Legume cover crops in high tunnels: Field evaluation for soil health and controlled environment freezing tolerance

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Abstract

This thesis explores legume cover crops as a possible management tool for nitrogen fertility and soil health maintenance in high tunnels. Projects include: 1) a two-year field evaluation of three fall planted cover crop mixes for winter annual production, 2) a controlled environment freezing tolerance study of hairy vetch (*Vicia villosa*) and red clover (*Trifolium pratense*) using simulated high tunnel conditions, and 3) a one-year field evaluation of three spring planted cover crop mixes. Cover crop mixes used in projects 1 and 3 consisted of: a) red clover monoculture (*T. pratense*), b) Austrian winter pea/winter rye 1:1 biculture (*Pisum sativum* and *Secale cereal*), and c) hairy vetch/tillage radish/winter rye 4:1:15 mix (*V. villosa*, *Raphanus sativus*, and *S. cereal*). Winter annual legume results show a wide range of biomass nitrogen additions (19.7 to 365.0 kg N ha^{-1}), with no negative impact cash crop yield or soil health measures.

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Chapter 1: Literature Review

Introduction

In the past decades, there has been a rapid increase in high tunnel construction in the United States due to a combination of expanded access to local produce markets and a recent cost-share incentive through the USDA NRCS Environmental Quality Incentive Program (NSAC, 2015; Huff, 2015). High tunnels increase marketable crop quality and yield and allow growers to claim a price premium for off-season, local produce. However, published and anecdotal data reports that soil health decline in high tunnels may negatively affect long-term yield (Warren et al., 2015; Bross, personal communication). Legume cover crops in rotation with cash crops are a potential management tool for soil health maintenance and nitrogen provisioning in high tunnels. Legume cover crops are of interest to those concerned about soil health and fertilizer input reduction, especially organic growers, who are directed by the National Organic Program to use cover crops in their crop rotations and to minimize nutrient surfeits. This thesis explores cover crops as a management tool for soil health in organically managed high tunnels. This literature review will describe specific microclimate and management practices employed in high tunnels, soil health vulnerabilities under common high tunnel management, legume cover crops as a possible tool for high tunnel growers, and current knowledge gaps that discourage legume cover crop implementation.

High tunnels

Construction Trends

High tunnels, sometimes called hoop houses, greenhouses, or poly tunnels, are semi-permanent, covered structures used to protect crops from extreme environmental conditions and extend the growing season (NRCS, 2015). The basis of these structures is a single sheet of polyethylene film stretched over a wooden or metal frame (Carey et al., 2009). Complexity can be built into this simple system by varying ventilation, insulation, and structural strength depending on needs dictated by climate and grower access to resources and capital. Ventilation options range from hand-cranked side curtains to automated ventilation of the sides or eaves of the structure, with or without an exhaust fan (Wells, 1996; Huff, 2015). Automated ventilation is particularly advantageous during spring and fall, when heat needs to be conserved during cool, cloudy weather but could rise to a damaging degree with sunny weather. Insulation may be increased by thickness of polyethylene film or by using two layers of polyethylene inflated by fans instead of the standard one layer (Lamont, 2005; Huff, 2015). Growers in colder climates may also opt to insulate the soil perimeter with polystyrene foam board to prevent lateral frost movement into the growing space, thus decreasing days per year of frozen soil (Adams and Todd, 2014). The structure may have corner posts or all posts sunk into the ground with or without concrete footings, depending on the risk of high winds. In places with heavy snowfall, growers may shorten the distance between posts to add strength and/or choose a roof shape designed to shed snow (Blomgren et al., 2007; Huff, 2015). Given the wide range of construction options, price can vary widely from $2.95 per square foot to $6.67 per square foot (Huff, 2015).

High tunnels are a relatively new technology that are now used worldwide. As of 2009, the majority of high tunnel production was in eastern Asia and the Mediterranean, with a combined 514,600 hectares under high tunnel production in the top five countries – China, Spain, Japan, Italy, and Korea, while the United States had the 10th largest area under high tunnel production, with 5,000 hectares (Lamont, 2009). Usage in the United States has increased since 2009, in part due to a federal cost share initiative through the National Resource Conservation Services (NRCS) Environmental Quality Incentives Program (EQIP). Between 2010 and 2014, 12,000 high tunnels have been built nationwide using EQIP assistance (NSAC, 2015). As of 2013, 1,241 of these EQIP supported high tunnels were in the Upper Midwest (Huff, 2015).

Microclimate

Growers use high tunnels because they provide favorable changes in microclimate. A polyethylene film covering can increase daily maximum and minimum air and soil temperature, though the amount of increase varies widely by location and season. A study in New Jersey found a 9-22% increase of nighttime air temperature and a 54-59% increase of nighttime soil temperature on average from March 29 through May 16 for two years (Both et al., 2007). In some cases, shade cloth rather than clear polyethylene can be used to reduce temperatures in high tunnels during excessively hot periods; a study in Kansas found that shade cloth during summer months reduced daytime air temperature by 0.4°C and daytime soil temperature by 3.4°C (Zhao and Carey, 2009).

In the Upper Midwest, increased temperatures in high tunnels allow growers to produce crops earlier in the spring and later in the fall, though generally not year-round, unless growers use a supplemental heating source. Growers may choose to use row cover inside the high tunnel to further insulate plants (Huff, 2015). The extended warm growing season also allows for production of specialty crops in temperate climates, such as figs, artichokes, and ginger (Orzolek, 2013). Additionally, high tunnels reduce wind speed by 34-41%, even when well ventilated, which reduces evapotranspiration potential (Zhao and Carey, 2009).

Production Trends

High tunnels in the United States are most often used to grow high value food crops, such as tomatoes, cucumbers, sweet peppers, melons, and leafy greens, although flowers and berry crops are also grown (Carey et al., 2009; Knewtson et al., 2010a; Reid et al., 2013; Wien, 2013; Foust-Meyer and O'Rourke, 2015). High tunnels are of particular importance to Upper Midwest growers, who use them mainly for warm season vegetables in summer and cool season greens in early spring and late fall (Carey et al., 2009; Knewtson et al., 2010a). High tunnels provide growers with an important opportunity to sell local produce at times when it would be impossible to grow in the open-field. Researchers found that in northern Minnesota high tunnels can extend the harvest season up to four weeks for raspberries (Yao and Rosen, 2011). High tunnels can also lead to earlier harvest for summer crops; several studies have shown that high tunnel tomatoes and peppers can be ready to harvest 2-4 weeks before they can be harvested from an open field (O'Connell et al., 2012; Reeve and Drost, 2012; Rudisill et al., 2015).

In addition to earlier and later production, high tunnels have been shown to increase overall marketable yield of high value crops. Several studies have demonstrated that both heirloom and modern tomato varieties produce earlier fruit and can command a price premium, especially when grown organically (Blomgren, Frisch, & Moore, 2007; O'Connell, Rivard, Peet, Harlow, & Louws, 2012; Rogers & Wszelaki, 2012). High tunnels can also decrease the incidence of diseases that reduce marketability, largely by excluding rain drops which transfer soil borne rot pathogens that move via soil splashing (Rogers and Wszelaki, 2012).

The increase in both yield and quality of produce is so dependable that high tunnels are considered as effective as crop insurance in managing income risk for small scale farmers, despite the large investment required to build the structure (Belasco et al., 2013). This dependable income is due not only to reliable yield but also to reliable markets where growers can sell produce for a sufficient price year after year. Research in Minnesota shows that grocery stores are experiencing increased demand for both organic and local foods, with wholesale purchasing only limited by available product (DiGiacomo, 2008); therefore growers can assume a continued market for high quality, local and organic produce grown in high tunnels.

Management Practices

Although the polyethylene film on a high tunnel is removable, many high tunnel growers keep the plastic high tunnel year-round until it needs to be replaced due to wear. A survey of high tunnel growers in the Great Plains states found that 96% of growers surveyed leave the plastic sheet on their high tunnels year-round (Knewtson et al., 2010a). An informal survey of growers in Minnesota found that out of 15 growers surveyed, only one planned to leave their high tunnel uncovered for the upcoming winter (Perkus, unpublished survey). The long-term protection from the polyethylene film provides opportunities and challenges. In situations where the polyethylene film is left on for many seasons, there is no opportunity for large rain events to leach nutrients or to flush excess salts from the soil profile.

In leaving high tunnels covered, growers also create a space for winter crop production; however, the winter growing environment is more challenging than in the spring, summer, and fall. Temperature fluctuations in high tunnels are more extreme in the winter than in the summer. The plastic captures heat well under sunlight, but provides little insulation against heat loss in the absence of sun, leading to air temperatures above freezing during the day and well below freezing at night. One study in Northern Minnesota found that between December and March, the average daily maximum temperature was 3.3°C in the high tunnel compared with -5.4°C in the open field, and the average daily minimum temperature was -15.6°C in the high tunnel compared with -21.4°C in the open field (Yao and Rosen, 2011). If high tunnel sides are opened to minimize high day temperatures, soils lose moisture quickly, as water evaporates readily into low humidity winter air. In the winter, there are few options to replenish soil moisture because irrigation sources are drained to minimize pipe damage from freezing. Both large temperature fluctuations and low soil moisture are challenges to winter production in high tunnels, yet 65% of growers in Virginia and 61% of growers in the Great Plains produce crops in at least one winter month (Knewtson et al., 2010a; Foust-Meyer and O’Rourke, 2015).

In all seasons, a consequence of using polyethylene film to capture heat is that it also shields out precipitation, requiring growers to supply irrigation through the growing season. Many growers use trickle irrigation or drip tape, though some do rely on hand watering (Montri and Biernbaum, 2009; Knewtson et al., 2010a). Irrigation through drip tape allows for increased water use efficiency, as well as the opportunity to apply soluble fertilizers in irrigation water (Montri and Biernbaum, 2009). However, irrigation under dry conditions can lead to high soil salinity when salts introduced through irrigation water remain in the top few inches of soil as water is taken up by plants or evaporates before it can infiltrate any deeper. The risk of soil salinization is higher when the salt content of the irrigation water is higher, such as when a soluble fertilizer is used (Gluck and Hanson, 2013).

Current fertility practices in organically-managed high tunnels rely heavily on manure and compost inputs, similar to organically-managed open fields (Montri and Biernbaum, 2009; Knewtson et al., 2010a). Manure and compost supply nutrients for crops, but usually contain much higher levels of phosphorus (P) than needed for optimum plant growth (Magdoff, 1993; Nelson and Janke, 2007). Manure applications in high tunnels have been shown to increase P above levels required for crop production (Reeve and Drost, 2012; Rudisill et al., 2015). While excess P rarely causes crop production issues, loss of excess P to the environment can result in eutrophication in freshwater (Nelson and Janke, 2007; Rosen and Allan, 2007).

Because high tunnels yield high value produce and provide off-season growing options, growers often grow more crops per year in high tunnels compared to neighboring open fields. This increase in cropping intensity requires more irrigation and fertility, which can compound issues of salinity and P loading. Increased cropping intensity also increases tillage frequency, which can degrade soil structure, reduce organic matter, decrease mineralizable N and decrease microbial biomass (Magdoff, 1993; Balesdent et al., 2000; Pikul Jr et al., 2006; Larsen et al., 2014).

Soil health

High tunnels offer growers increased production in length of season, crop yield, and crop quality. However, this technology is not without its challenges. The unique microclimate created by high tunnels coupled with intensive rotations to maximize high value production space leads to soil health vulnerabilities. This literature review will use the NRCS's characterization of soil health, defined as "the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans" (NRCS, 2017).

High tunnel producers report a decline in production after a few years (Bross, personal communication), and research variety trials have documented this yield decline phenomenon in high tunnels over a period of 3 years (Warren et al., 2015). This decline is hypothesized to be a result of diminishing soil health due to intensive management, combined with increased disease pressure in some cases (Montri and Biernbaum, 2009; Rudisill et al., 2015). A survey of high tunnel growers in the Great Plains states found that 14% of growers were experiencing soil health problems and 32% were unsure if they were experiencing problems (Knewtson et al., 2010a). For organic growers in particular, maintaining soil health is not just a matter of sustaining high production. The National

Organic Program mandates that farmers use practices that maintain or improve soil health across physical, chemical, and biological characteristics (NOP 205.203a, 2017).

The term soil health encompasses a wide range of abiotic and biotic factors as well as ecological processes. In order to quantify soil health, a number of individual measurements are taken that serve as proxies for holistic soil health, including soil salinity, organic matter, and soil biological activity, which are all at risk in intensively managed high tunnel environments (Doran and Jones, 1996; Idowu et al., 2009; Morrow et al., 2016).

Soil Organic Matter

Soil organic matter (SOM) is the fraction of soil made up of plant, animal, and microbial origin (Soil Science Society of America, 2008), and is a key ingredient in soil health (Bezdicek et al., 1996). SOM has been shown repeatedly to improve physical, chemical, and biological aspects of soil health by improving water infiltration and water holding capacity, tilth, pH buffering capacity, electrical conductivity (EC), cation exchange capacity (CEC) and nutrient availability, and a loss of SOM results in a decrease in these key attributes (Magdoff, 1993; Rosen and Allan, 2007; Miltner et al., 2012). SOM is strongly impacted by different management practices over the long-term and has been shown to decrease under tillage and removal of plant residue (Rosen and Allan, 2007; Idowu et al., 2009). A hypothesized potential route for soil health degradation in high tunnels is through the loss of organic matter, due to warm soil temperatures, low moisture, and a reduced period of frozen soil (Montri and Biernbaum, 2009). However, some studies have found that high tunnel systems can maintain or increase soil organic carbon, with the use of compost and animal or green manure amendments (Knewtson et al., 2012; Reeve and Drost, 2012; Rudisill et al., 2015).

A key function of SOM in agriculture is to supply nutrients in organic form that will be mineralized by microbes and then potentially taken up by plants (Doran and Parkin, 1996). Nitrogen supply is of particular interested to growers, and nitrogen cycling activity has been shown to increase with increasing soil C, supplied by SOM (Bowles et al., 2014). The fraction of organic matter with nutrient supplying capacity is called active or labile (Soil Science Society of America, 2008). This fraction can be measured with physical or chemical methods and usually focuses on the amount of organic carbon. The physical methods rely on size or density fractionation and measure what is assumed to be chemically active due to small particle size and no strong association with soil particles. The chemical methods either use a chemical to mimic microbial metabolism, such as potassium permanganate, or measure the products of microbial metabolism, such as evolved CO_2. The method used to measure carbon from labile soil organic matter highlights different fractions, which respond differently to management changes (Morrow et al., 2016).

Permanganate Oxidizable Carbon

Permanganate oxidizable carbon (POXC) is a method that measures chemically reactive soil organic carbon, considered to be labile soil organic carbon. POXC has also been found to be an excellent and relatively simple indicator of overall soil health, as it correlates well with other soil carbon pools including particulate organic carbon, microbial biomass carbon, and soil organic carbon (Culman et al., 2012; Morrow et al., 2016). POXC is an organic matter fraction that is sensitive to management changes in agricultural environments; therefore, it is a useful measure to assess the effect of a particular management strategy on soil health (Culman et al., 2013).

Soil Biological Activity

Soil biological activity is an important indicator of overall soil ecology, but is also important for plant growth as soil organisms break down and mineralize organic matter into plant available nutrients. Soil biological activity includes measurements of soil organism quantity, functional groups, and the rate of chemical processes mediated by microbes. There is extreme heterogeneity both spatially and temporally within soil ecosystems, therefore these measurements can be highly variable and challenging to interpret (Wienhold et al., 2006; Schipanski et al., 2010; McDaniel et al., 2014). A concern for high tunnel growers is a decrease in soil biological activity. However, evidence does not show conclusively that high tunnels reduce soil biological properties in all instances, for example, green manure and chicken manure applied in high tunnels for three years increased microbial activity measure by FDA analysis (Rudisill et al., 2015). Soil biological activity is often quantified using microbial biomass and potentially mineralizable nitrogen, assays commonly included in soil health assessments (Idowu et al., 2009; Morrow et al., 2016).

Microbial biomass

Microbial biomass measures the mass of carbon and nitrogen contained within microbes and provides an estimate of the quantity of microbes per unit of soil, and is considered to be an indicator of mineralizable nutrient supply (McDaniel et al., 2014).

This assay is limited in that it cannot identify microbial species diversity or richness, but it is often used in soil health indices as an indicator of soil biological activity (Karlen et al., 2006). Approximately 40% of microbial biomass becomes stabilized in soils as non-living soil organic matter (Miltner et al., 2012). Microbial biomass C:N can change with crop residue quality, generally more diverse crop rotations enhance microbial N status (McDaniel et al., 2014). Microbial variation in C:N is a result of variable microbial N status, which sometimes does not correlate well with other soil or management parameters (Bowles et al., 2014).

