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Corn (*Zea mays* L.) and Cover Crop Response to Corn
Density in an Interseeding System and Subsequent Dry
Bean (*Phaseolus vulgaris* L.) Yield

presented by

Dieudonné Nkundizana Baributsa

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of the requirements for the

Ph.D. degree in CROP AND SOIL SCIENCE

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**CORN (*Zea mays* L.) AND COVER CROP RESPONSE TO CORN DENSITY IN
AN INTERSEEDING SYSTEM AND SUBSEQUENT DRY
BEAN (*Phaseolus vulgaris* L.) YIELD**

By

Dieudonné Nkundizana Baributsa

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ABSTRACT

CORN (*Zea mays* L.) AND COVER CROP RESPONSE TO CORN DENSITY IN AN INTERSEEDING SYSTEM AND SUBSEQUENT DRY BEAN (*Phaseolus vulgaris* L.) YIELD

By

Dieudonné Nkundizana Baributsa

Reliable cropping strategies are needed to enhance legume cover crop utilization as a nitrogen (N) source for corn (*Zea mays* L.) and dry edible beans (*Phaseolus vulgaris* L.). Nitrogen can be applied as a fertilizer or provided by leguminous cover crops. Planting corn with interseeded legume cover crops into a killed leguminous stand and planting dry bean the subsequent year can reduce N input costs for corn and dry bean. It is important to know how leguminous cover crops affect corn yield when used as the sole N source, the effect of interseeding, and the maximum plant density at which this approach is feasible. This study evaluated (1) the effect of four corn densities (37 500 to 75 000 plants ha⁻¹) in an interseeding system on corn yield and on red clover (*Trifolium pratense* L.) or Chickling vetch (*Lathyrus sativus* var. AC Greenfix,) dry matter; (2) the effect of mineral N fertilizer versus organic N provided by plowed red clover on corn yield and N status at various corn densities; (3) the effect of corn density and interseeding on N status in plant tissues of corn and cover crops; and (4) the effect of these management practices on soil mineral N (NO₃⁻ and NH₄⁺), subsequent dry bean yield, and N status of dry bean. Results suggest that interseeded cover crops did not affect corn yield at any plant density.

Across years, yield of corn planted into plowed red clover was similar to that of corn fertilized with mineral N. During optimum growing conditions, corn fertilized with mineral N outyielded corn planted into plowed red clover, however the reverse was seen in dry years. Interseeded cover crop biomass decreased as corn density increased. However the subsequent spring, red clover biomass was similar regardless of previous corn density.

Across years, corn grain N concentration decreased as corn density increased and was highest in corn fertilized with mineral N. Interseeding cover crops did not reduce corn grain N concentration. Overall, grain N content of corn planted into plowed red clover was lower compared with fertilized corn. By fall, interseeded cover crops accumulated more N at low corn density compared with higher plant densities. The subsequent spring, red clover accumulated significant N ranging from 58 to 162.3 kg ha⁻¹.

Interseeding cover crops did not influence soil mineral N. During optimum growing conditions, dry bean yield following corn interseeded with red clover was similar to yield of dry bean fertilized with 45 kg ha⁻¹ of mineral N. Similar trends were seen for bean seed N accumulation. Red clover can be interseeded at high corn densities (up to 75000 plants ha⁻¹) and accumulate sufficient N the subsequent spring to meet the N demand of the following dry bean crop. These results are valuable to conventional, organic and low-input or low-resource farmers as they seek to maximize production while reducing the cost of N fertilizer.

Dedicated to my

daughter Jemima Baributsa,

son Dieubeni Baributsa,

wife Lydia Munyantore, and

mother Elina Nyirabagenzi.

In memory of my

father Aaron Baributsa Muhire

and

brother-in-law Twenge Munyantore

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Literature Review

Introduction

There is a growing interest in cover crops and green manure in both developing and developed nations due to cost and availability of agricultural inputs, and concerns about environmental pollution. Cover crops can provide ground cover, increase soil organic matter and water infiltration, reduce soil erosion, increase the population of soil microbes, suppress weeds, and reduce insect pests and diseases (Sarrantonio, 1994; Dinesh et al., 1999; Peachy et al., 1999; Ross et al., 2001; Smeltekop et al., 2002; Hendrickson, 2003). Cover crops are classified as leguminous and non-leguminous. The major advantage of legume cover crops compared to grass cover crops is the nitrogen (N) contribution to the soil through symbiotic fixation. In contrast, some non-legume cover crops are used to scavenge soil N that might be lost due to leaching and are effective in increasing soil organic matter by supplying C through increased biomass production (Sainju et al., 2000; Chambliss et al., 2003; Snapp et al., 2005). Legume cover crops may convert up to 227 kg of atmospheric N ha⁻¹ year⁻¹ and can provide all or part of the N needed by the subsequent crop (Cavigelli, 1998; Griffin et al., 2000; Diver et al., 2001). Legume cover crops can influence carbon (C) and N mineralization, wet aggregate stability, and light fraction¹ which can be used to estimate soil organic matter (Biederbeck et al., 1998).

¹ Soil organic matter quality can be estimated by the characterization of a light fraction. The light fraction is material that will float on the top of dense salt solution. The light fraction is generally considered to be material rich in plant nutrients, relatively large in size compared to other soil organic matter components and insoluble in water.

Studies have been conducted to assess the effect of monocropping or interseeding legume cover crops on subsequent corn (*Zea mays* L.) yield (Jeranyama et al., 1998; Hively and Cox, 2001; Abdin et al., 1998). Research has shown that yield of corn following a cover crop was higher compared with where a cover crop had not been established (Vyn et al., 1999; Balkcom and Reeves, 2005). Most of these studies have looked at the effect of cover crop and/or various rates of fertilizer on succeeding corn yield. However little or no research has focused on the effect of cover crop versus N fertilizer on corn yield and quality at various corn densities. Similarly, no research has looked at the effect of interseeded red clover (*Trifolium pratense* L.) in various corn densities on subsequent dry bean yield and quality.

Cover crops can be intercropped/interseeded or used in crop rotation as the sole crop. Incorporating a cover crop through rotation is not economically viable in many developing countries because of the loss of income from that field for an entire growing season. In many developing countries of the tropics, land constraint and economics are factors limiting the inclusion of cover crops into the rotation system. Interseeding is an alternative to rotating cover crops with cash crops. Interseeding presents the advantage of producing two or more crops on the same land at the same time, while diversifying food supply. However, recent interest in incorporating cover crops into cropping systems through interseeding has revealed some production or management challenges. Among the challenges are the choice of cover crops (some do not perform well because they are not shade tolerant), growth habit (some are annual and cannot be used late

in the fall for biomass production), time of interseeding (when interseeded early, cover crops can compete with the main crop; interseeded late, cover crops do not perform well) and crop density in interseeding system (not knowing the right crop density for intercropping). Some of these challenges have been studied extensively and others have not. For example, it is well documented that red clover is shade tolerant and could be used in an intercropping system with dense canopy crops such as corn (Bowman et al., 1998; Sattell et al., 1998; Peet, 1995). Studies have been conducted on the time of cover crop establishment in corn. Thompson and Wagner (2000) obtained satisfactory results when interseeding red clover and nitro alfalfa (*Medicago sativa*) when they were established after the second cultivation of the corn crop. Mutch and Martin (1998) suggested that cover crops should be interseeded into corn between V4-V6 growth stages. However, other factors such as density of the main crop in an interseeding system with a cover crop have not been addressed.

Planting corn in a plowed legume cover crop at various corn densities can help to understand how high corn density can be increased without using N fertilizer in the production system. Interseeding cover crops into corn at various corn densities can help assess cover crop growth, biomass and N content during and after the growing season. Planting dry bean (*Phaseolus vulgaris*, L.) after interseeded cover crops can help assess cover crop effect on dry bean yield and quality.

