampled in spring due to winter decomposition.
‡Means followed by the same letter in a column within each year are not sig. different at alpha=0.10.

Table 4.04. Main effects of cover crop and N strategy† on corn grain yield, Lansing, MI, 2015 – 2016.

	Grain Yield			
Main effect	2015		2016	
Cover crop	Mg ha^{-1}			
No cover	15.8	a‡	13.6	a
Radish	15.4	a	13.5	a
Oat	15.5	a	13.0	b
N Strategy				
Zero N	12.4	c	10.8	b
Urea [PPI]	15.9	b	14.0	a
PL [PPI] + V11	16.4	a	13.8	a
5x5 starter + V4	16.2	ab	13.7	a
5x5 starter + V11	16.4	a	13.7	a
5x5 starter + V4/V11	16.3	ab	13.9	a
ANOVA				
Cover (C)	0.1309		0.0311	
N strategy (N)	<.0001		<.0001	
C x N	0.2262		0.3271	

†All strategies requiring N fertilizer received total maximum return to N rate of 179 kg N ha^{-1} in all years.
‡Means within each column followed by same lower case letters are not significantly different at alpha=0.10.

Table 4.05. Correlation coefficients (r) (n=10) relating indicators of soil microbial alpha diversity† indicators (inverse Simpson diversity [ISD], Chao1 richness [Chao] and Shannon evenness [Shannon]) or soil parameters‡ (soil labile amino acid [SLAN], CO_2 respiration, soil NO_3-N and NH_4-N) to weather factors observed during the 14 d period prior to cover crop planting, autumn cover crop harvest, corn planting, corn tasseling, and corn harvest in Lansing, MI, in 2015 - 2016.

	ISD	Chao	Shannon	SLAN	Respiration	NO_3^-	NH_4^+
Max. Soil Moist.	0.81^{**}	0.02	$0.55^{0.10}$	-0.26	-0.12	$-0.62^{0.06}$	-0.78^{**}
Min. Soil Moist.	0.82^{**}	0.06	0.54	-0.28	-0.17	-0.64^{*}	-0.79^{**}
Precipitation	0.18	-0.28	0.25	0.25	0.38	-0.19	0.03
Max. Air Temp.	-0.52	-0.11	-0.29	0.27	0.39	0.35	$0.60^{0.07}$
Min. Air Temp.	-0.48	-0.05	-0.30	0.32	0.52	0.40	0.63^{*}

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

†Soil parameters were an average of means observed across 1) replications at cover planting 2) whole plot replications in the autumn and spring, and 3) subplot replications within the zero-N strategy at corn R1 and R6.

‡Soil alpha diversity indicators were an average of means observed across 1) replications at cover planting 2) whole plot replications in the autumn and spring, and 3) subplot replications combined across soil sampling zones at corn R1 and R6.

Table 4.06. Effect of cover crop and no N fertilizer on soil labile amino N (SLAN) and soil respiration (CO_2) means observed in the autumn and the ensuing May at corn planting, Lansing, MI, 2014 - 2016.

Treatment	Autumn SLAN†		Autumn CO_2‡		Spring SLAN		Spring CO_2	
	kg N ha^{-1}		mg CO_2 kg^{-1}		kg N ha^{-1}		mg CO_2 kg^{-1}	
2014 – 2015								
No Cover	115	b§	15.7	a	108	a	16.2	a
Radish	132	a	19.8	a	129	a	19.9	a
Oats	125	ab	17.8	a	118	a	19.5	a
P > F	.0716		0.5054		0.1369		0.5190	
2015 – 2016⁋								
No Cover	157	b	14.7	b	142	a	15.5	b
Radish	152	b	23.6	a	155	a	24.1	a
Oats	179	a	21.5	a	158	a	22.0	a
P > F	0.0973		0.0896		0.5037		0.0358	

†Mean SLAN was 142 and 150 kg N ha^{-1} at cover crop planting in 2014 and 2015, respectively.
‡Basal CO_2 respiration was 35.6 and 35.9 mg kg^{-1} at cover crop planting in 2014 and 2015, respectively.
§Means followed by the same letter in a column within each year not sig. different at alpha=0.10.
⁋Field soil moisture content at sampling time was 1.8 and 1.7 x 10-7 mg kg-1 in the autumn of 2015 and May 2016, respectively. Moistures not presented for autumn of 2014 and spring of 2015 as a dry method sampling protocol was used.

Table 4.07. Main effects of cover crop and N strategy† on basal soil respiration combined across observations at flowering (R1) and physiological maturity (R6), Lansing, MI, 2015.

Main effect	Respiration	
Cover crop	mg kg^{-1}	
No cover	18.1	b‡
Radish	20.2	a
Oat	20.9	a
N Strategy		
Zero N	19.8	a
Urea [PPI]	19.8	a
PL [PPI] + V11	20.0	a
5x5 starter + V4	18.5	a
5x5 starter + V11	19.9	a
5x5 starter + V4/V11	20.6	a
Timing		
Corn tasseling (R1)	21.6	a
Corn maturity (R6)	17.9	b
	ANOVA	
Cover (C)	0.0408	
N strategy (N)	0.7192	
C x N	0.2723	
Timing (T)	0.0089	
C x T	0.3177	
N x T	0.7337	
C x N x T	0.8163	

†All strategies requiring N fertilizer received total maximum return to N rate of 179 kg N ha^{-1} in all years.
§Means within each column followed by same lower case letters are not significantly different at alpha=0.10.

Table *4.08.* Cover crop and N management interaction (P=0.0374) on soil basal CO_2 respiration combined across R1 and R6 sampling times, Lansing, MI† 2016.