Potentially mineralizable nitrogen

Potentially mineralizable nitrogen (PMN) measures the microbially mediated process of soil nitrogen mineralization (Drinkwater et al., 1996), and quantifies the nitrogen supplying potential that results from the interaction between microbial activity and organic nitrogen in the soil (Doran and Jones, 1996). PMN is regarded as a good indicator of overall soil health (Morrow et al., 2016). PMN changes over short-term timescales in response to available organic nitrogen from incorporated plant residue (Idowu et al., 2009). Introducing a legume into crop rotations has been shown to increase PMN (Morrow et al., 2016), as has increasing crop diversity (McDaniel et al., 2014). However, PMN has been recently challenged as the best method to estimate N availability, as plants have been shown to sequester more N from the soil than is predicted by PMN alone (Osterholz et al., 2017). Nevertheless, PMN is still a useful measure for relating the interaction of N availability and microbial processing of one soil to another.

Soil salinity

Electrical conductivity (EC) is the method used to measure soil salinity (Smith and Doran, 1996). As soil salinity increases beyond the level a species can tolerate, the plant is unable to take up water and nutrients from the soil. Salt sensitive plants experience salt stress in mildly salty soils with an EC of 2.0 dS cm^{-1}. Tomatoes and peppers, both of which are high value crops commonly grown in high tunnels, experience salt stress at 2.5 dS cm^{-1} and 1.5 dS cm^{-1}, respectively (Blomgren et al., 2007). Soil salinity is at risk of elevation in high tunnels compared with open fields because rain is excluded by the polyethylene cover and leaching is prevented (Blomgren et al., 2007; Montri and Biernbaum, 2009). Studies have shown an increase in EC over 3 and 8 years of high tunnel production for both conventional and organic management (Knewtson et al., 2012; Rudisill et al., 2015), as well as an increase over a single growing season (Gluck and Hanson, 2013).

Cover crops

Cover crops are plants that are deliberately grown in rotation with cash crops, providing numerous and well documented benefits to cropping systems such as reduced erosion, reduced nitrate leaching, improved soil tilth, decreased weediness, increased soil organic matter, increased active soil C, increased nutrients for crop uptake, and increased microbial growth and reproduction (Magdoff, 1993; Mendes et al., 1999; Tonitto et al., 2006; Miltner et al., 2012; Schipanski et al., 2014). In winter annual cover cropped systems, mineralizable nitrogen is higher in early summer than at other times of the year (Mendes et al., 1999), synchronizing with summer annual vegetable nitrogen needs.

Cover crops have even been shown to have beneficial effects even during winter months in far north regions, such as reducing soil nitrate (Baggs et al., 2000).

Cover crops can be incorporated into cropping rotations either spatially or temporally and are grown as monocultures or mixtures, ranging in complexity from bi-cultures to multi-species mixes. Cover crop mixtures can have many benefits over monocultures, such as increased cover crop biomass (Ranells and Wagger, 1996), which has been shown to lower weed pressure (Blesh, 2018). Incorporating more cover crop species increases plant diversity, which has been shown to enhance microbial communities and C and N cycling (McDaniel et al., 2014). Legume cover crops in particular are often mixed with a grass to provide support for vining plants, such as hairy vetch, (Ranells and Wagger, 1996) and to increase nitrogen fixation by depleting soil N (Schipanski and Drinkwater, 2011).

Legume cover crops are of particular interest because of symbiotic biological nitrogen fixation, which brings new nitrogen into the cropping system without synthetic fertilizer (Tonitto et al., 2006). This characteristic is immensely important for farmers who do not add synthetic fertilizers either by choice or circumstance. Presence of inorganic nitrogen in soil decreases legume fixed nitrogen, though sandier soils are a stronger predictor of increased BNF compared to inorganic N availability (Schipanski et al., 2010), so agricultural systems that do not use synthetic nitrogen benefit more from biological nitrogen fixation. A recent winter annual cover crop study in the Upper Midwest found nitrogen credits in the first year after cover crop termination to range between 35-268 kg N ha^{-1} (Ginakes, 2017). However, benefits of legume cover crops extend beyond the first year; research has shown that the nitrogen credit derived from 7-

year alfalfa can provide sufficient N fertility for two full years of corn production after alfalfa termination (Yost et al., 2014).

The benefits of legume cover crops make them an attractive tool to maintain or improve soil health and provide nitrogen fertility in high tunnels. This alternative to compost and manure provides growers with a tool to provision nitrogen without adding additional phosphorus (Rosen and Allan, 2007). Studies have shown that when managed properly, legume cover crops can provide the total amount of N required by the cash crop, thus eliminating the need to import any N rich material that might also elevate phosphorus (Parr et al., 2011). While this approach is relatively new in high tunnels it has been attempted by researchers and growers alike. Researchers in North Carolina found that a winter annual mix of cereal rye (*Secale cereale*) and hairy vetch (*Vicia villosa*) in a high tunnel provided an estimated 53-93 kg N ha^{-1} available in the first growing season (O'Connell et al., 2012).

A survey of high tunnel growers in the Great Plains states found that 18% of growers were already experimenting with using winter annual cover crops in rotation and the majority of growers do not produce cash crops during three months in winter (Knewtson et al., 2010a). Thus, there is an established window where legume cover crops could be implemented and a desire among farmers to incorporate legume cover crops into high tunnel rotations.

Winter annual cover crops may be a promising approach to help alleviate soil health challenges, yet there is little known about legume cover crop winter survival, both within and outside of high tunnel environments. Guidelines exist for geographical ranges where winter annual cover crops are likely to be successful; however, at the northern extremes of these ranges, winter survival is highly variable from year to year. Hairy vetch (*Vicia villosa*) has survived open field conditions well in Pennsylvania and New York but poorly in Maine (16.9-37.7% survival) (Jannink et al., 1997; Teasdale et al., 2004; White et al., 2017). One controlled environment study investigated red clover (*Trifolium pratense*), and found that 50% of cold acclimated plants survived freezing temperatures of -14°C, though for non-cold acclimated plants 50% survived at -3°C (Bertrand et al., 2016). Such temperatures are common in Minnesota winters, indicating that cover crop survival is possible, but not guaranteed. Fall sown high tunnel crops experience temperatures that fluctuate beyond what plants experience in the open field, which may affect legume cover crop winter-hardiness in a high tunnel environment. Thus, winter survival may be a serious barrier to using winter annual cover crops in high tunnels in the Upper Midwest, and there are currently no accepted management strategies to overcome this obstacle. Several high tunnel management publications advise using cover crops (Belina et al., 2012, USDA NRCS, CODE 325-CPS-1), yet there is little research to support best management practices of cover crop use in high tunnels in the Upper Midwest.

Summary

As more high tunnels are approaching 5-10 years in production, peer reviewed and anecdotal evidence suggests soil health problems are driving a decrease in production after a few high yielding years. Therefore, this thesis aims to 1) investigate legume cover crops and winter management strategies that are viable for high tunnels in the Upper

Midwest, and 2) evaluate legume cover crops as a management tool to maintain or improve soil health and supply nitrogen fertility in high tunnels.

Chapter 2: Evaluation of three winter annual legume cover crop mixes for soil health and fertility in high tunnels

Introduction

High tunnels, also called hoop houses or poly tunnels, are semi-permanent structures covered with 6-mil polyethylene, used to create protected growing environments for plants grown directly in the soil (NRCS, 2015). The polyethylene cover captures heat and increases daytime temperatures, creating a longer growing season and a hotter summer season (Both et al., 2007; Zhao and Carey, 2009; O'Connell et al., 2012; Ward and Bomford, 2013). This technology is used in temperate climates throughout the world and in the United States (Carey et al., 2009; Lamont, 2009). Growers use these structures to produce horticultural crops, and in the Upper Midwest, common crops are tomatoes (*Solanum lycopersicum*), peppers (*Capsicum spp.*), and cucumbers (*Cucumus sativus*) in the summer and lettuce (*Lactuca sativa*) and spinach (*Spinacia oleracea*) in the spring and fall (Carey et al., 2009; Knewtson et al., 2010a; Huff, 2015). High tunnels can produce a higher marketable yield and reduce post-harvest disease, as well as allowing growers to harvest local produce when it would be impossible to do so in an open field (Waterer, 2003; Kadir et al., 2006; Blomgren et al., 2007; Yao and Rosen, 2011; Reeve and Drost, 2012).

Though expensive to construct, high tunnels provide a steady income for small growers with profits so dependable that they are as effective as crop insurance in mitigating economic risk (Belasco et al., 2013). In 2010, the National Resources Conservation Service enacted a cost-share program for high tunnels under the Environmental Quality Incentives Program (EQIP), to help growers afford construction of these structures, which range from $2.95 to $6.67 per square foot (Huff, 2015; NRCS, 2015). With EQIP assistance, growers across the United States erected 12,000 new high tunnels in the first four years of the program, with 1,241 of these in the Upper Midwest (Huff, 2015; NSAC, 2015).

Growers crop these high value growing spaces intensively, with more growing degree days in the high tunnel than in the open field (Both et al., 2007; Wildung and Johnson, 2012). With more intensive cropping comes increased tillage and increased nutrient demands. These management strategies, coupled with high temperatures and irrigation under protected conditions, leaves high tunnel soils vulnerable to degradation through loss of organic matter and increase soil salinity (Hajime et al., 2009). Soil health degradation is a major concern for growers, especially those who are USDA Organic certified and directed by the National Organic Standards to “maintain or improve the physical, chemical, and biological condition of soil” (USDA, 2017). In addition to the above concerns, organic growers use primarily compost and animal manure for nitrogen fertility (Knewtson et al., 2010a), which often leads to excess phosphorus and salinity (Montri and Biernbaum, 2009).

A potential management tool to mitigate these soil health issues and provide nitrogen without importing excess phosphorus is legume cover crops. Legume cover crops have been shown in some cases to provide sufficient nitrogen for subsequent crops (Drinkwater et al., 1998; Tonitto et al., 2006; Parr et al., 2011; Finney et al., 2016; White et al., 2017), and fields with a history of legumes in rotation show lower levels of phosphorus than fields without legumes in rotation (Schipanski and Drinkwater, 2011). Legume cover crops can also increase soil organic matter and increase soil biological activity related to nitrogen mineralization (Drinkwater et al., 1998; Marriott and Wander, 2006; Idowu et al., 2009). Studies have shown that using a green manure or legume cover crop in high tunnels can increase soil organic matter, supply sufficient N for a summer crop, and increase microbial activity (Montri and Biernbaum, 2009; O'Connell et al., 2012; Rudisill et al., 2015). However, it is unknown whether legumes can deliver these well-known benefits in high tunnel systems further north, such as in Minnesota.

This study aims to i) identity productive legume cover crop mixtures to use as winter annual cover crops in high tunnels in cold climates, ii) quantify the effect of different legume cover crop mixtures on soil health in high tunnels, and iii) assess the effect of legume cover crops on cash crop productivity.

Materials and Methods

Site description

This experiment was conducted over two years from August 2015 to September 2017 at three sites in Minnesota: North Central Research and Outreach Center in Grand Rapids, MN (lat. 47.242539, long. -93.492791); West Central Research and Outreach Center in Morris, MN (lat. 45.593615, long. -95.878379); and Rosemount Research and Outreach Center in Rosemount, MN (lat. 44.717434, long. -93.099067). Existing high tunnels at these sites were used, which varied in environmental, soil, and construction characteristics (Table 1). Baseline soil samples were collected at all three sites in September 2014 and sent to Midwest Laboratories (Omaha, NE) for analysis.

Experimental design

Plots were arranged in a randomized complete block design with a split plot at each site, with three blocks in each high tunnel. Cover crop treatment was the main plot factor and cover crop planting date was the split plot factor. Cover crop planting date was applied as a split plot rather than a factorial design in order to evenly distribute the effect of observed low temperature and high moisture observed along the edges of the high tunnels. Cover crop treatments consisted of: 1) red clover monoculture [(13.5 kg/ha); *Trifolium pratense*, Albert Lea Seed, MN], 2) winter pea/rye 1:1 biculture [(84.1 kg/ha); *Pisum sativum* and *Secale cereale*, Albert Lea Seed, MN], 3) hairy vetch/tillage radish/rye 4:1:15 mix [(84.1 kg/ha); *Vicia villosa*, *Raphanus sativus*, and *S. cereale*, Albert Lea Seed, MN], and 4) a bare-ground, weeded control. Two planting date treatments consisted of an early planting with cover crops interseeded between pepper rows in late August, or a late planting, with cover crops broadcast after pepper removal in mid-September. Subplots were 2.3 m x 1.4 m at Grand Rapids and 2.3 m x 2.1 m at Morris and Rosemount, and cover crop by planting date treatment combinations remained in the same plot location in both years. Decagon EM50 digital data loggers (Decagon Devices, WA) were installed and collected data every 15 min at each site with one air temperature sensor per site (Fig. 1). All site management practices followed USDA organic standards.

Site mangement

Cover crops were either interseeded in between pepper rows in late August or broadcast after pepper removal in mid September (Table 2). Legume seed was inoculated with N-Dure inoculant (Verdesian Life Sciences, NC) by moistening seeds with a 1:4 sucrose solution, mixed with the recommended rate of inoculant, and allowing seeds to air dry overnight. After broadcasting, seed-soil contact was improved by raking seeds until they were no longer visible on the surface, then seeds were watered in. Cover crops were watered overhead as needed. After 7-10 weeks of growth, cover crops were covered with 42.4 g m^{-2} spun-bonded polypropylene row cover (Ken-Bar, NY), propped up with low tunnel wire hoops, and side curtains were closed. Each site was watered for 1 hr prior to shutting off irrigation for the winter. Morris and Rosemount did not receive additional water until spring. Grand Rapids received two applications of 0.8 cm of water in 2016 and 2017. In spring, once temperatures were consistently above -5°C at night, row cover was removed and automatic side curtains were used. In May, cover crops were terminated with a riding mower at Grand Rapids and Rosemount in 2016 and with a walk-behind flail mower (BCS America, OR) at Morris in 2016 and at all sites in 2017. Cover crops were left on the soil surface for 2-5 days, then tilled into the soil with a rototiller to 20 cm (Table 2).

Sweet Sunrise bell peppers (Johnny's Selected Seeds, ME) were started in the greenhouse 8-10 weeks before planting. Peppers were transplanted 5-10 days after cover crop termination in staggered double rows with 45 cm spacing between plants and 90 cm spacing between rows. Peppers were irrigated with drip irrigation and weeded weekly by hand. No fertilizer was added to any plots for the duration of the experiment, however shortly before cover crop planting in Rosemount in 2015 the tunnel received 22.7 kg of 9-23-30 synthetic fertilizer. High tunnel curtains were set to open when the internal temperature exceeded a threshold of 23.9°C, and curtains closed when internal air temperatures fell below this thresholds. Peppers were trellised using a Florida weave system with stakes every four plants. Pepper harvest began when individual fruits were 90% yellow (Table 2). Peppers were harvested every 7-14 days and sorted, counted, and weighed according to USDA standards for marketable (combined Fancy, No. 1, and No. 2) and unmarketable (USDA, 2005). Harvest continued until pepper plants were removed in mid-September (Table 2).

Cover crop sampling and analysis

Cover crops were sampled on the same day as termination in 2016 and 2017 (Table 2). Two random 0.1 m^2 quadrats per plot were sampled by clipping cover crops to ground level and pooled. Samples were sorted according to plant type, then dried at 60°C for 72 hr, ground to 1 mm, and run on a Vario PYRO cube combustion analyzer (Elementar, Germany) for %C and %N. Cover crop coverage for the interseeded treatment was adjusted to account for bare soil from pepper rows where no cover crops were planted (30.5 cm wide).

Soil sampling and analysis

In order to quantify changes in soil properties resulting from cover crops, soil was sampled four times over the growing season: 1) At cover crop termination, 2) 2 weeks after tillage, 3) 5 weeks after tillage, and 4) final pepper harvest (Table 2). For each treatment, eight random soil cores within pepper rows were taken to 20 cm deep and pooled. Fresh soil was sieved to 2 mm, stored at 4°C, and analyzed within 2 weeks of collection. Dry soil was dried at 35°C for 72 hr and ground to 2 mm before analysis.

Extractable nitrogen (exN), potentially mineralizable nitrogen (PMN), and microbial biomass (MB) analyses were conducted on fresh soils. ExN was determined by shaking 10 g fresh soil and 40 mL 1M KCl for 1 hr at 240 rpm, then filtering with Whatman No 1 filter paper. PMN was determined with a 28 day aerobic incubation, where 10 g fresh soil was held at field capacity moisture in the dark at 37°C. After 28 days, samples were shaken with 40 mL 1M KCl for 1 hour at 240 rpm, then filtered using Whatman No 1 filter paper. MB analysis used the direct chloroform extraction method. Two 10 g fresh soil subsamples were extracted with 0.5M K_2SO_4 after one subsample received 0.5 mL chloroform. Both chloroformed and non-chloroformed samples were shaken for 4 hours at 150 rpm, and then filtered with Whatman No 1 filter paper. Chloroformed extracts were bubbled with a forced air vacuum apparatus for 25 min to remove any traces of chloroform. ExN, PMN, and MB extracts were analyzed for total organic carbon and total nitrogen on a TOC-L/TN analyzer (Shimadzu, Japan).