Interseeding system

Interseeding is a system of producing various crops in the same area at the same time. The interseeding system has been used by low-input, small-scale agricultural systems for many years (Andrews and Kassam, 1976; Foulds, 1987; Vandermeer, 1992). In many developing countries, the lack of agricultural inputs such as mineral fertilizer and the reduction of farm size due to population growth are among factors that have increased the use of intercropping. Further advantages of interseeding are a diversified food supply and a more equal distribution of the field work during the cropping season. Interseeding helps to better utilize farm labor, time, and equipment since plants have different management requirements in term of planting and harvesting time. In the low input agriculture of the tropics, food crops are mainly intercropped to reduce risk in crop failure caused by unfavorable weather conditions as well as pests and diseases (Trenbath, 1993; Carruthers et al., 1998; Sauerborn et al., 2000; Maluleke et al., 2005). In developed countries, intercropping food crops has received less attention from farmers and scientists due to difficulties of planting and harvesting two different food crops (Hartwig and Ammon, 2002). However, in recent decades the search for crop management practices that increase sustainability of natural resources (reduce environmental pollution) is one of the driving forces for the use of interseeding (Baumann et al., 2002).

Intercropping adds a spatial diversity to species across the field. In various farming systems, intercropping has been used to optimize crop use of water, nutrients and sunlight (Ghaffarzadeh, 1999; Sullivan, 2003a). In an intercropping

study, Zhang and Li (2003) showed that N uptake by intercropping of wheat (*Triticum aestivum*) and corn was greater than in sole cropping under the same N supply. Furthermore, corn improved the uptake of iron by peanut (*Arachis hypogaea*), and faba beans (*Vicia faba*) enhanced N and P uptake by intercropped corn. Intercropping is more advantageous than monocropping due to less competition for resources between plants within the same species (Sullivan, 2003a). Plants with different architectures (plant height: tall vs. short, rooting system: deep vs. shallow, canopy density: dense vs. light, and crop cycle: long vs. short maturity) are used for their potential complementarity in sunlight and water utilization to maximize crop production.

To achieve complementarity and reduce competition between intercrops, four things need to be considered: spatial arrangement, plant density, maturity dates, and plant architecture (Kantor, 1999; Akunda, 2001; Beuerlein, 2001; Sullivan, 2003a).

a. Spatial arrangement:

In intercropping systems, crops can be arranged in three different ways:

- **Row intercropping:** growing two or more crops at the same time with at least one crop planted in rows.
- **Strip intercropping:** growing two or more crops together in strips wide enough to permit separate crop production using machines, but close enough for the crops to interact.
- **Mixed intercropping:** growing two or more crops together in no distinct row arrangement.

b. Plant density:

In intercropping systems, crop density is reduced from its full rate. Reducing the plant density decreases competition and increases output of both crops when compared to growing the same crops in monoculture on the same area of land. In comparing sorghum (*Sorghum bicolor*) and soybean (*Glycine max*) yield in sole and intercropping systems at various densities, Akunda (2001) found that recommended crop density had the highest yield with intercropping, whereas sole crops had highest yield at the highest crop density. The challenge comes in knowing the optimum plant density for companion crops.

c. Maturity dates:

Intercrops with different maturity dates present the advantage of variations in resource demands for nutrients, water, sunlight and labor. Competition is lessened when one crop matures before its companion crop. Competition between corn and legume cover crops is reduced when cover crops are interseeded at corn laybay, approximately four weeks after planting corn (Bowman et al., 1998; Jeranyama et al., 1998; Mutch and Martin, 1998). Interseeded cover crops grow slowly under corn and then rapidly as more light is available when corn dries down and after harvest.

d. Plant architecture:

Plant architecture between and among species has to be considered to reduce competition for better use of nutrients, water and sunlight. For example using a legume and cereal is the common practice to maximize the use of energy for crop production, since both have different nutrient requirements. The goal is to

capitalize on the beneficial interactions between crops while avoiding negative interactions. The Land Equivalent Ratio (LER) is used to measure the effect of both positive and negative interactions between crops. The LER compares the yield from growing two or more crops together with yield from growing the same crops in monoculture. Intra and inter-species competition in the interseeding system may affect yield and crop quality of companion crops (Baumann et al., 2001; Blackshaw et al., 2001; Sullivan, 2003a).

The success of any intercropping system depends on the balance of positive and negative interactions between crop components. Interseeding can provide a significant management challenge to both conventional and organic growers due to different management practices and the economics related to each system. Incorporating cover crops in crop production might require a change in the cropping system. For instance, planting a cover crop into an established corn crop in June or late in fall, may delay the planting of the succeeding crop in order to allow greater cover crop biomass accumulation in the following spring. This may require planting a short season crop such as vegetables instead of field crops. Interseeding may also present the challenge of cover crop establishment and weed control in conventional and organic farming. Herbicide use in conventional systems may hinder the germination of the cover crop. For example, it is not recommended to establish a clover stand in corn treated with a preemergence broadleaf herbicide such as Atrazine (Knorek and Staton, 2004). In organic production, mechanical weed control after the establishment of the cover crop may be the most pertinent issue. Another

disadvantage is the timing of establishment. If established early, the cover crop can compete with the main crop for nutrients and moisture, and if planted too late, the main crop may limit the growth of the cover crop (Stute, 2000; Miles and Nicholson, 2003).

Cover crops

Cover crops are grown to provide ground cover, whereas green manures are grown for soil improvement purposes and incorporated while they are green or soon after they flower. Cover crops should exhibit as many positive good characteristics as possible, including fast germination and emergence, competitiveness with weeds rather than the major crops, tolerance to adverse climatic and soil conditions (drought), easy suppressibility, fertility benefits and low cost of establishment (Roos, 2006). Weed reduction by cover crops can be directly proportional to cover crop growth and canopy production, as shown by Fisk et al. (2001), who suggested that the quantity of ground cover produced by weeds was inversely proportional to that produced by the crops. Cover crops are grown primarily to prevent or reduce soil erosion. In crop production, cover crops may be selected to maximize benefits of biomass and N production.

Cover crops are used also for other purposes, either as catch crops or smother crops. When used as catch crops, cover crops are established after harvesting the main crop and are used to reduce nutrient leaching from the soil profile, primarily nitrate (McLenaghan et al., 1996; Sullivan, 2002; Weinert et al., 2002; Kristensen and Thorup-Kristensen, 2004). Legumes are not as effective as

non-legumes in sequestering and recycling soil inorganic N and should be grown where little inorganic N is left in the soil (Sainju et al., 1998; Isse et al., 1999; Sainju et al., 2000; Thorup-Kristensen et al., 2003). However, in a study on sequestering residual NO₃ by cover crops, red clover was as effective as rye (*Secale cereale*, L), oilseed radish (*Raphanus sativus* [L.] var *oleiferus* Metzg [Stokes]) and oat (*Avena sativa* L.), suggesting that N management in the corn cropping sequences could be improved by intercropping red clover (Vyn et al., 2000). A catch crop is usually planted a few weeks before or just after the main crops is harvested. Planting cereal rye following corn harvest helps to scavenge residual N, thus reducing the possibility of groundwater contamination (Brooks et al., 2006). In this instance, the rye catch crop also functions as a winter cover crop. When used as a smother crop, cover crops control weeds (Ross et al., 2001; Miles and Nicholson, 2003). These cover crops are selected based on the ability to compete with weeds, good seed germination and good plant vigor. Cover crop ability to control weeds varies from one species to another (Ross et al., 2001). Various studies have shown that cropping systems using annual cover crops and red clover can significantly reduce weed dry weight and density (Singer et al., 2000; Fisk et al., 2001; Ross et al., 2001). Fitting a cover crop into a crop production system can be challenging. Important cover crop characteristics are fast-growth, drought-tolerance, shade tolerance and minimal management requirement. Species with the potential to reduce pest populations are chosen, while those that harbor diseases or arthropod pests of the cash crops are avoided. In a study conducted in the U.K, red clover was successfully

used as a mean to reducing pest damage on winter wheat (Brooks et al., 2006).

In Canada, Studies have shown that the use of legume cover crops that are resistant to grasshoppers could reduce the overall need for insecticides because these crops will not likely become epicenters of grasshopper outbreaks (Olfert et al., 1995).

A cover crop may be grown in a pure stand or mixed with other crops during all or part of the year. It may be a fallow cover crop, winter cover crop, summer green manure crop, living mulch, catch crop or a forage crop (Diver et al., 2001). Specific terms have been used to refer to the incorporation of cover crops into cropping systems (Agboola, 1982; Davis, 1997; Nafziger, 2002).

- *Double-cropping*: also known as sequential cropping, refers to planting a second crop after the first crop is harvested.

- *Intercropping*: also known as interseeding or underseeding is growing two or more crops together on the same field at the same time.

- *Monocropping, monoculture or sole cropping*: is growing a cover crop as a single crop in a field.