	Cover					
N strategy	No Cover		Radish		Oat	
			mg CO_2 kg^{-1}			
Zero N	21.6	a‡	21.1	abc	22.2	a
Urea [PPI]	19.3	a	24.6	a	18.0	a
PL [PPI] + V11	20.8	a	24.7	a	22.4	a
5x5 starter + V4	22.5	a	18.5	c	23.9	a
5x5 starter + V11	17.6	a	22.6	ab	23.4	a
5x5 starter + V4/V11	18.9	a	19.7	bc	22.4	a
$P > F$	0.3287		0.0597		0.2330	

†All strategies requiring N fertilizer received total maximum return to N rate of 179 kg N ha^{-1} in all years.
‡Means within each column followed by same lower case letters are not significantly different at alpha=0.10.

Table 4.09. Effect of cover crop on alpha diversity indicators† (inverse Simpson [ISD], Chao1 Richness [Chao] and Shannon evenness [Shannon]) sampled in the autumn and spring prior to corn planting, Lansing, MI, 2014 – 2016.

	2014-15											
	Fall						Spring					
Treatment	ISD		Chao		Shannon		ISD		Chao		Shannon	
No Cover	392	a‡	5581	a	0.848	a	326	ab	3786	a	0.856	a
Radish	190	b	3874	a	0.827	a	290	b	3011	a	0.866	a
Oat	380	a	5821	a	0.857	a	379	a	4313	a	0.851	a
P > F	0.0470		0.3670		0.1531		0.0695		0.1806		0.3319	
	2015-16§											
No Cover	339	a	4032	a	0.859	a	350	a	3924	b	0.864	a
Radish	343	a	3828	a	0.864	a	340	a	4208	a	0.854	b
Oat	367	a	4339	a	0.860	a	371	a	4362	a	0.856	b
P > F	0.4458		0.4287		0.8873		0.1417		0.0305		0.0985	

†Diversity indices were assessed for operational taxonomic units (OTUs) based on a 97% sequence identity.
‡Means followed by the same letter in a column within each year are not sig. different at alpha ≤ 0.10.
§2015 ISD normalized using exponential transformation. Untransformed means are presented.

Table 4.10. Main effects of cover crop and N strategy[†] on alpha diversity indicators (inverse Simpson diversity [ISD], Chao1 richness [Chao] and Shannon evenness [Shannon]) across soil sampling zones (rhizosphere and bulk soil) and sample timings (corn tasseling and harvest), Lansing, MI, 2015- 2016.

	—2015—						—2016—					
Treatment	ISD		Chao		Shannon		ISD		Chao		Shannon	
Cover Crop												
No cover	340	a[‡]	4525	a	0.851	a	324	a	3715	a	0.858	a
Radish	354	a	4546	a	0.856	a	320	a	3789	a	0.855	a
Oat	355	a	4436	a	0.853	a	333	a	3908	a	0.855	a
N Strategy												
Zero N	356	ab	4714	a	0.854	a	329	ab	3930	ab	0.862	a
Urea [PPI]	347	b	4527	a	0.853	a	336	a	3680	bc	0.862	a
PL [PPI] + V11	372	a	4608	a	0.855	a	331	a	4141	a	0.856	abc
5x5 starter + V4	341	b	4286	a	0.853	a	309	c	3686	bc	0.849	c
5x5 starter + V11	341	b	4451	a	0.854	a	329	ab	3797	bc	0.856	ab
5x5 starter + V4/V11	340	b	4427	a	0.850	a	319	bc	3589	c	0.853	bc
Zone												
Bulk soil	343	b	4382	b	0.852	a	342	a	3888	a	0.859	a
Corn rhizosphere	357	a	4623	a	0.855	a	310	b	3719	a	0.853	b
Sample time												
Corn tassel (R1)	342	b	4510	a	0.850	b	291	b	3861	a	0.843	b
Corn maturity (R6)	357	a	4495	a	0.856	a	361	a	3747	a	0.870	a
	ANOVA (*P* > *F*)											
Cover (C)	0.3460		0.7785		0.3653		0.6247		0.6834		0.4827	
N strategy (N)	0.0242		0.5059		0.5827		0.0124		0.0970		0.0302	
C x N	0.8190		0.5083		0.4326		0.7487		0.4082		0.7117	
Zone (Z)	0.0112		0.0341		0.1048		<.0001		0.1543		0.0242	
C x Z	0.1921		0.2677		0.6214		0.6658		0.4137		0.2639	
N x Z	0.7730		0.7478		0.6892		0.2744		0.1833		0.4358	
C x N x Z	0.7675		0.6087		0.4783		0.7570		0.4796		0.4114	
Timing (T)	0.0238		0.9043		0.0005		<.0001		0.3286		<.0001	
C x T	0.4758		0.4015		0.9450		0.8872		0.7064		0.7028	
N x T	0.1981		0.6802		0.1492		<.0001		0.9340		0.0041	
C x N x T	0.9578		0.4999		0.5223		0.4173		0.8466		0.8244	
Z x T	0.0907		<.0001		<.0001		<.0001		<.0001		0.4922	
C x Z x T	0.0091		0.0413		0.9131		0.1669		0.5067		0.9546	
N x Z x T	0.3786		0.9459		0.9282		<.0001		0.7223		0.0159	
C x N x Z x T	0.8802		0.5765		0.6152		0.7955		0.9288		0.7904	

†All strategies requiring N fertilizer received total maximum return to N rate of 179 kg N ha^{-1} in all years.
‡Means within each column followed by same lower case letters are not significantly different at alpha=0.10.

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