Permanagate oxidizable carbon (POX-C), pH, and EC measurements were taken on dry soil. POX-C analysis reacted 2.5 g dry soil with 2 mL 0.2M $KMnO_4$ in 18 mL distilled water. After shaking for 2 min at 120 rpm, samples were incubated for 10 minutes in the dark at room temperature, and then 0.5 ml of supernatant was transferred to 49.5 mL water. This diluted sample was analyzed for absorbance at 550 nm on a SpectraMax 190 Microplate Reader (Molecular Devices, CA). Electrical conductivity (EC) and pH measurements were made on a 1:1 solution with distilled water using an economy pH/EC meter (Spectrum Technologies, IL).

Statistical analysis

Data were analyzed by site-year as a split plot design with cover crop mix as a whole plot factor and cover crop planting date as a sub-plot factor. Blocks were treated as replications. For measurements taken more than once over the course of a season, time was treated as a split-split plot factor. Analysis of variance was conducted using the split plot and split-split plot function in the R package "agricolae," to determine main effects and interactions (de Mendiburu, 2017; R Core Team, 2017). Square root transformations were used when data failed to meet assumptions of normality. P values are reported if less than 0.10, but only p-values below 0.05 were investigated further. Means were reported by the main factor only (cover crop treatment), unless the split plot factor (plant date) or the interaction was significant ($p > 0.05$). Mean separation was performed using Fisher's LSD ($p = 0.05$).

Results

Cover crop biomass

Total biomass (cover crop and weed biomass combined) varied by cover crop treatment at all site-years, and plant date was a significant factor at all sites in Y1 and Grand Rapids in Y2 (Table 3). Total biomass was higher in Y2 than Y1 for all sites, with a maximum of 14,602 kg ha^{-1} for the pea mix at Morris in Y2 (Table 4). Treatments that included rye (pea mix and vetch mix) had the highest total biomass for each site-year, except for the early planted vetch mix treatment at Rosemount in Y1. Cover crop treatment was a significant factor for legume biomass for all site-years except Morris in Y2 ($p=0.086$), and plant date was a significant factor at Grand Rapids for Y1 ($p=0.056$)

and Y2 (p=0.087) (Table 3). In Y1, this difference corresponded with early planted red clover producing more biomass than late planted red clover with 412 kg ha^{-1} and 88 kg ha^{-1}, respectively (Table 4). Across site-years legume biomass was variable, though red clover always had among the highest (Table 4). At Grand Rapids, vetch produced more biomass than pea in both Y1 and Y2, though at Morris and Rosemount there were no consistent differences between vetch and pea.

Weed biomass varied by cover crop treatment at all site-years except at Rosemount in Y2 (p = 0.055, Table 3). Weed biomass varied by plant date only at Morris in Y1, but the cover crop treatment x plant date interaction was significant at Grand Rapids in Y1 and Y2 and at Rosemount in Y1 (Table 3). In Grand Rapids for Y1 and Y2, late planted clover had the highest weed biomass with 1,044 kg ha^{-1} and 2,148 kg ha^{-1}, respectively. In both years at Rosemount and Morris, cover crop treatments with rye (pea mix and vetch mix) reduced weeds to a level statistically similar to the bare control 80% of the time. Two notable exceptions are the pea mix treatments in Morris and Rosemount in Y2, which had the highest weed biomass for their respective site-years. Commonly observed weeds at each site were common chickweed (*Stellaria media*) at Grand Rapids, groundsel (*Senecio vulgaris*) at Morris, and lamb's quarters (*Chenopodium album*) and shepherd's purse (*Capsella bursa-pastoris*) at Rosemount.

Legume %N was affected by cover crop treatment at Grand Rapids in both Y1 and Y2, where clover had lower %N compared to pea and vetch in both years (Table 3). Legume %N was not affected by plant date or the plant date x cover crop treatment interaction for any site-year (Table 3). Legume %N values ranged from 3.80% to 5.30%, even though at termination time none of the legume species reached 50% flowering at any site-year (Table 5). Total weighted C:N varied by cover crop treatment at Morris in Y1 and Y2 and at Grand Rapids in Y1, with a trend at Grand Rapids in Y2 ($p = 0.063$) (Table 5). In these cases red clover treatments always had the lowest C:N, ranging from 11.57 to 17.02, and pea mix always had the highest C:N, ranging from 18.93 to 30.91 (Table 5).

Total treatment biomass nitrogen varied by cover crop treatment for all site-years, with additional differences by plant date at Grand Rapids in Y1 and Y2, and the cover crop treatment x plant date interaction was significant at Rosemount in Y1 and at Grand Rapids in Y2 (Table 5). Total treatment biomass N was higher in Y2 than in Y1 (Table 5). For Grand Rapids in Y1 and Y2 and Rosemount in Y1 when differences between cover crop treatment x plant date were significant, the later planted treatments contained more nitrogen than earlier planted treatments of the same cover crop mix (Table 5). Across all site-years, all treatments with cover crops contained more nitrogen than the bare control except for the red clover treatment at Morris in Y2 (Table 5). The highest biomass nitrogen contribution came from the pea mix treatment in Morris in Y2 with 365 kg N ha^{-1}. This treatment also had the highest total biomass of any treatment for any site-year. Three other treatments across site-years also produced more than 200 kg N ha^{-1}.

Soil analyses

Three soil analyses, 1) extractable nitrogen (ExN), 2) microbial biomass carbon (MBC), and 3) permanganate oxidizable carbon (POXC), were investigated over multiple sampling time points throughout the cash crop season; potentially mineralizable nitrogen (PMN) was measured once at 2 weeks after tillage and pH and electrical conductivity

(EC) were measured once at the final pepper harvest. For the three analyses investigated over time, sampling time was a significant factor for all site years except for MBC at Morris and Rosemount in Y2 and for POXC at Morris in Y2 (Table 6).

Extractable nitrogen varied by the cover crop treatment x sampling time interaction at all site-years except Rosemount in Y1 (Table 6). At Grand Rapids and Morris in Y1 and Y2, ExN was the highest at tillage time (0 weeks) in the bare control treatment, likely due to a lack of growing plant material (Fig. 2a). At Grand Rapids in Y1 and Y2 and Morris in Y1, ExN increased from 2 weeks post tillage to 5 weeks post tillage, a time in pepper growth which requires substantial nitrogen. The range of ExN observed at all site-years, approximately 10-50 mg N kg^{-1} soil, was similar across site-years except for a global high observation, 225-275 mg N kg^{-1} soil, at Rosemount in Y1 at tillage time (0 weeks). This was likely due to mineral fertilization that took place a week before cover crops were planted in Rosemount in August 2015 only. For all sites in Y1, early planted treatments had higher ExN values 2 weeks post tillage compared to their late planted counterparts (Fig. 2b). This was not observed in Y2. MBC varied by cover crop treatment at Grand Rapids in Y1 and by plant date at Morris in Y1 (Table 6). All sites show a steep decrease in MBC from tillage to 2 weeks post tillage in Y1, but not in Y2 (Fig. 3).

POXC varied by cover crop treatment at Grand Rapids in Y2 (Table 6). At the final sampling time point, 20 weeks post tillage, pea mix and vetch mix treatments had higher POXC than red clover or the bare control (Fig. 4b). Plant date was a significant factor for POXC at Grand Rapids in Y1 and Y2 and Morris in Y2, and a trend for Rosemount in Y1 (p=0.076) (Table 6). At Grand Rapids in Y1 and Y2, the early planted treatment had higher POXC at most sampling time points compared to the late planted treatment, and this trend was reversed for Morris and Rosemount in both years (Fig. 4b).

In Y1, potentially mineralizable nitrogen (PMN) was not different among cover crop treatment for any site, though it differed by plant date at Morris and Grand Rapids (Table 6). At these sites, the late planted treatment had lower PMN than the early planted treatments, with a reduction of 36-73%. In Y2, cover crop treatment was significant at Grand Rapids, and a trend at Morris (p=0.052). At these sites, the pea mix had the highest PMN, 67.6 mg N kg^{-1} soil and 87.0 mg N kg^{-1} soil respectively, and the no cover crop control had the lowest PMN, 48.1 mg N kg^{-1} soil and 62.1 mg N kg^{-1} soil respectively (Table 7). At Rosemount in Y2, the cover crop treatment x plant date interaction was significant with the highest PMN in the late planted vetch mix, 70.9 mg N kg^{-1} soil, and the lowest PMN in the late planted bare control, 38.6 mg N kg^{-1} soil.

Soil pH differed across sites, but did not differ by cover crop treatment or plant date to any biologically meaningful extent, although there were small statistically significant differences at Grand Rapids (Table 6, table 7). Electrical conductivity (EC) was different across sites, but not affected by cover crop treatment or plant date (Table 6). EC values were highest at Morris in Y1 with a range of 1.04 to 1.53 mS cm^{-1}, determined using the 1:1 method (Table 7). The EC threshold at which salt sensitive plants are affected for silty loam soils using the 1:1 method is 1.4-2.5 dS cm^{-1} (Whitney, 1988). Only Morris Y1 soils were slightly saline, all other site-years were non-saline.

Pepper yield

Marketable yellow pepper yield was not affected by cover crop treatment, plant date, or the cover crop treatment x plant date interaction (Table 8) except at Rosemount in Y1 where the red clover treatment produced more than the no cover crop control, with 0.83 kg plant^{-1} and 0.53 kg plant^{-1}, respectively (Table 9). Unmarketable fruit was not affected by cover crop treatment, plant date, or the cover crop treatment x plant date interaction (Table 8), except at Rosemount in Y2 where the early planted red clover and vetch mix treatment showed reduced weight of unmarketable fruit relative to the late planted treatments (Table 9).

Discussion

Cover crop biomass

A major question in this experiment was whether cover crops could survive and produce significant biomass under high tunnels in Minnesota. Most high tunnel growers in the Upper Midwest avoid growing between Dec-Feb (Knewtson et al., 2010; Perkus, unpublished survey), and all cover crops died in a trial year of this experiment at Morris and Grand Rapids in 2014 (Chapter 4). This study found that cover crops did survive and were able produce significant biomass, as all cover crop treatments with cover crops (red clover, pea mix, and vetch mix) had higher biomass than the bare control in all site years (Table 4). Cover crop survival may be attributed to a combination of management strategies evolved from the 2014 trial year and consulting with a team of four seasoned farmer advisors. The management strategies included keeping sides closed all winter, using row cover inside the high tunnel, and heavy watering before irrigation lines were closed.

While total biomass for cover crop treatments with cover crops was significantly different from the bare, weed free control, this was not true for all legume species within the cover crop treatments. Pea biomass produced in Grand Rapids in Y1 and Y2, 2-9 kg ha^{-1} and 32 kg ha^{-1} respectively, was not statistically different from the 0 kg ha^{-1} legume biomass produced in the no cover crop control (Table 4). However, at both Morris and Rosemount in Y1 and Y2, pea biomass was higher than the no cover crop control. This suggests that Austrian winter pea is winter hardy for high tunnel environments in zones 4a and 4b but not in 3b. Open field studies in central New York (zone 5a) and Pennsylvania (zones 6a and 6b) both found that Austrian winter pea winter killed whereas red clover survived (Hively and Cox, 2001; White et al., 2017). All other legumes, except hairy vetch at Rosemount in Y1, produced significantly higher legume biomass than the 0 kg ha^{-1} legume biomass produced in the no cover crop control. This exception was likely due to rapid growth of the tillage radish in the vetch mix, which shaded out the vetch, after the Rosemount high tunnel was fertilized in August 2015 only (Fig. 5). Legume biomass varied across cover crop treatments in part due to different cover crop mixture composition, with red clover comprising 100% of the red clvoer treatment, pea comprising 50% of the pea mix treatment, and vetch comprising 20% of the vetch mix treatment.

Another desired function of cover crops is to suppress weeds, particularly for organic production. For RC treatments where plant date or plant date x cover crop treatment interaction was significant (all sites in Y1 and GR in Y2), weed biomass was always higher in the late planted treatment than the early planted treatment. This suggests that red clover is more effective at weed suppression when planted two weeks earlier. This is likely due to increased clover biomass in early planted treatments, which may be biologically significant though only statistically detected in Grand Rapids in Y1, as increased cover crop biomass has been shown to reduce weed biomass (Finney et al., 2016).

Legume %N was only different across legume species at Grand Rapids, where clover had lower %N than either vetch or pea in both years of the study. This indicates some limitation on nitrogen fixation at Grand Rapids, possibly related to colder temperatures. Overall, legume %N values were higher than generally reported in the literature with an average of 4.40% for pea, 4.00% for clover, and 4.73% for vetch. The full range of total weighted C:N found in this experiment ranged from 10.60 to 30.91 (Table 5). C:N values in this range predict net nitrogen mineralization as opposed to net nitrogen immobilization (Vigil and D.E, 1991; Hodge et al., 2000). None of the cover crop treatments tested in this experiment carry the risk of immobilizing nitrogen and reducing the amount of plant available nitrogen for the subsequent cash crop. Additionally, higher %N has been found to be positively correlated with mineralizeable nitrogen (Vigil and D.E, 1991), suggesting the treatments with cover crops in this study likely supply high plant available N.

Total treatment biomass N was higher in Y2 than in Y1, with no external N inputs besides biological nitrogen fixation in legumes. Growing legume cover crops in high tunnels is a viable means of introducing more nitrogen into the system without relying on fertilizers that can increase EC and phosphorus to damaging levels for crops and the ecosystem downstream. Research has shown hairy vetch and Austrian winter pea to fix 69-174 kg N ha^{-1} and 62-130 kg N ha^{-1}, respectively (Parr et al., 2011). A meta-study concluded that organic system cash crop yields are not reduced relative to conventional systems when cover crops supply more than 110 kg N ha^{-1} (Tonitto et al., 2006), a threshold which was exceeded by two thirds of cover crop treatments with legumes in Y2. In cases where planting date within a cover crop treatment produced different amounts of biomass N, the later planted cover crops produced more than the earlier planted cover crops. Data suggest that total biomass is higher in late planted cover crops as well, but this trend is not significant. Higher biomass N in some later planted cover crops shows that while earlier planted crops had more time to grow, the additional two weeks of time alone did not increase biomass N.

Soil analyses

The soil data collected over the growing season allude to complex belowground dynamics related to cover crop planting date that are not fully captured by the data collected in this study. For all sites in Y1, early planted treatments had higher ExN and MBC values 2 weeks post tillage compared to their late planted counterparts (Fig. 2b, fig. 3b). This was not observed in Y2. This difference cannot be explained by total biomass, legume biomass, or weed biomass, all of which were higher in the late planted treatments for those site years (Table 4). C:N and legume %N are not different by plant date in Y1 at these sites, and there was no consistent pattern of higher total treatment biomass nitrogen either (Table 5). In Y1 this time point, PMN values (also taken at 2 weeks post tillage) were higher for the early planted treatments at Morris and Rosemount (Table 7). In Y1, there is some below ground difference in the planting date treatments that cannot be explained by the above ground cover crop measurements.

Cover crop treatment had an effect on POXC at Grand Rapids in Y2 only (Table 6). High organic matter at Morris (4.4%) and Rosemount (4.1%) are likely buffering any differences from residue additions. Plant date had a stronger effect on POXC in both years; data suggests early planted treatments often had higher POXC values in Grand Rapids, while in Morris and Rosemount later planted treatments often had higher POXC values (Fig. 4b). Soil samples were taken within the pepper row, and late planted cover crops were sown after pepper plants were removed, so there is likely more residual cover crop root mass within the pepper rows for late planted treatments compared to early planted treatments. This may explain the higher POXC observed in late planted treatments in Rosemount and Morris, and is supported by studies that observed higher POXC with more organic carbon inputs (Culman et al., 2012; Rudisill et al., 2015). However, this explanation seems to contradict the dynamics observed in Grand Rapids.

None of the cover crop treatments increased EC relative to the bare control. Legume cover crops do have the potential to increase EC slightly when biologically fixed nitrogen in plant tissue is mineralized in soil to NH_4^+ and nitrified to NO_3^-, and the degree to which legume cover crops can increase EC is driven by the amount of new nitrogen brought into the soil system via biological nitrogen fixation. In this study, cover crops were used as a source of N for two years without increasing EC. However this is a different result from another high tunnel cover crop study which did find a small EC increase (0.03 dS m^{-1}) in cover cropped treatments relative to a bare control after three years of production, though this difference is likely not biologically significant (Rudisill et al., 2015). Legume cover crops may increase EC slightly in certain conditions, but not to a damaging degree.