- *Relay intercropping*: refers to the planting of second crops when the standing crop is at its reproductive stage.

- *Living mulch*: is a system where the cover crop is established prior to row crop establishment and the row crop is established directly into all or a portion of the suppressed or actively growing cover crop species. A living mulch is a cover crop that is intercropped with an annual or perennial cash crop.

- ***Alley cropping***: Alley cropping is the planting of trees or shrubs in two or more sets of single or multiple rows with agronomic, horticultural, or forage crops.

Cover crops may be annual, biennial, or perennial herbaceous plants.

a. Annual cover crops:

They complete their life cycle in one growing season and are divided into summer and winter annuals.

- ***Summer annual cover crops:***

These cover crops are established during a portion of the summer growing season and include oats, sorghum-sudan grass (*Sorghum sudanense*), field peas (*Pisum sativum*), sweet clover (*Melilotus spp.*), velvet bean (*Mucuna pruriens*) and buckwheat (*Fagopyrum esculentum* Moench). Summer annual cover crops germinate and mature without a cold snap and usually tolerate warm temperatures (Bowman et al., 1998; Creamer and Baldwin, 1999). Warm-season cover crops can be used to fill a niche in crop rotations, to improve the conditions of poor soils or to prepare land for a perennial crop (Sullivan, 2003b). Legume cover crops such as cowpeas (*Vigna unguiculata*), annual sweet clover, or velvet beans may be grown as summer green manure crops to add organic matter along with N. Non-legumes such as sorghum-Sudan grass, Pearl Millet (*Pennisetum glaucum*), or buckwheat are grown to provide biomass, smother weeds, and improve soil tilth (Sullivan, 2003b). Since in the temperate climate most of summer annual cover crops are killed by frost, no herbicide control is needed the following spring except for buckwheat, which needs to be killed in a timely manner to prevent it from shedding seed.

- Winter Annual cover crops:

Winter annual cover crops include cool-season legume such as some clovers, vetches, medics (*Medicago spp.*), and field peas and non-legumes such as rye, wheat, barley (*Hordeum vulgare*), and ryegrass (*Lolium multiflorum*). Winter annual cover crops are more cold-tolerant and require cold temperature to set seed (Bowman et al., 1998). These winter cover crops are selected for their tolerance to cold and are planted in late summer or fall to provide soil cover during the winter. Cereal cover crops such as winter rye and winter wheat are used as cover crops to allow the production of biomass for ground cover and to absorb excess nitrate from the soil (Sullivan, 2003b; De Bruin et al., 2005). Some cereal cover crops can be incorporated, mowed or harvested for silage. If cover crops are used as mulch, they will provide soil moisture conservation in case of water stress during the growing season. If drought conditions occur directly before crop establishment, there is a risk of crop water stress due to depletion of soil moisture by the cover crops. The major benefits of a winter cover crop are soil structure improvement, soil protection in the winter, reduced nitrate leaching and the addition of organic matter and N in the case of legumes.

b. Biennial cover crops:

A biennial cover crop grows vegetatively during its first year and then sets seed during its second year (Bowman et al., 1998). Biennial cover crops include legumes such as sweetclover. Red clover is a perennial that acts like a biennial. Annual ryegrass has a biennial tendency in cool regions because it will regrow quickly and produce seed in late spring if it over-winters (Bowman et al., 1998).

c. Perennial cover crops:

Perennial cover crops are usually used in perennial crops such as orchards to provide permanent covers (Hartwig and Ammon, 2002). They provide excellent weed control and serve as a food source as well as refuge for beneficial insects. There are various types of perennials, including short-lived perennials and long-lived perennials. Red clover, which is a short-lived leguminous perennial, can be established in summer, fall or frost-seeded late in the winter. Red clover can be killed in the spring of the following year by tillage or herbicides. White clover (*Trifolium repens* L.), a longer-lived leguminous perennial that grows more slowly than red clover, is often used as living mulch in vegetable systems (Bowman et al., 1998). Other leguminous perennial cover crops include perennial forages such as Kura clover (*Trifolium ambiguum* L.) and alfalfa that are used in annual crops to enhance the survival and improve the efficiency of natural enemies of pests (Schmidt et al., 2004).

Biennial or perennial legumes can fit many different niches, sometimes grown for a short period between cash crops. They can be interseeded into other crops and left to grow after cash crop harvest and used as forage. In this case they are functioning more as a rotation crop than a cover crop but are providing many benefits, such as erosion and weed control, organic matter and N production. They also can break weed, disease and insect cycles. Deep-rooted biennial and perennial legume cover crops are not recommended for the most severely drought-prone soils, as their excessive use of soil moisture will negatively affect yield of the following crops (Biederbeck et al., 2000).

Red clover

Red clover has high cold tolerance, good N fixing capabilities, and good shade tolerance (Bowman et al., 1998). Sattell et al. (1998) have grouped red clover in two varieties/cultivars based on early flowering or late flowering types: Mammoth red clover and Medium red clover. '*Mammoth Red clover*' is one of the most common late flowering, or single cut, varieties and is used at high elevations or where the growing season is short. It is a winter-hardy perennial that grows in a round clump without flowering stems the first year. '*Medium Red clover*' is an early flowering type or double cut (because it can be cut several times in a year for hay). It produces tall, erect flowering stems with leaves at the nodes the spring after it is planted. Although they are perennials, early flowering red clovers most often are treated as winter annuals (turned under or killed in spring) when used as a cover crop. In Michigan, Knorek and Staton (2004) classify red clover into three common cultivars: Michigan mammoth, Canadian mammoth (also known as Altaswede clover) and medium red clover. Their research suggests that Michigan mammoth performs better than the other red clovers when frost-seeded into well-fertilized wheat. Canadian mammoth clover does not tolerate the increased shading and competition from well-fertilized wheat.

While red clover has low to moderate drought tolerance, it does not tolerate flooded soil. Red clover can be planted in spring, summer and fall. Generally, red clover is frost-seeded in small grains like wheat by the middle of March. Red clover may also be sown in early summer, usually intercropped with

a cash crop. Red clover seedlings are very competitive with weeds. In fall, red clover can be established before or after the harvest of the cash crop.

Traditionally, red clover is drilled at 9-14 kg ha⁻¹ and overseeded at 11-20 kg ha⁻¹ (Bowman et al., 1998). In Michigan, the recommended red clover seeding rate is 11-13 kg ha⁻¹ (Knorek and Staton, 2004), whereas Sattell et al. (1998) suggest 17- 28 Kg ha⁻¹ for Oregon. Early seeding gives best stands, and if red clover is allowed to go to seed, it will produce enough seed to re-establish itself.

For plow-down, red clover can be killed in the fall or the spring by tillage or herbicide application. Timing is important when killing red clover in the spring. Red clover should be allowed to grow as long as possible in the spring to add additional nutrients to the soil and suppress weeds, but it can also use up soil moisture and hurt the following cash crop if dry conditions exist. Red clover fixes N and releases it slowly to the following crop. Research results have shown that red clover can provide all or most of the N needed by a subsequent corn crop (Vyn et al., 1999; Hively and Cox, 2001). Red clover can produce 2-3 tons of biomass, can accumulate 79-168 kg ha⁻¹ of N, and also can increase microbial activity and accelerates the decomposition of surface crop residues (Doran et al., 1987; Bowman et al., 1998; Drury et al., 1999).

Chickling vetch (*Lathyrus sativum* L.)

Chickling vetch (grass pea, guaya in Ethiopia, Keshari in India, garbanzo in Venezuela, pois carré in France, etc.) is an annual legume crop grown in different parts of the world (Campbell, 1997; IPBO, 2006; Small, 1999) as food

and sometimes as animal fodder or green manure. In North America, chickling vetch is used primarily as a green manure alternative to summer fallow in small grain production systems to reduce wind and water erosion and to improve soil (Small, 1999). Chickling vetch seeds have a protein content of 25-28% (Bellido, 1994). In the past decades, chickling vetch has received more attention as a multi-use crop in arid regions, because it is drought tolerant and adaptable to marginal soils (Biederbeck et al., 1993; Biederbeck and Bouman, 1994; Campbell, 1997).