Pepper yield

Cash crop yield is a vitally important factor for high tunnel growers, who rely on large, high quality yields from high tunnel structures to mitigate economic risk (Belasco et al., 2013). Compared with a yield goal of 0.7-1.0 kg plant^{-1}, approximately 22,000 to 25,000 kg ha^{-1}, for open field production in Missouri (Trinklein, 2006), some site-years reach this production standard while others do not. Baseline soil tests indicate that nutrient deficiencies were not the cause of low yield, with the possible exception of Boron (B) in Rosemount (Table 1). One cause for lower production in this study is that pepper plants were removed in all sites in mid-September, before production was complete. In both years at all sites, plants still had mature green fruits, immature green fruits, and flowers. Mature green fruits can be sold at a lower market value, allowed to ripen off-plant, or left to ripen on plants as long as temperatures remain warm in the high tunnel.

Overall, neither cover crop treatment nor plant date consistently affected marketable yield or average weight of marketable fruit. Increased weight of marketable fruits is desirable because larger fruits are more likely to meet the highest USDA grade, fancy, which can be sold for a higher price (USDA, 2005). Where treatment differences were observed in total yield and marketable pepper weight, cover crops were beneficial relative to the no cover crop control. This experiment demonstrates that cover crops in the high tunnel are unlikely to reduce yield relative to bare fallow, so high tunnel growers can choose a species/mixture of cover crop(s) based on other criteria, without sacrificing yield. Growers can also choose to plant the cover crops in early or mid-September in Minnesota high tunnels without affecting bell pepper yield.

Conclusions

High tunnels are a profitable tool for growers, but are vulnerable to certain soil health problems due to their unique microclimate and intensive production. This study shows that winter annual cover crops can be successfully grown in high tunnel environments in zones 3b-4b, though Austrian winter pea should be avoided in zone 3b. Winter annual cover crops in high tunnels can provide a large amount of nitrogen, up to 200 kg N ha^{-1}, and can in some cases provide weed suppression, both important issues in organic agriculture. With regard to soil health, the data show subtle differences that are largely obscured by high variability. Where differences are observed, cover crops improve soil health characteristics compared to a bare control. However, no one cover crop treatment, plant date, or cover crop treatment x plant date interaction performed consistently better or worse across all site-years. Finally, pepper production was not reduced in plots with cover crops compared to plots without cover crops. This study confirms that legume cover crops in high tunnels in Minnesota are a potential management tool for growers looking for a low P and low EC nitrogen source, but it lacks the power to discern any conclusive soil health benefits.

The dominant management strategy in organically managed high tunnels is intensive cropping using compost and/or manure to meet fertility needs and replenish soil organic matter (Knewtson et al., 2010a). This study shows that farmers can use winter cover crops in high tunnel rotations to diversity management strategies without fear of reduced cash crop yield relative to a bare fallow. Due to the length of the growing season necessary for successful winter annual legume cover crops this is not a strategy that farmers would choose every year in every high tunnel because it would require taking both fall and spring months out of cash crop production. Nevertheless, employed selectively in several-year rotations, winter annual legume cover crops are a feasible, organic alternative to compost and manure to meet fertility needs and replenish soil organic matter.

Tables and Figures

Table 1. Site characteristics.

	Site		
	Grand Rapids	**Morris**	**Rosemount**
High tunnel dimensions and area	15.2 m x 6.4 m 97.3 m^2	14.6 m x 9.1 m 132.9 m^2	14.6 m x 9.1 m 132.9 m^2
High tunnel orientation (long side)	N/S	NE/SW	E/W
High tunnel covering	Single layer polyethylene	Single layer polyethylene	Double layer polyethylene
Previous management	Experiment described in Chapter 4	Experiment described in Chapter 4	Soil covered with landscape fabric, various crops planted in pots aboveground
USDA Hardiness Zone	3b	4a	4b
Soil type	Shooker very fine sandy loam	Byrne silt loam	Waukegan silt loam
% OM	2.9	5.9	5.3
Nitrate-N (ppm)	25	20	120
Bray-P (ppm)	341	282	63
K (ppm)	253	424	423
Mg (ppm)	236	709	651
Ca (ppm)	1563	3471	3131
Na (ppm)	65	68	45
S (ppm)	38	135	121
Zn (ppm)	5.7	8.5	5.9
Mn (ppm)	13	35	46
Fe (ppm)	58	24	54
Cu (ppm)	1.8	3.2	1.1
B (ppm)	1.0	2.2	0.7

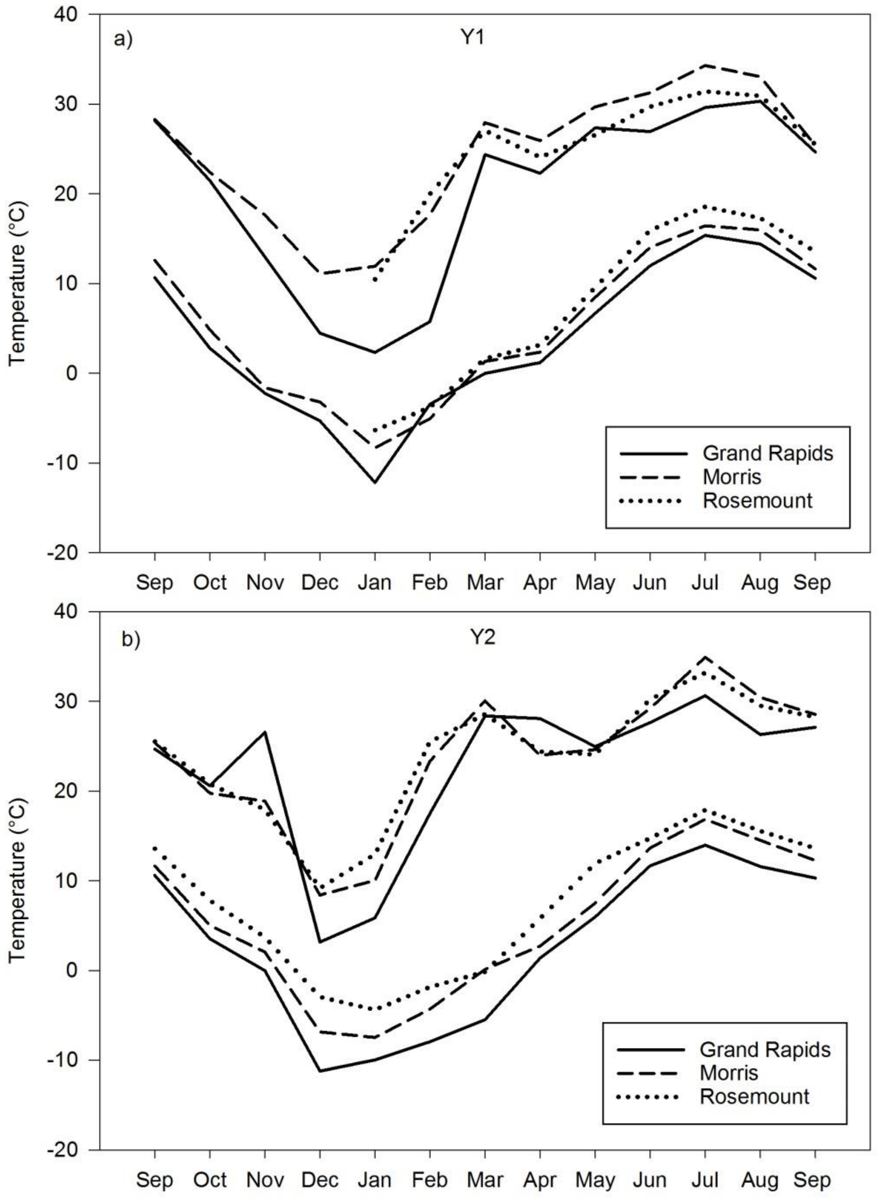

Figure 1. Monthly average high and low temperatures for all sites in a) Y1 and b) Y2.

Table 2. Dates of field operations.

	Y1			Y2		
	Grand Rapids	**Morris**	**Rosemount**	**Grand Rapids**	**Morris**	**Rosemount**
Early planted cover crop	Aug 28, 2015	Aug 24, 2015	Sept 2, 2015[a]	Aug 25, 2016	Aug 30, 2016	Sept 2, 2016
Late planted cover crop	Sept 16, 2015	Sept 18, 2015	Sept 22, 2015	Sept 16, 2016	Sept 14, 2016	Sept 20, 2016
Cover crop termination/ soil sample 1	May 17, 2016	May 6, 2016	May 4, 2016	May 18, 2017	May 4, 2017	May 2, 2017
Soil sample 2	June 6, 2016	May 25, 2017	May 23, 2016	June 5, 2017	May 23, 2017	May 19, 2017
Soil sample 3	June 27, 2016	June 14, 2016	June 16, 2016	June 26, 2017	June 12, 2017	June 9, 2017
First pepper harvest	Aug 25, 2016	Aug 11, 2016	Aug 5, 2016	Aug 3, 2017	Aug 7, 2017	July 28, 2017
Final pepper harvest/soil sample 4	Sept 16, 2016	Sept 13, 2016	Sept 12, 2016	Sept 15, 2017	Sept 13, 2017	Sept 18, 2017

[a] No existing peppers, seeds broadcasted.

Table 3. Significance of F test for total plot biomass, legume biomass, weed biomass, legume %N, total treatment C:N, and total treatment biomass N, analyzed by site-year. Analysis of variance conducted using split plot model with cover crop treatment (CCT) as the main factor and plant date (PD) as the split plot factor. Total biomass, legume biomass, and weed biomass were square root transformed to fit assumptions of normality and equal variance. P values below 0.10 are reported, all other p values are not significant (NS).

		Year 1			**Year 2**	
	Grand Rapids	**Morris**	**Rosemount**	**Grand Rapids**	**Morris**	**Rosemount**
Total biomass (kg ha^{-1})						
CCT	< 0.001	< 0.001	< 0.001	< 0.001	0.001	< 0.001
PD	0.004	0.015	0.061	0.015	NS	NS
CCT x PD	NS	0.051	0.004	0.023	NS	NS
Legume biomass (kg ha^{-1})						
CCT	0.001	< 0.001	0.002	< 0.001	0.086	0.039
PD	0.056	NS	NS	0.087	NS	NS
CCT x PD	0.009	NS	NS	NS	NS	NS
Weed biomass (kg ha^{-1})						
CCT	< 0.001	< 0.001	0.026	< 0.001	0.003	0.055
PD	NS	0.029	NS	NS	NS	NS
CCT x PD	0.046	0.067	0.009	0.032	NS	NS
Legume %N						
CCT	0.010	NS	NS	0.042	NS	NS
PD	NS	NS	NS	NS	NS	NS
CCT x PD	NS	NS	NS	NS	NS	NS
Treatment C:N						
CCT	0.040	0.005	NS	0.063	0.014	NS
PD	NS	NS	NS	0.071	NS	NS
CCT x PD	NS	0.089	NS	NS	NS	NS
Treatment biomass N (kg ha^{-1})						
CCT	< 0.001	< 0.001	< 0.001	< 0.001	0.001	0.001
PD	0.036	0.073	NS	0.043	NS	NS
CCT x PD	NS	NS	0.038	0.020	0.079	NS

Table 4. Total biomass (cover crop and weed), legume biomass, and weed biomass results, analyzed by site year. Data reported by cover crop treatment (main factor), unless plant date or plant date x cover crop interaction was significant ($p < 0.05$). Cover crop treatments are red clover (RC), pea mix (PM), vetch mix (VM), and no cover crop control (NCC); plant date treatments are early planted (Early) and late planted (Late). Letters represent significant differences (alpha = 0.05) between values for a single biomass type within a single site-year column. Mean separation performed using an LSD test using square root transformed data.

				Y1			**Y2**	
			Grand Rapids	**Morris**	**Rosemount**	**Grand Rapids**	**Morris**	**Rosemount**
Total biomass (kg ha^{-1})								
	RC	**Early**	705 d	812 d	1286 b	3698 bc	988 bc	1707 b
		Late	1131 c	768 d	1151 b	3565 c		
	PM	**Early**	1959 b	1644 bc	1254 ab	3873 c	14602 a	6095 a
		Late	2619 a	2412 a	1209 b	11830 a		
	VM	**Early**	1606 b	1310 c	726 c	2075 bc	4641 b	5234 a
		Late	1834 b	2217 ab	1761 a	7018 b		
	NCC	**Early**	0 e	0 e	0 d	0 d	0 c	0 c
		Late	0 e	0 e	0 d	0 d		
Legume biomass (kg ha^{-1})								
	RC	**Early**	412 a	668 a	1116 a	2185 a	140 ab	931 ab
		Late	88 b					
	PM	**Early**	9 c	109 c	539 b	32 c	558 a	3442 a
		Late	2 c					
	VM	**Early**	102 b	346 b	80 c	588 b	551 a	2895 a
		Late	147 b					
	NCC	**Early**	0 c	0 d	0 c	0 c	0 b	0 b
		Late	0 c					

Table 4 cont. on next page

Table 4 cont.

		Y1			Y2		
		Grand Rapids	**Morris**	**Rosemount**	**Grand Rapids**	**Morris**	**Rosemount**
Weed biomass (kg ha^{-1})							
RC	**Early**	293 b	14 b	24 bc	745 b	848 b	776 ab
	Late	1044 a	231 a	181 a	2148 a		
PM	**Early**	96 bc	14 b	25 bc	231 bcd	6934 a	2636 a
	Late	141 bcd	29 b	0 c	103 cd		
VM	**Early**	34 cd	0 b	77 bc	444 bc	591 b	1002 ab
	Late	45 cd	2 b	107 bc	34 cd		
NCC	**Early**	0 d	0 b	0 c	0 d	0 b	0 b
	Late	0 d	0 b	0 c	0 d		

Table 5. Legume percent nitrogen (%N), total treatment C:N (including legume cover crops, non-legume cover crops, and weeds), and total biomass nitrogen (N) (including legume cover crops, non-legume cover crops, and weeds) results. Data reported by cover crop treatment (main factor) unless plant date or plant date x cover crop interaction was significant ($p < 0.05$). Cover crop treatments are red clover (RC), pea mix (PM), vetch mix (VM), and no cover crop control (NCC); plant date treatments are early planted (Early) and late planted (Late). Letters represent significant differences (alpha = 0.05) between values of a single data type within a single site-year column. Mean separation performed using an LSD test on untransformed data for legume %N and total weighted C:N and square root transformed data for total biomass N. If no letters are reported, there were no differences between treatments.

		Y1			**Y2**		
		Grand Rapids	**Morris**	**Rosemount**	**Grand Rapids**	**Morris**	**Rosemount**
Legume %N							
RC		3.98 b	4.17	4.17	3.80 b	4.13	3.72
PM		4.59 a	3.84	4.03	5.30 a	4.27	4.39
VM		4.72 a	4.50	4.91	4.55 a	4.93	4.77
NCC		--	--	--	--	--	--
Total weighted C:N							
RC		17.02 b	11.57 b	11.09	13.82 b	12.17 b	11.90
PM		30.91 a	21.47 a	16.73	26.15 a	18.93 a	10.60
VM		20.38 b	21.23 a	12.70	21.97 ab	16.26 a	11.50
NCC		--	--	--	--	--	--
Treatment biomass N (kg ha^{-1})							
RC	**Early**	21.8 d	32.1 b	50.5 a	129.0 ab	33.8 bc	64.5 b
	Late	24.1 cd		52.3 a	109.3 bc		
PM	**Early**	38.1 b	53.0 a	56.1 a	65.0 c	365.0 a	268.1 a
	Late	43.7 ab		34.5 ab	204.3 a		
VM	**Early**	34.2 bc	43.5 ab	19.7 b	119.7 bc	124.8 b	201.3 a
	Late	58.1 a		58.2 a	139.0 ab		
NCC	**Early**	0.0 e	0.0 c	0.0 c	0.0 d	0.0 c	0.0 c
	Late	0.0 e		0.0 c	0.0 d		

Table 6. Significance of F test for extractable nitrogen (ExN), microbial biomass carbon (MBC), permanganate oxidizable carbon (POXC), potentially mineralizable nitrogen (PMN), pH, and electrical conductivity (EC). For ExN, MBC, and POXC, analysis of variance conducted using split-split plot model with cover crop treatment (CCT) as the main factor, plant date (PD) as the split plot factor, and sampling time point (TP) as the split-split plot factor, with 3 TPs for ExN and MBC and 4 TPs for POXC. For PMN, pH, and EC, analysis of variance conducted using a split-plot model with CCT as the main factor and PD as the split plot factor. P values below 0.10 are reported, all other p values are considered not significant (NS).

	Y1			**Y2**		
	Grand Rapids	**Morris**	**Rosemount**	**Grand Rapids**	**Morris**	**Rosemount**
ExN						
CCT	0.080	0.053	NS	0.051	NS	NS
PD	0.004	<0.001	NS	NS	NS	0.066
CCT x PD	NS	NS	NS	NS	NS	0.046
TP	<0.001	<0.001	0.004	<0.001	<0.001	0.019
TP x CCT	0.002	<0.001	NS	0.004	0.011	0.032
TP x PD	<0.001	<0.001	NS	NS	NS	0.083
TP x CCT X PD	NS	NS	NS	NS	NS	0.048
MBC						
CCT	<0.001	NS	NS	0.060	NS	NS
PD	NS	0.003	NS	0.067	0.062	NS
CCT x PD	NS	NS	NS	NS	NS	NS
TP	<0.001*	<0.001	<0.001	<0.001	NS	NS
TP x CCT	0.055	NS	NS	NS	NS	NS
TP x PD	NS	0.001	0.079	0.007	NS	<0.001
TP x CCT X PD	NS	NS	NS	NS	NS	NS
POXC						
CCT	NS	NS	NS	0.032	NS	NS
PD	0.007	0.008	0.076	0.045	NS	NS
CCT x PD	NS	NS	NS	NS	NS	NS
TP	0.024	<0.001	<0.001	<0.001	NS	<0.001
TP x CCT	NS	NS	NS	NS	NS	NS
TP x PD	NS	0.005	<0.001	NS	NS	NS
TP x CCT X PD	NS	NS	NS	NS	NS	NS
PMN						
CCT	NS	NS	NS	0.022	0.052	NS
PD	NS	0.007	<0.001	NS	NS	NS
CCT x PD	NS	NS	NS	NS	NS	0.033
pH						
CCT	0.022	NS	NS	NS	NS	NS
PD	NS	0.088	NS	0.043	NS	NS
CCT x PD	NS	NS	NS	NS	NS	NS

Table 6 cont. on next page

Table 6 cont.

	Y1			Y2		
	Grand Rapids	**Morris**	**Rosemount**	**Grand Rapids**	**Morris**	**Rosemount**
EC						
CCT	NS	NS	NS	NS	NS	NS
PD	NS	NS	NS	NS	NS	NS
CCT x PD	NS	NS	NS	NS	NS	NS

* Grand Rapids Y1 MBC data only has 2 sampling time points due to analysis failure.

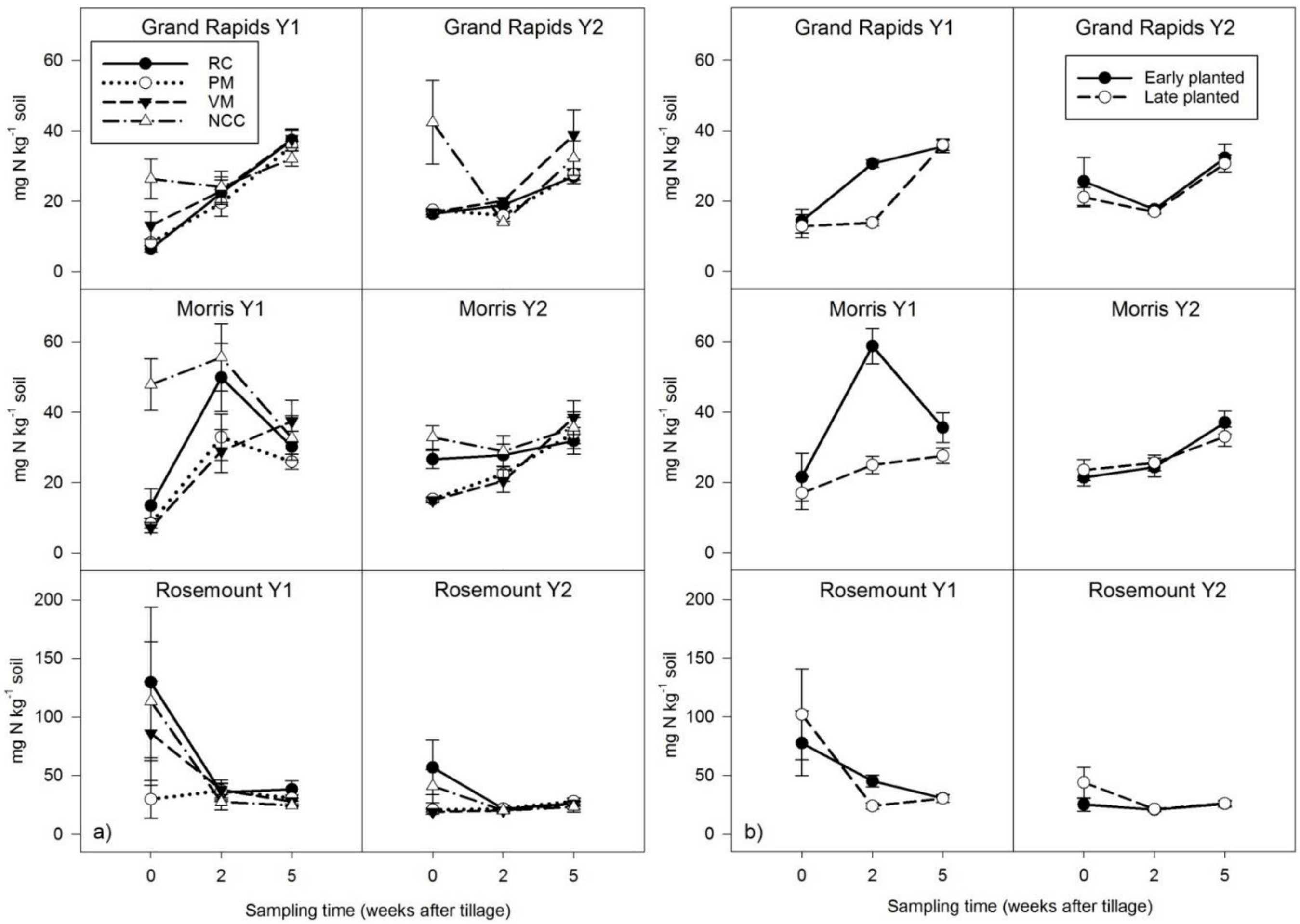

Figure 2. Extractable nitrogen (ExN) values over sampling time plotted by a) Cover crop treatment (main plot factor) and b) Plant date (split plot factor). Error bars represent 1 SE.

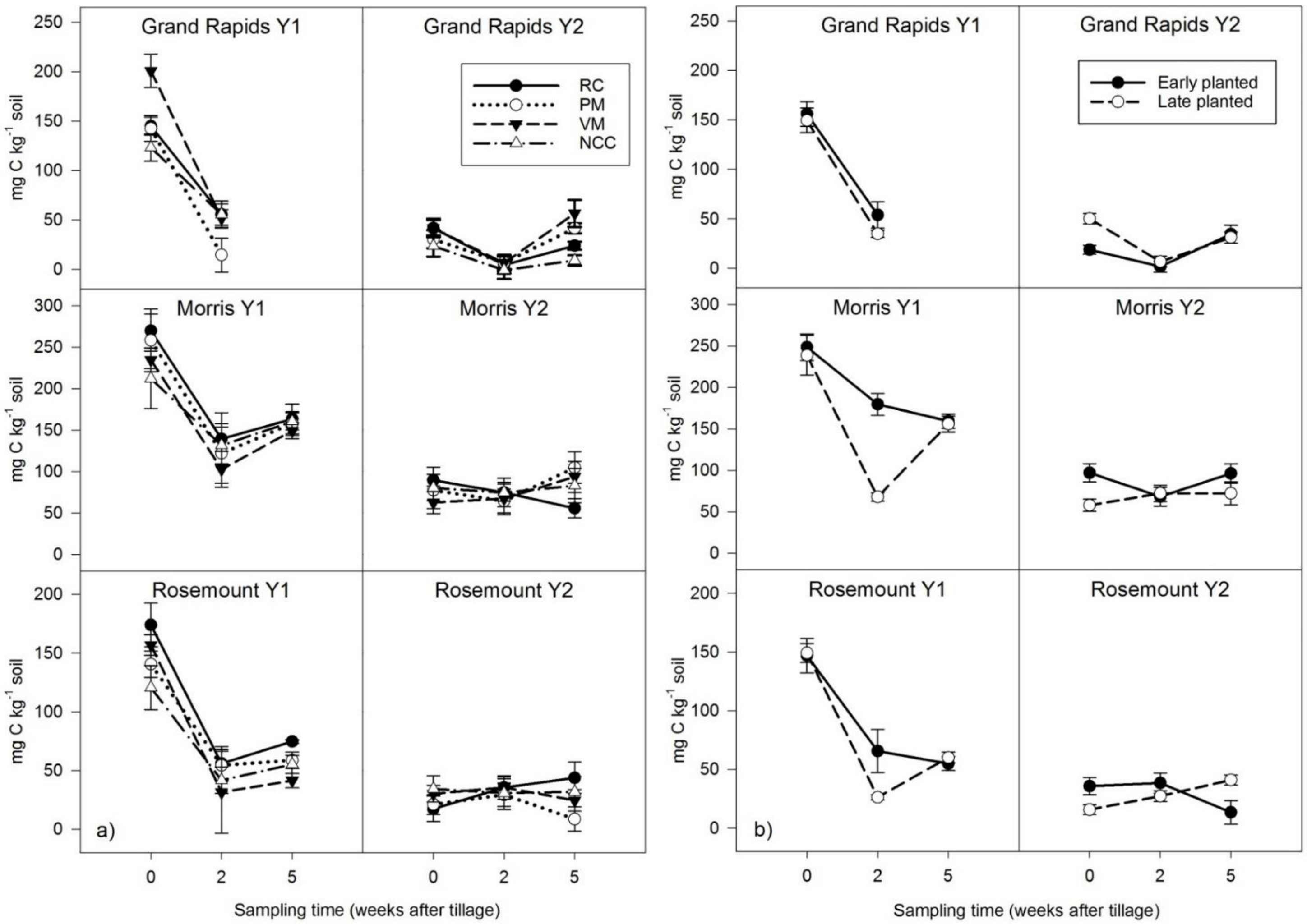

Figure 3. Microbial biomass carbon (MBC) values over sampling time plotted by a) Cover crop treatment (main plot factor) and b) Plant date (split plot factor). Error bars represent 1 SE.

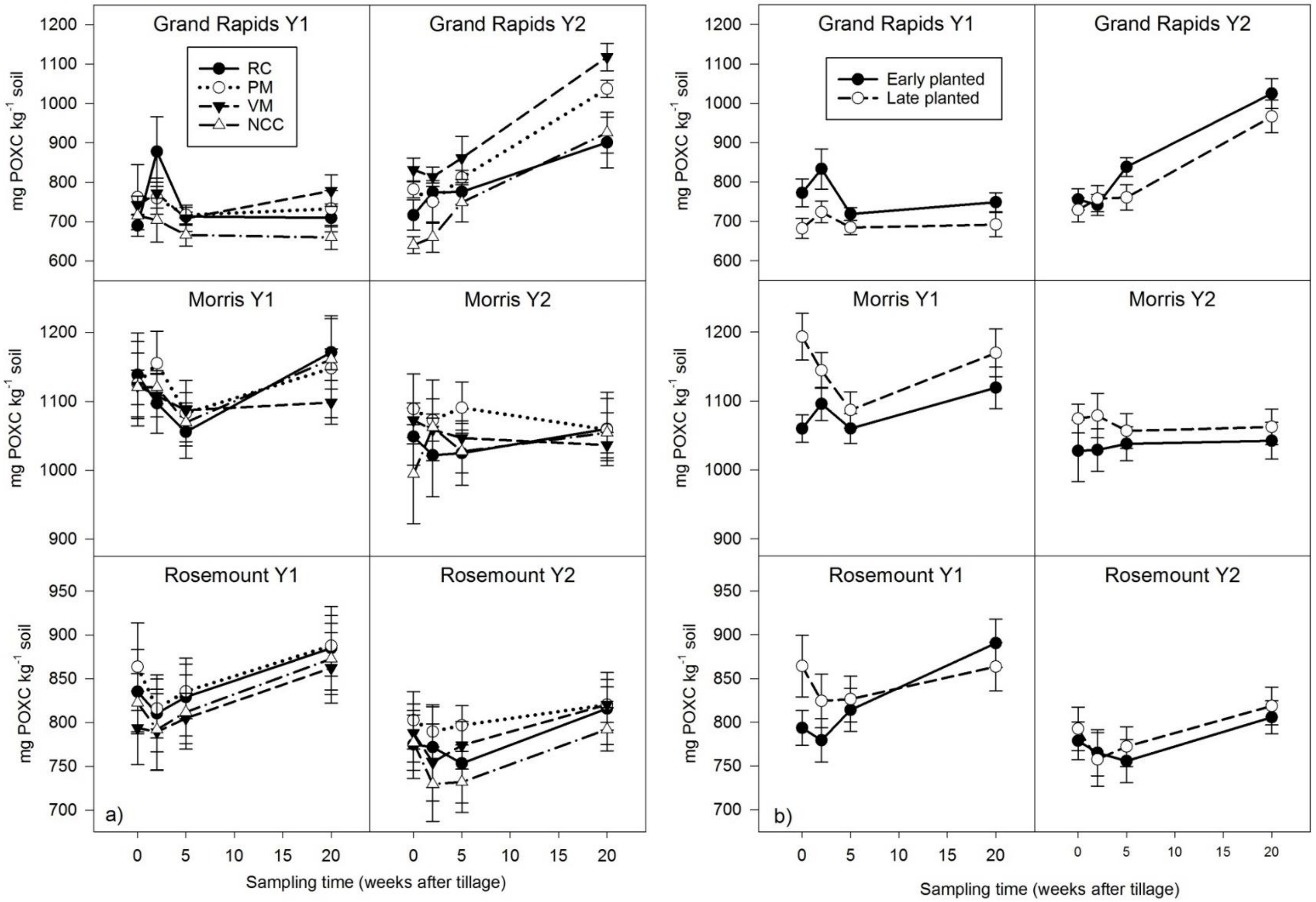

Figure 4. Permanganate oxidizable carbon (POXC) values over sampling time plotted by a) Cover crop treatment (main plot factor) and b) Plant date (split plot factor). Error bars represent 1 SE.

Table 7. PMN, pH, and EC results. Data reported by cover crop treatment (main factor), unless plant date or plant date x cover crop interaction was significant ($p < 0.05$). Cover crop treatments are red clover (RC), pea mix (PM), vetch mix (VM), and no cover crop control (NCC), and plant date treatments are early planted (Early) and late planted (Late). Letters represent significant differences (alpha = 0.05) between values within a single site-year column; mean separation performed using an LSD test. If no letters are reported, there were no differences between treatments.

		Y1			**Y2**		
		Grand Rapids	**Morris**	**Rosemount**	**Grand Rapids**	**Morris**	**Rosemount**
PMN (mg N kg^{-1} soil)							
RC	**Early**	11.5	67.0 ab	47.1 a	66.1 ab	68.5 b	40.8 bc
	Late		48.9 b	25.0 b			53.5 abc
PM	**Early**	17.1	74.0 ab	42.5 a	67.6 a	87.0 a	57.6 ab
	Late		45.2 b	24.1 b			44.1 bc
VM	**Early**	39.1	93.1 a	50.9 a	82.0 a	72.9 ab	45.6 bc
	Late		47.5 b	22.9 b			70.9 a
NCC	**Early**	10.3	49.1 b	44.5 a	48.1 b	62.1 b	41.6 bc
	Late		43.6 b	15.9 b			38.6 c
pH							
RC	**Early**	6.77 ab	7.33	6.78	6.77 ab	7.17	6.62
	Late				6.87 ab		
PM	**Early**	6.80 a	7.32	6.88	6.80 ab	7.20	6.80
	Late				6.90 a		
VM	**Early**	6.78 a	7.38	6.92	6.73 b	7.17	6.73
	Late				6.80 ab		
NCC	**Early**	6.73 b	7.23	6.93	6.80 ab	7.13	6.83
	Late				6.83 ab		
EC (dS cm^{-1})							
RC		0.32	1.53	0.42	0.22	0.89	0.37
PM		0.30	1.18	0.38	0.23	0.89	0.28
VM		0.40	1.04	0.42	0.27	0.83	0.31
NCC		0.38	1.53	0.52	0.23	0.76	0.32

Table 8. Significance of F test for pepper yield analysis of variance using a split plot model with cover crop treatment (CCT) as the main factor and plant date (PD) as the split plot factor. Data were square root transformed to fit assumptions of normality and equal variance. P values below 0.10 are reported, all other p values are considered not significant (NS).