Although chickling vetch is rich in protein and improves soil N, it contains significant amounts of an anti-nutritional compound β -N-oxalylamino-L-alanine, also known as β -N-oxalyl-L- α , β -diaminopropionic acid or ODAP (Chen et al., 2000; Grela et al., 2001). There are three categories of ODAP concentration in *Lathyrus spp*: low=0.06%, grass pea; medium=0.2 to 0.3%, chickling vetch; and high=1.0%, Indian varieties (Klassen, 2002). The scientific community speculates that ODAP probably confers resistance to pests or climatic extremes (Raloff, 2000). The ODAP is a neurotoxin amino acid known to cause muscular rigidity, weakness, and paralysis of the leg muscles (Munro, 2003). Soaking and heating before cooking are the two methods used to detoxify chickling vetch seeds in order to help reduce the risk of the neurotoxin (Raloff, 2000). When used as forage, the hay should be removed before it sets seed because it contains the neurotoxin and may cause problems if fed in large quantities to cattle over an extended period of time (DFS, 2003). Canadian plant breeders at the International Center for Agricultural Research in the Dry Areas (ICARDA) have

concentrated their efforts on developing low-ODAP cultivars. In recent years, a low-ODAP variety of chickling vetch called 'AC-Greenfix' was developed and released by the Semiarid Prairie Agricultural Research Centre (SPARC) in Swift Current, Saskatchewan, Canada (DFS, 2003). Agriculture and Agri-food Canada, which has been very active in developing low toxin varieties, has recommended that low toxin type be referred to as grass peas while high toxin types be referred to as chickling vetch (Klassen, 2002). AC Greenfix's low ODAP content, ability to overcome the problems of soil moisture depletion and to fix high amounts of N has led to its increased use and reduced the use of other more traditional green manures (Krause and Krause, 2003). AC Greenfix can produce 2242 to 4483 kg ha⁻¹ of dry matter (DFS, 2003). Pauly (2004) reported that chickling vetch grown in dry land areas produced enough N to meet the requirements of a subsequent cereal crop.

Various management practices have been suggested for maximum benefit from the cover crop (DFS, 2003). AC Greenfix planted in spring yields best results because it can tolerate temperatures as low as -6 to -3° C. Fall planting in August when moisture is sufficient or with adequate watering can also yield satisfactory results. AC Greenfix grows slowly for the first 30-40 days, with plants in full bloom by about 60 days. For maximum N production, the plants should be plowed under before pods begin filling, about 40-60 days after planting. The seeding rate for AC Greenfix is 80 kg ha⁻¹. AC Greenfix grows best and produces greater dry matter when planted in N-depleted soil (DFS, 2003). It should be clipped before seedpods begin to fill and will regrow if moisture is sufficient.

Because AC Greenfix is such a short season crop, it could fit in various cropping systems to provide N and organic matter.

Cover crop adoption and management constraints

Optimal success with cover crops depends on species selection, proper management techniques, and a good understanding of the agro-ecosystem (Roos, 2006). Cover crops present potential negative challenges related to cost of establishment (seed cost, seeding and killing methods), depletion of soil moisture, reduction of spring soil temperatures, allelopathy, and habitat for pests and disease (Ewing et al., 1991; Roos, 2006). Farmers will increase adoption or use of cover crops only if these negative aspects are minimized.

Seed cost, method of seeding, and method of killing the cover crop should be considered. The benefits of cover crops need to be assessed in terms of cash returns as it relates to a reduced need for inputs (mineral fertilizer, pesticides, etc.) as well as the long-term impact on soil improvement (soil structure, organic matter, etc.). The benefit of the cover crop use has to consider cover crop seed cost and establishment in comparison to N fertilizer cost reduction and crop yield and quality. Soil moisture is another critical factor when considering cover crop use. All cover crops require water for good growth; however, some cover crops such as AC Greenfix use less water than others (Biederbeck et al., 1993; Biederbeck and Bouman, 1994). Soil moisture use by cover crops is a concern especially in areas with less precipitation in the spring before the establishment of the main crop. Abdin et al. (1998) reported that crimson clover (*Trifolium*

incarnatum L.) competed with corn when the moisture was limiting. Blackshaw et al. (2001) showed soil water content reduction by sweetclover at the time of seeding spring wheat compared to tilled fallow. The earlier the cover crop is sown in the fall or the longer it is allowed to grow in the spring, the more water it will use (Ingels et al., 1996).

Additional management is required when cover crops are added to a cropping system. Turning cover crops under or suppressing them requires additional time and expense compared to having no cover crop at all. If a cover crop is not winter-killed, other methods such as mechanical or chemical control should be considered before the crop competes with the next cash crop. For plow down, herbicide use is necessary where tillage does not provide full control of the cover crop. Some cover crops need to be killed at a certain time in their life cycle to ensure that they do not set seed and become a weed problem in future years. For example, hairy vetch (*Vicia villosa* Roth) is not a good cover crop to use when small grains are included in the rotation. If the vetch ever goes to seed, it can become a terrible weed in a small grain crop (Sullivan, 2003b).

Some cover crops produce chemicals that can hinder weed germination and/or crop growth (Ohno and Doolan, 2001; Dhima et al., 2006). Several species of cover crops can affect the cash crop if the cash crop is planted too soon after the cover crop is plowed down. Cover crops with allelopathic properties can reduce the germination or establishment of the cash crop, and a large amount of dry matter left by non-legume cover crops can tie up N needed by the succeeding cash crop (Hamilton, 1998). To minimize yield reduction of

corn following winter-hardy cereal grain cover crops, it is recommended to terminate the cover crop more than 14 days prior to corn planting and use starter fertilizer (Singer and Kaspar, 2006). Duiker and Curran (2005) found that with adequate N, planting corn 7 to 10 days after killing rye does not reduce corn yield. In a study conducted in the USA, Blacksburg, Virginia, Vaughan and Evanylo (1998) found that corn yields were reduced due to N immobilization when rye biomass increased significantly. Cover crop residues can also lead to cooler soils in the spring.

Influence of legume cover crop on crop yield and N content

When used in monocropping or interseeding, legume cover crops can improve overall soil quality and contribute to yield improvement of the succeeding crop (Jeranyama et al., 1998; Vyn et al., 2000; Hively and Cox, 2001; Balkcom and Reeves, 2005). The use of cover crops such as annual medics can significantly reduce weed density and dry weight and thereby its incidence on the following crops (Fisk et al., 2001). Teasdale and Daughtry (1993) reported weed density and biomass reduction by hairy vetch compared with a fallow treatment. A leguminous cover crop interseeded with a row crop such as corn can supply N for the subsequent crop. Bruulsema and Christie (1987) found that yield of corn following red clover and alfalfa was comparable to yield obtained by application of 90 to 125 kg of N ha⁻¹. Yield of a succeeding corn crop was increased following intercropped legume cover crops compared to continuous corn. Interseeded legume cover crops reduced fertilizer needs of the subsequent corn

crop by 18 to 36 kg ha⁻¹ (Jeranyama et al., 2000). Corn following interseeded medium red clover and Dutch white clover produced greater yield compared to corn following no cover crop or rye seeded after soybean [*Glycine max* (L.) Merr.] harvest (Hively and Cox, 2001).

Few research studies currently exist on dry bean, following a cover crop. Some studies have been conducted to evaluate the effect of cover crops on a subsequent snap bean crop. Yield of snap beans following a legume cover crop was similar to yield of beans supplied with 90 kg ha⁻¹ of N fertilizer (Skarphol et al., 1987). Furthermore, research conducted in Maryland found that bean following cover crops required less N in some cases (Peet, 1995). Beans following hairy vetch, Austrian winter pea and crimson clover covers did not respond to additional N, whereas bean succeeding wheat and wheat/legume mixes needed additional N to achieve their highest yield. Bean yield in cropping systems with cover crops were higher compared to those grown without cover crops, particularly in the drier year of the experiment. However, in an investigation of the effect of conventional system, rye and hairy vetch cover crops on snap bean, Mwaja et al. (1996) found snap bean yield was higher in the conventional management. An investigation of the impact of interseeded cover crops in various corn densities on dry beans yield is critical to assess their effects on bean yield and quality.

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Chapter One

Corn and Cover Crop Response to Corn Density in an Interseeding System

Abstract

Reliable cropping strategies are needed to enhance legume cover crops utilization as a nitrogen (N) source for crop production. Interseeding legume cover crops into corn (*Zea mays* L.) can affect corn yield and cover crop dry matter. This study evaluated (1) the effect of corn density (37 500 to 75 000 plants ha⁻¹) on corn yield and cover crop dry matter when corn was interseeded with red clover (*Trifolium pratense* L.) or AC Greenfix (*Lathyrus sativus* L.) and (2) the impact of N source [fertilizer vs. plowed red clover] on corn yield at various corn densities.

Four-year data suggest that interseeded cover crops did not affect corn yield at any corn density. Overall, yield of corn planted into plowed red clover was similar to corn yield supplied with N fertilizer. Interseeded cover crop dry matter (DM) decreased as corn density increased. However, red clover DM the subsequent spring was similar regardless of corn density. Interseeded cover crops produced less DM compared with monoculture cover crops. Results show that cover crops can be interseeded into corn densities up to 75 000 plants ha⁻¹ without corn yield reduction and still produce substantial dry matter the subsequent spring regardless of corn density.

Introduction

In recent decades, farmers in the temperate regions have increasingly been interested in management practices that maintain soil productivity and environmental quality and improve farm profitability (Baumann et al., 2001). Interseeding legume cover crops has been investigated as one way to achieve these goals (Scott et al., 1987; Mutch and Martin, 1998; Smeltekop et al., 2002; Brooks et al., 2006). Legume cover crops may be used in an interseeding system to increase nutrient cycling, weed suppression, or enhance cropping-system diversity (Sarrantonio, 1994; Diver et al., 2001; Oswald et al., 2002; Mutch et al., 2003). Interseeding a legume cover crop into wheat (*Triticum aestivum* L.) and oats (*Avena sativa* L.) is common (Hesterman et al., 1992; Ross et al., 2005; Singer et al., 2006). Similarly, cover crops have been interseeded into corn (*Zea mays* L.), cassava (*Manihot esculenta*, L), sorghum (*Sorghum bicolor*) and cabbage (*Brassica oleracea*) (Some et al., 1992; Bellinder et al., 1996; Jeranyama et al., 1998; Chikoye et al., 2001; Khan et al., 2002).

When cover crops are well established, interseeded cover crops can reduce weed growth and density (Liebman and Dyck, 1993; Singer et al., 2000). Mutch et al. (2003) suggested that the major advantage of frost-seeding legume cover crops is ragweed (*Ambrosia artemisiifolia*) suppression, no reduction of companion wheat yield, and the ability to supply nitrogen (N) to the subsequent crop. Various studies have been conducted to assess the effect of monocropping or interseeding legume cover crops on subsequent corn yield. Yield of corn following intercropped legume cover crops was higher compared to continuous

corn (Jeranyama et al., 1998). Corn following interseeded medium red clover (*Trifolium pratense* L.) and Dutch white clover (*Trifolium repens* L.) produced greater yields compared with corn following no cover crop or rye (*Secale cereale*, L.) seeded after soybean [*Glycine max* (L.) Merr.] harvest (Hively and Cox, 2001).

In evaluating corn response to a preceding cover crop, Vyn et al. (1999) found that corn yield was consistently higher following red clover compared with oilseed radish (*Raphanus sativus* [L.] var *oleiferus* Metzg [Stokes]), annual ryegrass (*Lolium multiflorum*), and where a cover crop had not been established. Balkcom and Reeves, (2005) observed that corn yield following sunn-hemp (*Crotalaria juncea* L.) with no additional N fertilizer was greater than corn yield planted in fallow and supplied with 56 kg N ha⁻¹. Planting mucuna [*Mucuna pruriens* (L.) D.C.] and pigeon pea (*Cajanus cajan* L.) after corn reduced N and P fertilizer needs in the subsequent year and increased corn grain yield by 37.5 and 32.1%, respectively (Sogbedji et al., 2006). In Michigan, corn yield following interseeded medics (*Medicago polymorpha* and *M. scutellata* L.) was higher compared with corn yield without medic (Jeranyama et al., 1998). These studies investigated the effect of cover crop and/or various rates of fertilizer on succeeding corn yield. However, little or no research has examined the effect of cover crop versus N fertilizer on corn yield at various corn densities.

The success of any intercropping system depends on the balance of positive and negative interactions between companion crops. Various factors play a key role in the interseeding system, including companion crop species,

time of interseeding, crop density, and cover crop species. When grown together, cash crops and cover crops compete for nutrients, water and light.

Scott et al. (1987) observed no reduction in corn yield when it was interseeded with various cover crops. Kura clover (*Trifolium ambiguum* M. Bieb.), with adequate suppression, can be managed as living mulch in corn with little or no grain yield reduction (Zemenchik et al., 2000). Several studies have suggested that competition in an interseeding system is determined by the time of interseeding, while others suggest it is determined by the cover crop species that is used. Research results have shown corn yield reduction when cover crops were interseeded at corn planting (Exner and Cruse, 1993; Jeranyama et al., 1998). In West Africa, Sogbedji et al. (2006) found that relay intercropping mucuna and pigeon pea into a maize crop does not cause maize grain yield loss if established 50 to 60 days after corn planting. No reduction in corn yield was reported when cover crops were interseeded 28 days after corn planting or between corn growth stages V4 and V6 (Jeranyama et al., 1998; Mutch and Martin, 1998). In contrast, Abdin et al. (1998) suggested that corn yield was affected by cover crop species and not by the time of interseeding. Increased plant density in an interseeding system can increase competition of companion crops (Ross et al., 2003). To increase output and reduce competition between companion cash crops in an interseeding system, plant density may be reduced (Akunda, 2001). However, when interseeding cash crops and small seeded cover crops such as red clover, cover crop density may be increased (Bowman et al., 1998). Since cover crop full growth in interseeding systems is after harvest of the

cash crop, it would be interesting to know the impact of increased plant density of the cash crop on the growth of the cover crop. Another challenge is knowing the optimum plant density for companion crops (Blaser et al., 2006).

Cover crop species and cultivars within species differ in their ease of establishment in an interseeding system (Singer et al., 2006). In intercropping seven legumes of *Medicago* spp, Alford et al. (2003) observed that only Black medic (*M. lupulina* L.) did not reduce corn yield. All the other cover crops significantly reduced corn yield. Crimson clover (*Trifolium incarnatum* L.) accumulated enough biomass to produce higher corn yield compared with other legume cover crops due to its ability to tolerate shade (Freeman et al., 2000). Cover crop growth, N accumulation and availability to a succeeding crop can be affected by environmental factors such as precipitation, temperature, length of growing season, and soil productivity (Hesterman et al., 1992; Dekker et al., 1994 ; Stute and Posner, 1995; Singer et al., 2006). Red clover, a common cover crop in Michigan, is used in several interseeding systems, because of its ease of establishment and shade tolerance (Mutch and Martin, 1998; Bowman et al., 1998). When interseeding various cover crops into corn, Thompson and Wagner (2000) recommended mammoth red clover and nitro alfalfa (*Medicago sativa*) because they were the easiest to establish and showed the most vigor. Rye and hairy vetch (*Vicia villosa* Roth) did not perform as well. Singer et al. (2006) observed greater dry matter with red clover diploid compared with tetraploid cultivars.

Red clover is used in various cropping systems (Hesterman et al., 1992; Mutch and Martin, 1998). When compared to alfalfa, black lentil (*Lens culinaris* Medik. subsp. *culinaris*) and chickling vetch (*Lathyrus sativus*, L) in relay and double cropping systems, red clover produced the most above ground dry matter and had the fastest growth rate (Martens et al., 2001). Interseeding a shade tolerant cover crop like red clover can result in more rapid establishment of the cover crop after the cash crop is harvested and extend the growing period for the cover crop. When left in the field after corn harvest, red clover may provide ground cover during the fall and spring, and supply N to the subsequent crop. A greater understanding of cover crop growth and performance in various plant densities is critical to assessing the potential for the use of cover crops in various management systems.