	Y1			**Y2**		
	Grand Rapids	**Morris**	**Rosemount**	**Grand Rapids**	**Morris**	**Rosemount**
Marketable fruit (kg plant^{-1})						
CCT	NS	NS	NS	NS	NS	NS
PD	NS	NS	NS	NS	NS	NS
CCT x PD	NS	NS	NS	NS	NS	NS
Unmarketable fruit (kg plant^{-1})						
CCT	NS	NS	NS	NS	NS	NS
PD	NS	0.075	NS	NS	NS	0.022
CCT x PD	NS	NS	NS	NS	NS	NS
Average marketable pepper weight (kg)						
CCT	NS	NS	NS	0.005	NS	NS
PD	NS	NS	NS	NS	NS	NS
CCT x PD	NS	NS	NS	NS	NS	NS

Table 9. Marketable yellow fruit per plant, unmarketable fruit per plant, and average weight of marketable yellow fruit results. Data reported by main factor, cover crop treatment, unless plant date or plant date x cover crop interaction was significant ($p < 0.050$). Cover crop treatments are red clover (RC), pea mix (PM), vetch mix (VM), and no cover crop control (NCC) and plant date treatments are early planted (Early) and late planted (Late). Letters represent significant differences (alpha = 0.05) between values within a single site-year, mean separation performed using an LSD test on square root transformed data. If no letters are reported, there were no differences between treatments.

		Y1			**Y2**		
		Grand Rapids	**Morris**	**Rosemount**	**Grand Rapids**	**Morris**	**Rosemount**
Marketable fruit (kg plant^{-1})							
RC		0.25	0.90	0.83 a	0.20	0.57	0.51
PM		0.12	0.88	0.70 ab	0.17	0.60	0.37
VM		0.27	0.93	0.70 ab	0.25	0.67	0.45
NCC		0.20	0.95	0.53 b	0.20	0.60	0.38
Unmarketable fruit (kg plant^{-1})							
RC	**Early**	0.03	0.13	0.08	0.03	0.10	0.00 b
	Late						0.07 a
PM	**Early**	0.02	0.12	0.07	0.07	0.08	0.07 a
	Late						0.07 a
VM	**Early**	0.03	0.13	0.08	0.10	0.08	0.00 b
	Late						0.07 a
NCC	**Early**	0.05	0.17	0.03	0.07	0.05	0.03 ab
	Late						0.03 ab
Average marketable pepper weight (kg)							
RC		0.16	0.24	0.17 a	0.13 b	0.18	0.16
PM		0.17	0.23	0.16 ab	0.13 b	0.18	0.15
VM		0.19	0.24	0.16 ab	0.15 a	0.19	0.15
NCC		0.16	0.24	0.15 b	0.13 b	0.19	0.15

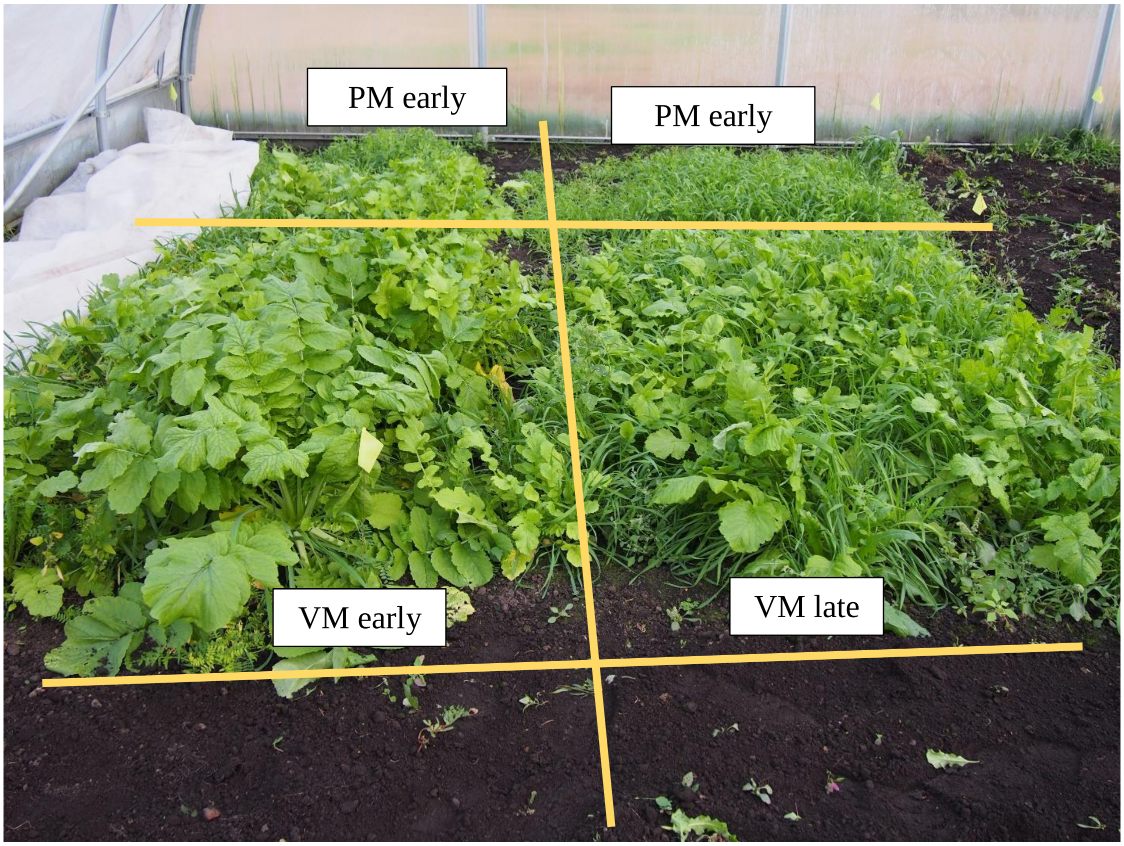

Figure 5. Y1 cover crops at Rosemount, October 30, 2015. Vetch mix (VM) early and late planted treatments show rapid radish growth, increasing low light stress compared to early and late planted pea mix (PM).

Chapter 3: Controlled environment freezing tolerance of hairy vetch and red clover with simulated high tunnel acclimation

Introduction

High tunnels are semi-permanent structures, covered with 6-mil polyethylene, used to extend the growing season in temperate climates (NRCS, 2015). High tunnels are popular throughout the world, particularly in East Asia and the Mediterranean, and have also gained popularity with fruit, vegetable, and flower growers in the United States (Carey et al., 2009; Lamont, 2009). With the help of a federal cost-share initiative through the National Resource Conservation Service's Environmental Quality Incentives Program (EQIP), 1,241 new high tunnels were constructed in the Upper Midwest between 2010 and 2013 (Huff, 2015).

Use of high tunnels has been shown to increase the length of the growing season, increase marketable yield and quality, and provide a price premium for locally produced off-season produce (Waterer, 2003; Kadir et al., 2006; Blomgren et al., 2007; Reeve and Drost, 2012). Growers opt to crop these high value spaces intensively, though in the Upper Midwest most growers leave the tunnel under bare fallow December-February, due to low temperatures and lack of irrigation (Knewtson et al., 2010a; Perkus, unpublished survey). Intensive cropping, high fertilization rates, irrigation under dry conditions, and higher soil temperatures have the potential to degrade high tunnel soils via loss of organic matter, increased soil salinity, and decreased soil biological activity (Hajime et al., 2009; Montri and Biernbaum, 2009; Rudisill et al., 2015). Growers are concerned that soils degraded in these ways can reduce yield over time (Bross, personal communication; Knewtson et al., 2010b; Montri and Biernbaum, 2009).

Legume cover crops are a potential management tool to mitigate soil degradation (Tonitto et al., 2006; Schipanski et al., 2014). Two legume cover crops of interest to growers in the Upper Midwest are hairy vetch (*Vicia villosa*) and red clover (*Trifolium pratense*). Both hairy vetch and red clover have been used successfully as winter annual cover crops in moderately cold climates (Hively and Cox, 2001; Delate et al., 2003; Schipanski and Drinkwater, 2011; Schipanski et al., 2014; Finney et al., 2016). However, winter hardiness and freezing tolerance for these species, particularly hairy vetch, in the far north is inconsistent (Jannink et al., 1997; Teasdale et al., 2004).

High tunnel environments further complicate prediction of winter hardiness in cover crops because rather than mirroring the gradually cooling daily maximum temperatures in the open field, high tunnel daily maximum temperatures are 4.9°C higher or more (Ward and Bomford, 2013). In late fall, as night temperatures in both the open field and high tunnel decrease below freezing, high tunnel day temperatures increase above freezing, increasing growing degree days relative to the open field (Yao and Rosen, 2011; Wildung and Johnson, 2012; Ward and Bomford, 2013). It has been reported that red clover survives freezing temperatures better after a cold acclimation period (Meyer and Badaruddin, 2001; Bertrand et al., 2016), and it is thought that the majority of plants benefit from an acclimation period before more extreme freezing temperatures (Xin and Browse, 2000; Gusta and Wisniewski, 2013). However, acclimating conditions commonly used in freezing studies are designed to mimic open field conditions, and it is unknown whether the high temperature fluctuation observed in high tunnels compared to the gradual cooling in the open field has an effect on freezing tolerance.

For growers who want to use winter annual legume cover crops in high tunnels to mitigate soil health issues, the problem is therefore two-fold – there is little information on cover crop winter hardiness and little information on how high tunnel environments affect winter hardiness. The purpose of this study was to compare the effect of simulated high tunnel conditions compared to typically applied acclimation conditions on hairy vetch and red clover survival and regrowth for a range of freezing temperatures using controlled environments.

Materials and Methods

Plant Material and Growing Conditions

This study was conducted at the University of Minnesota, St Paul in controlled environments from February to April in 2017. Species used in this study were red clover (*Trifolium pratense*) and hairy vetch (*Vicia villosa*) (Table 1). Prior to seeding, seeds were scarified by mechanical abrasion and inoculated with N-Dure Alfalfa/True Clover Combination inoculant and N-Dure Pea/Vetch/Lentil inoculant (Verdesian Life Sciences, Cary, NC), respectively. Two seeds were planted in 3.8 cm x 21.0 cm conical containers filled with Sunshine Natural and Organic Professional Growing Mix (Sun Gro Horticulture, Agawam, MA). Plants were allowed to germinate in a greenhouse for 3 weeks at 25°C day/10°C night under natural daylight supplemented with 12 hr 200 µmol m^{-2} s^{-1} lights. After 1.5 weeks, plants were thinned to one plant per pot. Plants were watered as needed and were fertilized weekly with 10 mL of an aqueous solution of 100-150-50 ppm, diluted from 2-3-1 fish and seaweed blend fertilizer (Neptune's Harvest, Gloucester, MA). Vetch plants were staked to prevent tangling, and trays were rotated on the greenhouse bench weekly to account for light, fan, and temperature variations.

Plants were placed in ten trays with 30 clover and 30 vetch seedlings in each tray. After 3 weeks of growth in the greenhouse, five trays each were moved into one of two growth chamber acclimation treatments for 3 weeks. Acclimation treatments were: 1) “Standard acclimation” (SA) with 2°C day/2°C night, following acclimation procedures from previous freezing tolerance studies (Hulke et al., 2008, 2012; Hoffman et al., 2010), and 2) “High tunnel simulation” (HTS) with one week of 20°C days/2°C nights followed by two weeks with 20°C days/-2°C nights, attempting to mimic high tunnel temperatures during mid to late fall in Minnesota. Light in both chambers was set to 10 hr days with 200 µmol m^{-2} s^{-1}.

After 21 days of acclimation, plants were placed in a shallow tub of water and allowed to soak from the base for 1 hour, then allowed to drain for 30 minutes to standardize media moisture. Plants were then subjected to freezing at one of five temperatures: -6°C, -9°C, -12°C, -15°C, or -18°C. For each temperature treatment, one tray of 30 vetch and 30 clover plants from each of the acclimation treatments were placed in a Tenney programmable freezer (model no. T20S, Thermal Product Solutions; New Columbia, PA) for 14 hrs. Over the 14 hr period, the temperature decreased by 2°C hr^{-1} from a base temperature of -2°C to the target temperature. The target temperature was held for an hour before rising immediately to 2°C at the end of the program (Fig. 1).

After freezing, plants were defrosted at 2°C for 48 hr in the SA chamber. Plants treated with standard acclimation were then returned to the greenhouse under optimal conditions 22°C day/22°C night with natural light in early April and plants treated with high tunnel simulation conditions were placed back into the HTS chamber with 20°C days/-2°C nights and 10 hr days with 200 µmol m^{-2} s^{-1} light. The plants recovered in these conditions for 3 weeks (Table 2).

Measurements

After a 21 day recovery period, plants were assessed for survival, with each individual plant receiving a score of either 0 for dead or 1 for alive in order to calculate percent survival for each treatment combination. Aboveground living biomass was collected from all surviving plants (plants scored as "1"). Living stems were cut at the soil surface and dried at 60°C for 72 hrs, then weighed. Dried vetch plant material was pooled into samples large enough for percent nitrogen and C:N analysis, then ground to a fine powder using a mortar and pestle. Three pooled samples per treatment combination, each consisting of 2-10 plants depending on treatment survival, were analyzed for percent nitrogen (%N) and C:N ratio on a Vario PYRO cube combustion analyzer (Elementar, Langenselbold, Germany).

Experimental Design and Statistical Analysis

This experiment was organized in a factorial design with three factors: plant species (red clover or hairy vetch), acclimation treatment (HTS or SA), and freezing temperature (-6°C, -9°C, -12°C, -15°C, or -18°C). Individual plants were treated as replicates. Sigmoidal curves were fit to survival data using a general linear model. To calculate LT_{50}, the temperature on the survival curve where y = 50% survival was identified. In some cases this point was outside the range of collected data. Dunn's test was used to calculate mean separation for biomass, due to unequal sample sizes. Tukey's

HSD test was used to calculate mean separation for percent nitrogen. All statistical analyses were conducted using R (R Core Team, 2017).

Results:

Survival and LT_{50}

Survival data for clover and vetch plants treated with high tunnel simulation (HTS) and standard acclimation (SA) showed differences in survival across the range of freezing temperatures (Fig. 2). The lowest freezing temperature used in this study did not result in 100% mortality of either plant species under HTS. Percent survival for SA clover ranged from 90% at -6°C to 0% at both -15° and -18°C, whereas clover in the HTS treatment had 100% survival at all temperatures except -18°C (46.7%). Percent survival for SA vetch ranged from 100% at -6°C, to 6.7% at -18°C, whereas vetch in the HTS treatment had 100% survival at all temperatures, except -15°C (96.7%) and -18°C (76.7%). Data suggests that acclimation treatment had a stronger effect on percent survival at each freezing temperature than did plant species and, at the evaluated growth stage, vetch may be more tolerant to freezing events than red clover.

LT_{50} values, the predicted temperature at which plants experience 50% mortality, were estimated based on the survival curves (Table 3). For HTS clover and vetch, the temperature at which plants experienced 50% mortality was below the observed temperature range because neither clover nor vetch in the HTS treatment experienced 100% mortality at any freezing temperature. For both species, the LT_{50} is lower for HTS plants than for SA plants. When comparing species within the same acclimation treatment, the 95% confidence intervals for SA clover and vetch overlap by 2.7°C, whereas the 95% confidence intervals for HTS clover and vetch overlaps by 6.7°C. This indicates that while both species treated with HTS react similarly to freezing temperatures, there may be a species difference regarding survival after SA treatment. An experiment with larger sample sizes may have the power to elucidate this difference.

Biomass and %N

For both clover and vetch, plant biomass collected three weeks after freezing was affected by both acclimation treatment and freezing temperature (Fig. 3). For both clover and vetch, warmer freezing temperatures resulted in higher biomass regrowth than colder freezing temperatures, and HTS plants had significantly more biomass than SA plants for almost every freezing temperature. The only exception was vetch frozen at -6°C, where HTS and SA plants did not show a difference for biomass accumulation.

Vetch tissue nitrogen (%N) varied by temperature (Table 4), with a high of 4.82 %N and a low of 2.96 %N. For HTS vetch, %N increased from a low of 2.96 %N at -6°C to a high of 4.04 %N at -18°C. This trend was inverted for the SA vetch, which decreased from 4.82 %N at -6°C to 3.15 %N at -12°C. No values are reported for SA vetch at freezing temperatures of -15°C and -18°C or for any clover treatments because plants did not survive with sufficient biomass for analysis.