Chickling vetch, an annual legume crop, is grown in different parts of the world as food and sometimes as animal fodder or green manure (Campbell, 1997; IPBO, 2006; Small, 1999). In past decades, chickling vetch has received more attention as a multi-use crop in arid regions, because it is drought tolerant and adaptable to marginal soils (Biederbeck et al., 1993; Biederbeck and Bouman, 1994; Campbell, 1997). Chickling vetch seeds have a protein content of 25-28% (Bellido, 1994). AC Greenfix, a variety of chickling vetch, is used in North America primarily as a green manure alternative to summer fallow in small grain production systems to reduce wind and water erosion, and to improve soil (Small, 1999). AC Greenfix has a seeding rate of 80 kg ha⁻¹ and the potential to produce 90-112 kg of N ha⁻¹ in 8-10 weeks after planting (DFS, 2003). A Study of AC

Greenfix in several locations across southwest Saskatchewan, found AC Greenfix forage yields averaged 2590 kg ha⁻¹ with a crude protein content averaging 19.63% (Biederbeck, 2005). Other studies by Rao et al. (2005) have shown that at full bloom (75 days after planting), AC Greenfix produced an average of 6415 kg ha⁻¹ of DM compared with only 2013 kg ha⁻¹ for lentil (*Lens culinaris* Med. cv. Indianhead). In evaluating cover crops in relay and double cropping, AC Greenfix was ranked second to alfalfa when comparing the fertilizer replacement value for oat following various cover crops (Martens et al., 2005). Various management practices have to be considered for maximizing AC Greenfix potential, including plowing it under before pods begin filling, about 40-60 days after planting. Because AC Greenfix is such a short season crop it could fit in various cropping systems to provide N and organic matter. Since cover crop performance and dry matter production varies from one region to another, between species and with crop management, research on AC Greenfix will help to assess its performance, in comparison to red clover, in the interseeding systems in Michigan.

As part of an on-going research effort in Michigan on incorporating cover crops into corn production, studies have been conducted on cover crop establishment in corn and wheat to investigate time of planting and performance of various cover crop species and cultivars (Jeranyama et al., 1998; Mutch and Martin, 1998). Interseeded cover crops appear to provide many benefits to crop production systems, but a greater understanding of establishment and species differences is needed in order to realize these potential benefits.

The objectives of this study were to assess the effect of (i) corn density in an interseeding system on corn yield and on red clover or AC Greenfix dry matter and (ii) nitrogen fertilizer versus nitrogen provided by plowed red clover on corn yield at various corn densities.

Materials and Methods

Site description

The research was conducted from 2002 to 2005 at the Kellogg Biological Station (KBS) in Hickory Corners, Michigan. The soil types at KBS were the Kalamazoo (fine-loamy, mixed, mesic Typic Hapludalfs) and Oshtemo (coarse-loamy, mixed, mesic Typic Hapludalfs) series (Crum and Collins, 2004). The experiment was conducted on a different field each year to permit planting on site following red clover plow down. Every year after wheat harvest, red clover was planted into wheat stubble in July-August except in 2001 when it was planted in corn stubble harvested the previous year. Red clover was chisel-plowed the subsequent spring before corn planting in order to serve as a N source for the non-conventional plots. The period before and after the winter allowed red clover to grow and produce relatively significant biomass for the following corn crop. Each year, prior to the establishment of corn, red clover was sampled using a 0.45 by 0.45 m quadrat for estimating per hectare dry matter (DM) and N content. Nitrogen concentration was determined using the Kjeldahl method. Nitrogen content was obtained by multiplying the DM and the N concentration.

Experimental design

The field experiment was a split-plot in a completely randomized design with four replications. The main plots were four corn densities: 37 500, 55 000, 65 000 and 75 000 plants ha⁻¹. Subplots were four management practices: (1) Conventional management, corn seeded into wheat stubble with N fertilizer applied (CMNF); (2) Corn seeded into plowed red clover, no N fertilizer, interseeded with AC Greenfix (PRIA); (3) Corn seeded into plowed red clover; no N fertilizer, interseeded with red clover (PRIR) ; and (4) Corn seeded into plowed red clover, no N fertilizer, not interseeded with cover crop (PRNI). Individual experimental units consisted of 6 rows of 4 by 4.5 m in 2002 and 2003 (due to small field size in 2002) and of 5 by 4.5 m 2004 and 2005.

Corn

The hybrid Great Lakes 4979 (Great Lakes Hybrids), relative maturity 99 days, was planted on 29 May 2002 and 06 June 2003; and Pioneer Hybrid 38P05, relative maturity 93 days, was planted on 30 May 2004 and 27 May 2005. The change in hybrid from Great Lakes 4979 to Pioneer Hybrid 38P05 was due to a discontinuation of seed production by Great Lakes Hybrids. The trials were planted at approximately 100,000 plants ha⁻¹. Two weeks after emergence, each plot was hand-thinned to target the appropriate plant density.

Corn was harvested from the four center rows of each plot on 17 October 2002, 29 October 2003, 10 November 2004 and 06 October 2005. Data collected in the four center rows included grain yield, plant height, days to flowering,

number of ears harvested and the number of plants harvested. In 2002 and 2003, prior to harvest two whole plants were collected from four center rows of each plot for total N analysis. In 2004 and 2005, ten plants were collected from the four center rows. Corn was harvested using a combine. Corn grain yield, test weight and moisture content were automatically measured by the GrainGage™, a HarvestData System™ mounted on a plot combine (Juniper Systems, Logan UT). Dry weight was determined by the method detailed by (Lauer, 2002). Grain yields were a summation of combine and hand harvested corn and were reported at 155 g kg⁻¹ moisture content. Corn height was measured from the soil surface to the tip of the tassel on five randomly selected plants from the four middle rows of each plot. Days to flowering were determined from planting to the day on which 50% of the plants had extruded tassels. Prior to harvest, the number of plants per plot and ears per plots were counted in all four center rows.

Soil sampling and agricultural inputs

Each year, eight soil cores were taken from every plot at a depth of 25 cm at the end of April. Soil samples were air-dried and sent to the MSU Soil and Plant Nutrient Laboratory for NPK and pH analysis, and fertilizer recommendations. Soil pH was 6.1 in 2002, 6.4 in 2003, 6.8 in 2004 and 6.9 in 2005. Each year, based on soil test results, either P or K or both were applied to the whole field a few days before planting. Nitrogen was applied only to CMNF plots as a starter fertilizer after planting corn. In 2002 and 2003, Urea was applied as a starter fertilizer a few days after planting at the rate of 23 kg ha⁻¹

whereas in 2004 and 2005, ammonium nitrate was applied as starter fertilizer at the rate of 28 kg ha⁻¹. In 2002, P and K were applied at the rate of 57 kg ha⁻¹ and 108 kg ha⁻¹ respectively, and lime at the rate of 1000 kg ha⁻¹. In 2003, P and K were applied a few days before planting at the rate of 50 kg ha⁻¹ and 82 kg ha⁻¹, respectively. In 2004, P and K were applied at the rate of 23 kg ha⁻¹ and 68 kg ha⁻¹, respectively. In 2005, only P was applied at the rate of 40 kg ha⁻¹. In mid-June of every year, soil samples were taken for nitrate analysis. Based on Preseed Nitrate Test results, supplemental N fertilizer was applied in CMNF up to a total of 140 kg ha⁻¹ every year.

Herbicides were used to control weeds. Each year, herbicides were broadcast in CMNF and applied in 25.4 cm bands in PRIR, PRIA and PRNI to reduce herbicide interference with the germination of cover crops that were interseeded later. In 2002, Acetochlor (1.79 kg ai ha⁻¹) was used one week after planting. The first application of herbicide did not totally control weeds. Three weeks later, a second application of herbicides, Atrazine (1.12 kg ai ha⁻¹) and Bromoxynil (0.42 kg ai ha⁻¹) was broadcast on CMNF plots, whereas PRIR, PRIA and PRNI plots were cultivated. In 2003, the preemergence herbicide S-metolachlor (1.42 kg ai ha⁻¹) and Flumetsulam (0.06 kg ai ha⁻¹) were applied four days after planting. In 2004, S-metolachlor (1.42 kg ai ha⁻¹) and Atrazine (0.56 kg ai ha⁻¹) were applied three days after planting. In 2005, Lumax (S-metolachlor 1.42 kg ai ha⁻¹; Atrazine 0.47 kg ai ha⁻¹; mesotrione 0.15 kg ai ha⁻¹) was applied directly after planting corn.

Cover crops

Red clover and AC Greenfix were interseeded on 11 July 2002, 14 July 2003, 06 July 2004 and 01 July 2005 when corn plants were V5-V7 growth stages. In 2004 and 2005, pure or monoculture cover crop plots were established at the time of interseeding to compare biomass in pure stand with biomass of interseeded cover crops. Red clover was broadcast with a hand-seeder at the seeding rate of 20.4 kg ha⁻¹ and AC Greenfix was hand-broadcast at the rate of 90 kg ha⁻¹. Before planting, AC Greenfix was inoculated with *Rhizobium leguminosarum*. Above ground biomass of red clover and AC Greenfix were hand-clipped at full bloom of AC Greenfix on 27 September 2002, 02 October 2003, 23 August 2004 and 08 August 2005 by removing plants from a random quadrat of 0.209 m² in each plot.