Discussion

Survival and LT_{50}

Both survival and LT_{50} were affected by acclimation treatment more so than by plant species. The factor driving plant survival and LT_{50} differences between HTS and SA may be that the HTS treatment included night temperatures below 0°C, whereas the

SA plants first encountered temperatures below 0°C during the freezing event. In this study, SA was used as a comparison for HTS based on prior controlled environment studies (Hoffman et al., 2010; Hulke et al., 2012; Hulke et al., 2008; Meyer and Badaruddin, 2001). SA does not replicate open field conditions observed in the Upper Midwest, and, for vetch particularly, HTS seems to approximate open field conditions better than SA. The LT_{50} for HTS vetch, -19.2°C, is similar to survival results from a field study which noted 100% survival at a site with absolute minimum temperatures ranging from -11°C to -13°C and 36-85% survival at a site with absolute minimum temperature of -20°C with little snow cover (Teasdale et al., 2004). These observations show greater freezing tolerance than the LT_{50} for the SA vetch, which predicted 50% survival at -11.3°C. Differences in freezing tolerance can be attributed to a large number of factors including plant age, light, soil moisture and humidity (Gusta and Wisniewski, 2013), so while conclusions cannot be drawn from comparison of plants in this study to open field observations, comparisons can direct future research to reexamine the utility of SA for estimating field freezing tolerance.

There were no species differences for the LT_{50} of HTS plants, but data suggested there may be a difference between the LT_{50} of SA plants. Plants physiologically prepare for freezing at different acclimation temperatures (Xin and Browse, 2000). The LT_{50} of SA vetch (-11.3°C) and clover (-7.6°C) suggests that an acclimation period of 2°C induces more freezing tolerance in vetch than it does in clover. The HTS conditions prior to freezing may improve freezing tolerance for hairy vetch and red clover by a nighttime temperature below freezing or by a warmer daytime temperature. Freezing tolerance correlates with sugar accumulation, and the warmer daytime temperature in the HTS

(20°C) compared with SA (2°C) may have allowed plants to prepare for freezing events by increased photosynthetic rate (Gusta and Wisniewski, 2013). This study does not have the power to distinguish the effects of a cooler nighttime temperature versus a warmer daytime temperature found in the HTS treatment.

Biomass and %N

In general, biomass regrowth patterns followed expectations, with plants frozen at warmer temperatures regrowing more biomass than plants frozen at colder temperatures, and HTS plants, which had lower LT_{50}s, regrowing more biomass than SA plants. SA and HTS vetch plants frozen at -6°C regrew statistically similar amounts of biomass 3 weeks after freezing, whereas at all other freezing temperatures, -9°C to -18°C, the HTS vetch regrew more biomass than the SA vetch. Because there was no difference in acclimation treatment for this freezing temperature, it seems that vetch plants at this growth stage can tolerate instances of -6°C equally well regardless of prior acclimation conditions.

Vetch tissue nitrogen results were somewhat unexpected, as plants at similar growth stages under the same growing conditions are expected to have the same %N. Plants frozen at colder temperatures were smaller, suggesting younger tissue. Younger plant tissues have higher %N (Hicks, 1928), which explains the pattern seen in the HTS plants where %N increased as freezing temperature and regrown biomass decreased. However, this does not explain the opposing trend seen in SA vetch where %N was highest at the warmest freezing temperature, which also had the highest biomass. The two mechanisms for nitrogen uptake in legumes are root uptake from the soil and nitrogen fixation by rhizobia bacteria in nodules, both belowground. For HTS plants, soil froze completely each night for 2 weeks during the acclimation period, while in SA plants the soil only froze once during the freezing treatment and roots experienced an even 2°C environment for 3 weeks. This suggests that SA nitrogen uptake was affected by freezing temperature alone rather than a combination of acclimation period and freezing temperature. Hairy vetch nodules have been shown to stop fixing nitrogen at temperatures of 2°C and below, but they recover the ability to fix nitrogen when temperatures rise (Dart and Day, 1971), so it is likely that SA nodules paused nitrogen fixation with little damage. HTS nodules likely suffered some stress or damage due to freeze/thaw cycles exhibited in acclimation and recovery. However, even cold susceptible strains of rhizobia have been found to survive 24hr instances of extreme cold (-80°C) at 33% (Drouin et al., 2000), so it is unlikely that rhizobia in HTS nodules died completely.

Conclusions:

Legume cover crops are a potential management tool for high tunnel growers who are concerned with soil health in their high tunnels. High tunnel maximum air and soil temperatures in fall are different from those in the open field, and these differences during the acclimation period may affect freezing tolerance of legume cover crops in high tunnels, which will experience similar night temperatures as the open field during nighttime freezing events. In this study, the high tunnel simulation acclimation treatment improved freezing tolerance for both hairy vetch and red clover. After HTS, LT_{50}s were lowered from -11.3°C to -19.2°C in vetch and from -7.6°C to -18.1°C in clover. Overall, freezing tolerance trends observed in this study must be reinforced through repetition and larger sample sizes, as the confidence intervals for the LT_{50} values in this study are larger than other reported studies (Meyer and Badaruddin, 2001; Hulke et al., 2008). Additionally, it should be noted that LT_{50} values determined in this study arose from single freezing events, and freezing tolerance of plants exposed to long periods of freezing may produce different results (Gusta and Wisniewski, 2013).

This study illustrated the possibility that high tunnel acclimation conditions may be more favorable for freezing tolerance than the open field because these plants are exposed to warmer daytime temperatures that improve biomass growth as well as mild freezing temperatures before colder freezing events. This result requires validation in the field before recommendations to growers are made, and, if confirmed, this would allow growers in cold climates to select legume varieties that would otherwise be marginally or not at all winter hardy in their open fields. Growers should be aware that hairy vetch does not fix nitrogen when soils are below 2°C, though plants will resume nitrogen fixation when soils warm up. Further research is required to assess the nitrogen fixing capacity of nodules experiencing high tunnel conditions compared to those in an open field. Additionally, a colder range of freezing temperatures should be tested to verify survival curves and LT_{50} for HTS red clover and hairy vetch.

Figures and Tables:

Table 1. Plant material.

Common name	Cultivar	Latin name	Seed source
Red clover (RC)	--	*Trifolium pratense*	Albert Lea Seed House, Albert Lea, MN,
Hairy vetch (HV)	‘Purple Bounty’	*Vicia villosa*	Allied Seed, Albany, OR

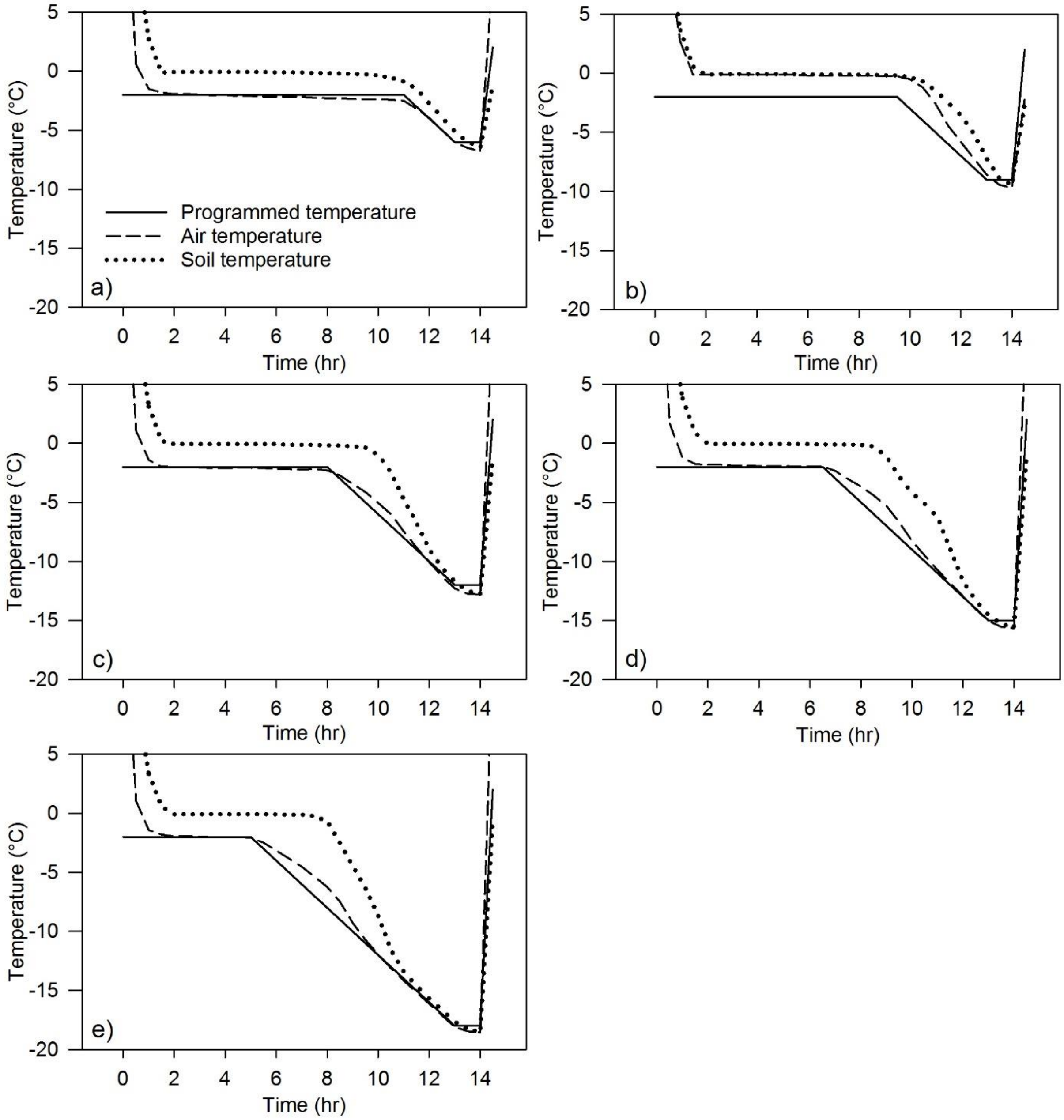

Figure 1. Freezing treatment temperatures over time for a) -6°C, b) -9°C, c) -12°C, d) -15°C, and e) -18°C. Solid lines represent programmed values, dashed lines represent an average of two air temperature sensors, and dotted lines represent an average of two soil temperature sensors.

Table 2. Environmental conditions for all stages of the two acclimation treatments, standard acclimation (SA) and high tunnel simulation (HTS).

			Acclimation Treatment	
Stage	**Length**	**Day length**	**SA**	**HTS**
Germination	3 weeks	12 hr	25°C day/10°C night	25°C day/10°C night
Acclimation	3 weeks	10 hr	2°C day/2°C night	20°C day/2°C night (1 week) 20°C day/-2°C night (2 weeks)
Freezing	1 day	10 hr	-6°C, -9°C, -12°C, -15°C, or -18°C	
Defrosting	2 days	10 hr	2°C day/2°C night	2°C day/2°C night
Recovery	3 weeks	10 hr	22°C day/22°C night	20°C day/-2°C night

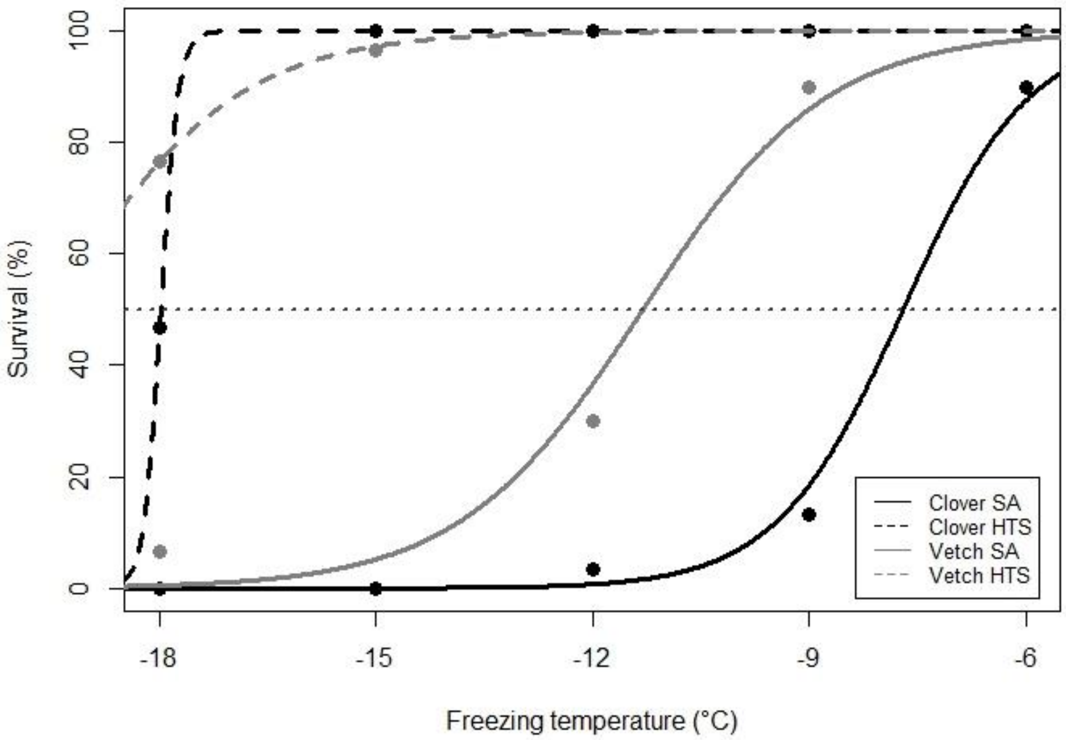

Figure 2. Survival of hairy vetch (grey) and red clover (black) grown under high tunnel simulation (dashed lines) or standard acclimation conditions (solid lines) at a range of freezing temperatures from -18°C to -6°C, with a horizontal dashed line representing 50% survival. Sigmoidal curves were fitted to data, for each point n = 30 plants.

Table 3. LT_{50} for clover and vetch plants grown under high tunnel simulation (HTS) and standard acclimation (SA), (95% confidence interval).

Species	HTS	SA
Clover	-18.1	-7.6
	(±3.3)	(±3.3)
Vetch	-19.2	-11.3
	(±4.5)	(±3.1)

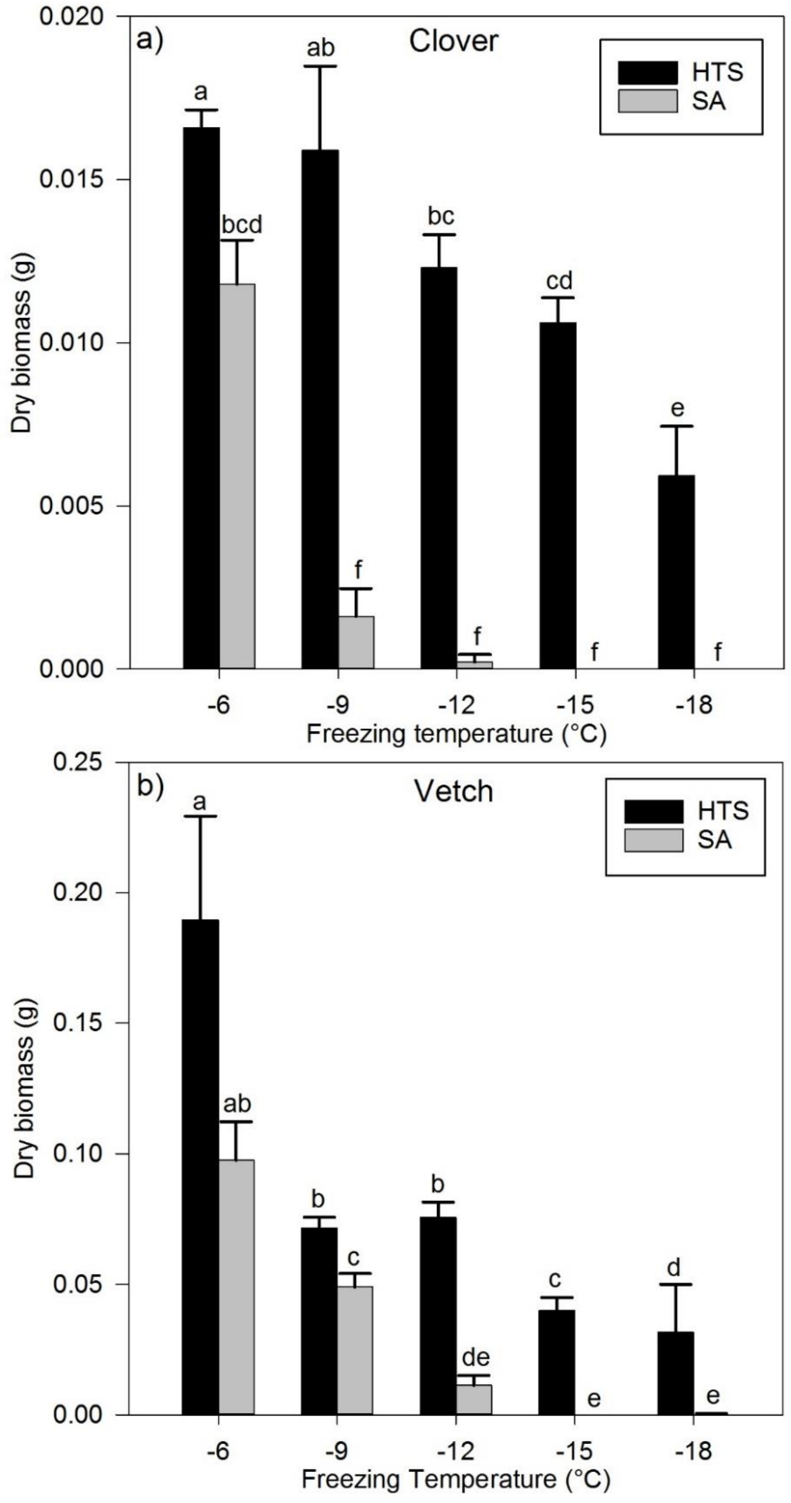

Figure 3. Dry biomass accumulated by a) clover and b) vetch plants, collected 3 weeks after freezing. N = 30 plants, error bars show 1 SE, and mean separation calculated using Dunn's test.