To assess cover crop density, the number of red clover and AC Greenfix plants was counted and reported on a plants m⁻² basis. Plant height was determined by measuring five randomly selected plants in each quadrat. After corn harvest, corn stalks were mowed to increase cover crop exposure to light. Plots were left undisturbed until the following spring. The subsequent spring, only red clover biomass was sampled on 02 June 2003, 02 June 2004 and 01 June 2005 because AC Greenfix did not survive the winter. After each sampling, cover crop biomass was oven dried at 60° C for 48 h to determine DM. Total DM of the cover crop was calculated by multiplying the yield per quadrat by the number of quadrats ha⁻¹.

Soil moisture measurements

Soil moisture was measured after interseeding the cover crops. The percent volumetric soil moisture content was measured using a Time Domain Reflectometer (TDR) (MESA Systems Co., Medfield, Massachusetts). Soil moisture was measured on 13 July, 19 July, 26 July, 2 August, 9 August, 16 August, 30 August, 13 September, 27 September and 11 October in 2004, and on 19 July, 26 July, 2 August, 9 August, 16 August, 31 August, 06 September and 13 September in 2005. The % volumetric soil moisture was measured in three directions (parallel, perpendicular and diagonal to corn row) at two different depths (0 to 18 and 18 to 36 cm) in tubes placed within one of the two center rows of the six-row corn plot. Soil moisture readings of the three directions were averaged at each depth since no difference was detected among directions.

Statistical analysis

All data were analyzed using Proc Mixed in Statistical Software Package SAS version 8.2 (SAS, 2001). Plant density and cropping system were considered fixed effects. Two error terms were considered in the analysis of the data, one associated with the whole plot (plant density) and the other associated with the subplot (management practices) and the interaction (plant density x management practices). When interaction effects were found to be significant, means separation was conducted for respective cell means. When main effects were significant while interactions were not, means separation was conducted for marginal means. Effects were considered statistically significant at $p = 0.05$.

Results and discussion

Weather patterns

Daily precipitation and monthly average temperature (minimum and maximum) were obtained from the Long-Term Ecological Research weather station (LTER-Weather, 2006). In 2002, there was a drought period in June and July, and the average rainfall in June was below the 30-year average (Figure 1a and Appendix A). Precipitation in June and July during the 2003 growing season were lower than the 30-year average (Figure 1a). Seasonal total precipitation in 2004 was the only year above the 30-year average (Appendix A) and was well distributed throughout the growing season (Figure 2). Precipitation in April, May and August during the 2005 growing season was lower than any other growing season and than the 30-year average (Figure 1a). The low rainfall in spring of 2005 helps explain the low red clover DM before corn establishment (Table 1, Figures 1a and 2). Although total precipitation in the 2005 growing season was lower than the 30-year average, rainfall occurred during critical corn growth stages (Figure 1a). Monthly average minimum temperature for April 2003 was lower compared to the 30-year average (Figure 1b and Appendix B). Monthly average maximum temperatures during the 2005 growing season from June to September were higher than the 30-year average (Figure 1b). A drought period combined with high temperature in 2005 prevented the germination of interseeded red clover.

Soil moisture in 2004 and 2005

Soil moisture varied across management practices and corn density at both 0 to 18 cm and 18 to 36 cm depths (Figures 3 and 4). No interaction was observed among sampling dates and depth with either management practices or corn plant densities. Comparisons among treatments were conducted at each sampling date for each treatment for an individual depth.

In 2004, across corn density, no significant difference was observed among management practices at 0 to 18 cm depth at each sampling date from 13 July to 30 August (Figure 3a, and Appendix C). On 13 and 27 September, soil moisture in PRIA and PRIR was significantly higher than in CMNF but not in PRNI. No difference was seen at the last sampling (11 October) among management practices. In 2004 at 18 to 36 cm, soil moisture was significantly higher in PRIA compared with CMNF and PRIR at all sampling dates (Figure 3b). No difference was seen between PRIA and PRNI. AC Greenfix appeared to be using less water compared to red clover. In 2005 at 0 to 18 cm during the first sampling, soil moisture in PRIR was significantly higher than in PRNI (Figure 3c and Appendix D). However, no significance difference was detected among treatments on the other sampling dates. Soil moisture decreased with time from the second sampling (26 July) up to the last sampling (13 September). In 2005 at 18 to 36 cm, no significant difference was observed among management practices from the first to the last sampling and soil moisture decreased with time (Figure 4d).

Across management practices, soil moisture at four corn densities varied with depth and weather conditions in 2004 and 2005 (Figure 4). In 2004 at 0 to 18 cm depth within the same date, soil moisture in plant density was not significantly different from 13 July to 9 August (Figure 4a). From 16 August to 11 October 2004 at 0 to 18 cm depth, soil moisture at 75 000 plants ha⁻¹ was significantly higher than at 55 000 plants ha⁻¹ but not different at 37 500 and 65 000 plants ha⁻¹. From 13 July to 11 October 2004 at 18 to 36 cm depth, soil moisture at 75 000 plants ha⁻¹ was higher than at 65 000 plants ha⁻¹ except on 2 August (Figure 4b). In addition, soil moisture at 75 000 plants ha⁻¹ was significantly higher than at 37 500 plants ha⁻¹ from 16 August to 11 October. In 2005 within the same date at both 0 to 18 cm and 18 to 36 cm depths, soil moisture in plant density was significantly higher at 55 000 plants ha⁻¹ compared at 37 500 plants ha⁻¹ from 19 July to 2 August (Figures 4c and d). From 9 August to 13 September within the same date at both 0 to 18 cm and 18 to 36 cm depths, no significant difference was seen in soil moisture at any plant density. In 2005, soil moisture decreased in all management practices and at all plant densities with time, as rainfall decreased (Figure 3c and d, and 4c and d).

Overall, adequate rainfall conditions in 2004 seemed to increase strong differentiation between treatments at 18 to 36 cm compared with 0 to 18 cm. At low or no rainfall no clear differentiation was observed at both 0 to 18 and 18 to 36 cm depths. No differentiation among treatments in dry conditions may be explained by corn plants accessing the deeper soil moisture in dry conditions that create less moisture near the surface (Zemenchik et al., 2000). Similarly,

Biederbeck and Bouman (1994) observed a substantial decrease in soil moisture at deeper depth during dry conditions.

Interseeding system and N source effect on corn yield

There was no interaction between management practice and corn density on corn yield; however, there was a year and management practices or corn density effect on corn yield (Table 2). Mean corn yield for management practices across corn densities varied from year to year and within years (Table 3). In 2002, corn yield of PRIR was significantly higher than PRIA at 55000 plants ha⁻¹. In 2002, corn yield of CMNF was lower at each plant density compared with those of corn planted into plowed red clover (PRIR, PRIA and PRNI). The differences in yield of CMNF compared with PRIR, PRIA and PRNI may be attributed to the rainfall pattern (Figure 2). In June and July of 2002, we had a period of drought. These dry conditions happened just after side-dressing N to CMNF plots. The lack of moisture probably reduced N uptake in CMNF plots and resulted in lower corn yields. In the same year at the nearby Long Term Ecological Research (LTER) at KBS, yield of corn following cover crops was significantly higher than those fertilized with N (LTER-Yields, 2002). Previous studies have shown similar observations of good crop performance following cover crops compared with non-cover crop plots when soil moisture was inadequate. In Maryland, during a dry year, beans planted after a cover crop had higher yields compared to those grown without cover crops (Peet, 1995). In 2003, no significant difference was observed in corn yield among all

management practices. Corn yield in 2003 was lower compared to 2002, 2004 and 2005. This was probably due to low rainfall below the 30-year average, for June, July and August (Figure 1a), and delayed planting because of slow growth of the cover crop due to a cold spring (Figure 1b). Planting occurred one week later compared to other growing seasons in order to allow red clover (used as source of N for PRIR, PRIA and PRNI) to grow during the spring. The delay in cover crop plow down may have also increased soil moisture depletion and hence adversely affected corn growth and yield. Corn yield of CMNF was significantly higher than PRIR, PRIA PRNI at 65 000 and 75 000 plants ha⁻¹ in 2004. In 2003 and 2004 no significant difference was observed between corn yield of PRIR, PRIA and PRNI (Table 3). In 2005, corn yield in CMNF was higher than in PRIA and PRIR at 55 000 plants ha⁻¹, PRNI at 65 000 plants ha⁻¹, and PRIA, PRIR and PRNI at 75 000 plants ha⁻¹.