Table 4. Percent nitrogen in vetch tissue collected 3 weeks after freezing (1 SE). N = 3 pooled samples, mean separation calculated using Tukey's HSD.

Acclimation treatment	**-6°C**	**-9°C**	**-12°C**	**-15°C**	**-18°C**
HTS	2.96 e (0.07)	3.21 de (0.07)	3.33 de (0.03)	3.53 cd (0.08)	4.04 bc (0.12)
SA	4.82 a (0.02)	4.30 ab (0.24)	3.15 de (0.06)	--	--

Chapter 4: Spring planted legume cover crops in high tunnels

Introduction:

In early October 2014, cover crops were planted in high tunnels at the North Central Research and Outreach Center in Grand Rapids, MN (lat. 47.242539, long. -93.492791) and the West Central Research and Outreach Center in Morris, MN (lat. 45.593615, long. -95.878379), to begin the experiment described in Chapter 2: Evaluation of three winter annual legume cover crop mixes for soil health and fertility in high tunnels. However, cover crops experienced total mortality over the winter, and these site-years could not be included in the high tunnel winter annual cover crop study. The following spring, cover crops were re-seeded in mid-April, to assess feasibility of spring planted cover crops in high tunnels in Minnesota. Spring planted cover crops offer growers an opportunity to incorporate cover crops into high tunnel rotations without sacrificing fall cash crop production. However, it is unknown whether this short growing season allows cover crops to produce enough biomass to provision sufficient nitrogen or influence soil health characteristics. The results of this pilot study are presented here.

Materials and methods

High tunnels at Grand Rapids and Morris varied in environmental and construction characteristics (Table 1). Plots were arranged in a randomized complete block design with three blocks in each high tunnel. The experimental factor was cover crop treatment (CCT), and treatments consisted of 1) Red clover monoculture [(13.5 kg/ha); *Trifolium pratense*, Albert Lea Seed, MN], 2) Winter pea/rye 1:1 biculture [(84.1 kg/ha); *Pisum sativum* and *Secale cereale*, Albert Lea Seed, MN], 3) Hairy vetch/tillage radish/rye 4:1:15 mix [(84.1 kg/ha); *Vicia villosa, Raphanus sativus,* and *S. cereale*, Albert Lea Seed, MN], and 4) weedy control. Cover crops were inoculated with N-Dure inoculant (Verdesian Life Sciences, NC) by moistening seeds with a 1:4 sucrose solution and mixing with the recommended rate of inoculant, then allowing seeds to air dry overnight. Cover crops were seeded on April 16, 2015 in Morris and April 17, 2015 in Grand Rapids by broadcasting into plots that were 2.3 m x 4.2 m in Morris and 2.3 m x 2.8 m in Grand Rapids. Plants were watered overhead as needed.

Cover crops were terminated in Morris on June 5, 2015 and in Grand Rapids on June 11, 2015 with a riding mower and a push mower, respectively. Cover crops at both sites were rototilled into the soil on the same day as termination to a depth of 15-20 cm. Sweet Sunrise bell peppers (Johnny's Selected Seeds, ME) were started in the greenhouse 10-12 weeks before planting. Peppers were transplanted 3-5 days after tillage in staggered double rows with 45 cm between plants and 90 cm between rows. Peppers were irrigated with drip irrigation and supplemental fertilizer, AgGrand 4-3-3 (AMSOIL Inc., WI), was applied via fertigation over 8 weeks starting in July. Fertility applications were based on recommendations for bell peppers in Minnesota (Rosen and Eliason, 2005) and modified with cover crop N contribution estimates calculated using biomass data. Fertility rates were 9.1 kg N ha^{-1} $week^{-1}$ for Morris, with a total recommendation of 112.1 kg N ha^{-1} due to high OM and a cover crop contribution estimated at 39.2 kg N ha^{-1}, and 15.4 kg N ha^{-1} $week^{-1}$ for Grand Rapids, with a total recommendation of 156.9 kg N ha^{-1} due to low OM and a cover crop contribution estimated at 33.6 kg N ha^{-1}. Peppers were hand weeded weekly and trellised in July using a Florida weave system with stakes every four plants. Pepper harvest began when individual fruits were 90% yellow. Peppers were harvested every 12-18 days and sorted, counted, and weighed according to USDA standards for marketable (combined Fancy, No. 1, and No. 2) and unmarketable (USDA, 2005). Harvest occurred from August 8, 2015 to September 18, 2015 at Morris and August 28, 2015 to September 16, 2015 at Grand Rapids.

Cover crops were sampled on May 28, 2015 at Morris and June 10, 2015 in Grand Rapids. Four random 0.1 m^2 quadrats per plot were collected by clipping cover crops to ground level and pooled. Samples were sorted according to plant type. Samples were dried at 60°C for 72 hr, ground to 1 mm, and run on a Vario PYRO cube combustion analyzer (Elementar, Germany) for %C and %N. Soils were sampled 1) At cover crop termination, 2) 2 weeks after tillage, 3) 5 weeks after tillage, and 4) final pepper harvest (15 weeks after tillage). For each treatment, eight random soil cores within pepper rows were taken to 20 cm deep and pooled, then dried at 35°C for 72 hr and ground to 2 mm before analysis. Soils were analysed for pH and EC using a 1:5 soil in water solution. Soils were analyzed for permanganate oxidizable carbon (POXC) using the method described in Culman et al., 2012. Baseline soil samples, collected at cover crop tillage, were sent to Midwest Laboratories (Omaha, NE) for analysis.

Data were analyzed as a randomized complete block design with site and cover crop treatment as the main effects. Blocks were treated as replications. For soil analyses, which were collected four times over the course of a season, time was treated as a split plot factor, and a split plot design was used for analysis. Analysis of variance was conducted using the anova function in the R base package (R Core Team, 2017) and split plot function in the R package “agricolae,” to determine main effects and interactions (de Mendiburu, 2017). Square root transformations were used when data failed to meet assumptions of normality. P values are reported if less than 0.10, but only p-values below 0.05 were investigated further. Mean separation was performed using Fisher's LSD ($p = 0.05$).

Results

Cover Crop biomass

Total treatment biomass (cover crop and weed biomass combined) differed by site (Table 2). Biomass was higher at Morris than Rosemount, but there were no differences in total treatment biomass between cover crop treatments and bare controls (Table 3). Legume biomass did not differ by site, though legume biomass did vary by treatment within sites (Table 2). At Morris, cover crop treatments containing legumes (RC, VM, and PM) produced more legume biomass than the weedy control (NCC), which had no planted legumes (Table 3). However, at Grand Rapids, only RC produced more legume biomass than NCC (Table 3).

Soil Analyses

POXC varied by time point at Grand Rapids only (Table 5), with the highest values observed at 2 and 5 weeks after tillage and the lowest values observed 0 and 15 weeks after tillage (Table 6). pH varied by time point at both sites, with an interaction between time point and cover crop treatment at Grand Rapids (Table 5), however these changes were slight and not biologically significant (Table 6). EC varied by time point at both sites (Table 5). At Grand Rapids, EC was elevated at 2 and 5 weeks post tillage, whereas at Morris EC was only elevated at 2 weeks post tillage.

Pepper yield

Pepper yield varied by site, but not by cover crop treatment (Table 7), with higher yields in Morris than Grand Rapids (Table 8).

Discussion

Spring planted cover crops offer a management alternative to fall planted winter annual cover crops, allowing growers to continue using their high tunnel for cash crop production through late fall. Legumes alone in this study produced 15.0 to 23.7 kg N ha^{-1} in Morris (zone 4a) and 8.5 to 30.7 kg N ha^{-1} in Grand Rapids (zone 3b), a contribution which in some cases can reduce the amount of fertilizer that grower would need to apply. In addition to the nitrogen supplying capability, VM was able to suppress weeds in Morris, making it an attractive option for growers.

POXC, a measure of labile carbon and an indicator of soil health (Morrow et al., 2016), is shown to increase 2 weeks after the addition of cover crop biomass in all cover crop treatments in Grand Rapids. This result may be in part due to tillage, as POXC has been shown to increase after tillage events as well as biomass additions (Culman et al., 2012). EC increased 2 weeks after tillage at both sites and at 5 weeks after tillage at Grand Rapids, but fell back to baseline levels by the end of the season. This suggests nutrient availability for the pepper cash crop during early growth without increasing EC after a season of production (Smith and Doran, 1996). These results illustrate the possibility of cover crops as a nutrient source without increasing EC from season to season, though this trend should be evaluated over consecutive years.

Pepper yield is low at Grand Rapids, likely due to the effect of a colder climate, which may be magnified by a late transplanting date and early removal date compared with standard grower practices. At both sites, a later transplanting date and an earlier removal date for peppers was chosen to maximize growth time for cover crops. There are no differences in pepper yield by cover crop treatment. This may be due to the fact that cover crop biomass additions were the same across cover crop treatments in conjunction with supplemental fertilizer additions that were applied at the same rate across treatments.

It is important to note that the timing of operations in this study are not ideal. Over 75% of growers in the Great Plains begin growing crops in their high tunnels by March, and over 90% of growers in the Upper Midwest begin growing crops in their high tunnels by April (Knewtson et al., 2010a; Perkus, unpublished survey). The early spring season gives growers an early yield for high value crops, such as tomatoes, with important price premiums (Blomgren et al., 2007; Rogers and Wszelaki, 2012). Using a spring planted cover crop maybe be a useful tool for growers with several high tunnels, who have more space than needed for early spring production, to introduce once into a several year rotation.

Conclusions

Spring planted cover crops produced similar total biomass to the weedy control, though only vetch mix at Morris reduced weeds relative to the weedy control. Legume biomass alone added 8.5 to 30.7 kg N ha^{-1}, with the legumes in the red clover treatment in Grand Rapids producing more than pea mix or vetch mix. Cover crop treatment had no effect on soil properties, though an increase in POXC wass observed in Grand Rapids 2 weeks after cover crop and weed biomass was tilled in. EC also increased 2 weeks after tillage at both sites, and remained high at Grand Rapids 5 weeks after tillage. Cover crop treatment had no effect on cash crop yield at either site. Spring planted cover crops may be useful for increasing labile soil carbon in low organic matter soils, such as Grand Rapids, and legumes cover crop mixes can provide a small amount of nitrogen that could offset fertilizer needs while in some cases providing weed control. However, these benefits are possible rather than assured, depending on spring weather conditions and length of time growers are willing to take a high tunnel out of production.

Tables and Figures

Table 1. Site description.

	Site	
	Grand Rapids	**Morris**
High tunnel dimensions and area	15.2 m x 6.4 m 97.3 m^2	14.6 m x 9.1 m 132.9 m^2
High tunnel orientation (long side)	N/S	NE/SW
High tunnel covering	Single layer polyethylene	Single layer polyethylene
Previous management	Strawberries	Bare fallow
USDA Hardiness Zone	3b	4a
Soil type	Shooker very fine sandy loam	Byrne silt loam
%OM	1.9	4.4
Nitrate-N (ppm)	11	14
Bray-P (ppm)	342	271
K (ppm)	258	522

Table 2. Significance of F test for total plot biomass, legume biomass, weed biomass, legume %N, legume C:N, and legume biomass N. Analysis of variance conducted with the main factors site (Site) and cover crop treatment (CCT). Total biomass, legume biomass, and weed biomass were square root transformed to fit assumptions of normality and equal variance. P values below 0.10 are reported, all other p values are considered not significant (NS).

		p-value
Total biomass (kg ha^{-1})		
	Site	0.03
	CCT	NS
	Site x CCT	NS
Legume biomass (kg ha^{-1})		
	Site	NS
	CCT	<0.001
	Site x CCT	NS
Weed biomass (kg ha^{-1})		
	Site	0.091
	CCT	0.017
	Site x CCT	NS
Legume %N		
	Site	NS
	CCT	<0.001
	Site x CCT	NS
Legume C:N		
	Site	NS
	CCT	0.001
	Site x CCT	NS
Legume biomass N (kg ha^{-1})		
	Site	NS
	CCT	0.029
	Site x CCT	NS

Table 3. Total biomass, legume biomass, and weed biomass results. Data reported by cover crop treatments: red clover (RC), pea mix (PM), vetch mix (VM), and no cover crop control (NCC). Letters represent significant differences (alpha = 0.05) between values for a single biomass type within each site. Mean separation performed using an LSD test using square root transformed data.

		Grand Rapids	Morris
Total biomass (kg ha^{-1})			
	RC	2270	2905
	PM	1610	2859
	VM	2117	2326
	NCC	1601	2218
Legume biomass (kg ha^{-1})			
	RC	858 a	579 a
	PM	207 ab	293 a
	VM	154 ab	322 a
	NCC	0 b	0 b
Weed biomass (kg ha^{-1})			
	RC	15.8	26.1 a
	PM	11.3	16.9 ab
	VM	8.1	8.1 b
	NCC	17.9	24.9 a

Table 4. Legume percent nitrogen (%N), legume C:N, and legume biomass nitrogen (N) results. Data reported by cover crop treatment: red clover (RC), pea mix (PM), vetch mix (VM), and no cover crop control (NCC). Letters represent significant differences (alpha = 0.05) between values of a single data type for each site. Mean separation performed using an LSD test. If no letters are present, there were no differences between treatments.

		Grand Rapids	Morris
Legume %N			
	RC	3.66 b	4.02 b
	PM	4.74 a	4.96 ab
	VM	5.41 a	5.56 a
	NCC	--	--
Legume C:N			
	RC	10.9 a	10.1 a
	PM	9.3 ab	9.8 a
	VM	7.9 b	7.5 b
	NCC	--	--
Legume biomass N (kg ha^{-1})			
	RC	30.7 a	23.7
	PM	9.7 ab	18.1
	VM	8.5 ab	15.0
	NCC	0.0 b	0.0

Table 5. Significance of F test for permanganate oxidizable carbon (POXC), soil pH (pH), and electrical conductivity (EC). Analysis of variance conducted as a split plot with cover crop biomass (CCT) as the main factor and sampling time point (TP) as the split plot factor. P values below 0.10 are reported, all other p values are considered not significant (NS).

		Grand Rapids	Morris
POXC (mg C kg^{-1} soil)			
	CCT	NS	NS
	TP	0.022	NS
	CCT x TP	NS	NS
pH			
	CCT	NS	NS
	TP	0.038	0.017
	CCT x TP	0.004	NS
EC (μS cm^{-1})			
	CCT	NS	NS
	TP	<0.001	<0.001
	CCT x TP	NS	NS

Table 6. Permanganate oxidizable carbon (POXC), soil pH (pH), and electrical conductivity (EC) results. Data reported by cover crop treatment: red clover (RC), pea mix (PM), vetch mix (VM), and no cover crop control (NCC). Letters represent significant differences (alpha = 0.05) between values of a single data type for each site. Mean separation performed using an LSD test. If no letters are present, there were no differences between treatments.

		Grand Rapids	Morris
POXC (mg C kg^{-1} soil)			
	0	752 b	1178
	2	844 a	1189
	5	777 ab	1175
	15	730 b	1158
pH			
	0	7.0 b	7.4 a
	2	7.1 ab	7.3 b
	5	7.0 b	7.5 a
	15	7.2 a	7.4 a
EC (µS cm^{-1})			
	0	134 b	340 b
	2	212 a	558 a
	5	189 a	318 b
	15	160 b	351 b

Table 7. Significance of F test pepper yield reported as marketable yellow fruit per plant (kg). Analysis of variance conducted with the main factors site (Site) and cover crop treatment (CCT). P values below 0.10 are reported, all other p values are considered not significant (NS).

		p-value
Pepper yield		
	Site	<0.001
	CCT	NS
	Site x CCT	NS

Table 8. Pepper yield reported by cover crop treatment: red clover (RC), pea mix (PM), vetch mix (VM), and no cover crop control (NCC). There were no differences between treatments for each site.

		Grand Rapids	Morris
Marketable yellow fruit per plant (kg)			
	RC	0.34	0.80
	PM	0.31	0.73
	VM	0.30	0.66
	NCC	0.33	0.80

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