Mean corn yield at four corn densities across management practices varied with year (Table 3). In 2002 and 2003, no significant increase in corn yield was observed for CMNF with increased plant density. When comparing treatments planted into plowed red clover, no yield increase was observed for PRIR and PRNI with increased plant density in 2003 (Table 3). In 2003, only two replications were usable for data collection and analysis due to poor corn stand caused by wildlife damage. Corn grain yield tended to be greater at higher plant density, but was significantly different at 75 000 plants ha⁻¹ for PRIA in 2003 and CMNF in 2004. No significant increase in corn grain yield was observed beyond 65 000 plants ha⁻¹ for PRIR, PRIA and PRNI in 2004. In 2005, corn grain yield

was numerically higher at 75 000 plants ha⁻¹ in all management practices, but not significantly different from 65 000 plants ha⁻¹ in PRIR and PRNI and from 55 000 plants in CMNF.

The four-year average corn yield across plant densities suggest increased corn yield with increased corn density in both corn planted into plowed red clover and corn supplied with N fertilizer (Table 4). No corn yield increase is observed beyond 55 000 plants ha⁻¹ for PRIR and PRNI. PRIR and CMNF showed corn grain yield increases up to 65 000 plants ha⁻¹. The four-year average corn yield suggests no corn yield reduction with interseeding at any plant density and no difference between PRIR, PRIR, PRNI and CMNF at any plant density (Table 4). This is in agreement with Abidin et al. (1998) who reported no effect on corn yields due to interseeding red clover and other cover crops 10 days after corn emergence. When intercropping medics into corn three weeks after emergence, Jeranyama et al. (1998) did not observe any corn grain yield reduction.

Mean corn yield in management practices across plant density varied significantly from year to year (Table 5). However, the four-year average of corn yield in the four management practices suggest no significant difference between treatments receiving N from plowed red clover or from N fertilizer (Table 5). There was an interaction between year and management practices on corn yield due to dry conditions that occurred in 2002 and affected corn yield in CMNF (Table 2). Corn yield in CMNF was lower in 2002 and similar in 2003 when compared with corn yields of PRIR, PRIR, and PRNI (Table 5). However, in 2004 and 2005, corn yield in CMNF was significantly higher than corn yield of PRIR,

PRIA, and PRNI (Table 5). This is due probably to the higher and well distributed rainfall (Figures 1 and 2) that occurred in 2004; and rainfall that occurred when corn was at critical growth stages (Coffman, 1998) in 2005. This study suggests that in dry years, yield of corn following plowed red clover could be higher than those with non-cover crop plus N fertilizer, while the opposite will be seen when soil moisture is not a constraint. Corn following cover crops produced similar or higher yield than fertilized corn in various studies. Vyn et al. (2000) reported similar corn grain yield in corn planted into plowed red clover compared to corn supplied with 150 kg ha⁻¹. Griffin et al. (2000) showed that legume cover crops did not respond to additional N and supplied all N required by sweet corn. Vyn et al. (1999) also noted that red clover was the best cover crop with respect to N availability to succeeding corn when compared to other cover crops such as oilseed radish. Balkcom and Reeves (2005) showed higher corn yield following sunn-hemp compared to corn with no cover crop plus 56 kg ha⁻¹ of N fertilizer.

There was an interaction between year and management practices on days to flowering (Table 2). This was due to climatic conditions that occurred in 2005. Days to flowering, plant height, ears per plant, grain moisture and test weight of corn varied with management practice, corn density and year (Tables 5 and 6). Corn plants in the CMNF treatment flowered approximately two days later in 2002 and 2003, and one day later in 2004 than plants in the PRIR, PRIA and PRNI treatments. However, no difference was noticed in 2005 (Table 6). No difference was observed in days to flowering in relation to plant density in any growing season (Table 7). In 2005, all plants regardless of treatment flowered at

the same time. In 2005, corn flowered approximately 54 days after planting compared to 66 days on average for the 2002, 2003 and 2004 growing seasons. This was probably due to high temperatures in June and July, which were higher compared to the 30-year average (Figure 1b). Similar findings were obtained by Sarrantonio and Molloy (2003) who observed that sweet corn tasseled three weeks earlier when temperatures were high.

There was an interaction between year and management practices on corn height (Table 2). Dry conditions that affected corn growth in 2002 may account for this interaction and explain the difference among treatments (Table 6; Figure 1). In 2002, corn plants in CMNF were on average 17 cm shorter than corn planted into plowed red clover (Table 6). However no significant difference was observed among management practices during the 2003, 2004 and 2005 growing seasons (Table 6). Sarrantonio and Molloy (2003) observed greater height (20 cm difference) of sweet corn following red clover compared to non-clover in dry conditions and no difference when rainfall was sufficient. Abdin et al. (1998) observed no difference in corn height in some treatments when rainfall was non-limiting to crop growth. No consistency was observed in corn height, taken after tasseling, in relation to plant density, as no difference was seen in 2002 and 2003. In 2004, plants in the 75 000 plants ha⁻¹ treatment were significantly higher in plant height (1.83 m) compared to the 37 500 and 55 000 plants ha⁻¹ treatments, 1.73m and 1.72 m respectively (Table 7). In 2005, 55 000 plants ha⁻¹ was significantly higher in plant height (2.04m) compared to 37 500 plants ha⁻¹ (1.94 m).

The number of ears per plant was similar in management practices in 2002 and 2003 (Table 6). However in the following years, the CMNF treatment had a higher number of ears compared with PRIR in 2004 and with PRIR, PRIA and PRNI in 2005. In all four years, no significant difference was observed in ears per plant between interseeded (PRIR, PRIA) and no interseeded (PRNI) corn (Table 6). Similarly, in a study of interseeding various cover crops into corn, Abdin et al. (1998) found no consistent effects of interseeded cover crop treatments on corn grain yield components such as ears per plant. The four corn plant densities differed in number of ears per plant except in 2002 (Table 7). The number of ears per plant at 37 500 plants ha⁻¹ was significantly higher than all the other densities in 2003 (except at 55 000 plants ha⁻¹), 2004, and 2005. In 2005, the number of ears per plant at 55 000 plants ha⁻¹ was also significantly higher than at 65 000 and 75 000 plants ha⁻¹. There was an interaction between year and plant density on the number of ears per plant and this was probably due to climatic conditions (Table 2). A higher number of ears per plant was observed in 2004 and 2005 (data not shown), probably due to optimum conditions for corn growth. This study suggests that corn planted at a much lower density likely will have two or more ears per plant. This can be attributable to reduced stress at low corn density because plants can compensate for factors that influence resource capture at low corn density (Tollenaar et al., 2006). In a corn crowding study, Hashemi et al. (2005) observed a decrease in ears per plant in all hybrids as plant density intensified.

In two of the four years of this study, corn grain moisture in CMNF was significantly different from grain moisture of corn planted into plowed red clover (PRIR, PRIA and PRNI) (Table 6). In 2003, CMNF had higher grain moisture compared with corn planted into plowed red clover, whereas in 2004 it was lower. In 2005, CMNF grain moisture was significantly higher compared to PRIR and PRNI and not different from PRIA. No difference was detected in grain moisture among treatments in 2002. No significant difference was observed for grain moisture among plant densities in any year (Table 7). Overall, grain moisture was very high in 2003 and low in 2005. This is presumably due to weather which was cool in 2003 and dry and hot in 2005.

Corn grain test weight of CMNF was significantly lower compared with PRIR and PRIA in 2002 and with PRIR, PRIA and PRNI in 2003 (Table 6). However in 2004, the test weight of CMNF was significantly higher compared with PRIR, PRIA and PRNI. No difference was observed in 2005. This difference may have been due to hybrid types and growing conditions. Widdicombe and Thelen (2002) reported a significant variation in test weight among various hybrids and locations.

Corn density effect on interseeded cover crops in fall

Separate analysis was conducted for the first three years of cover crop data and the fourth year of AC Greenfix data because no data was available for red clover for the fourth year. No interaction was observed between corn density and cover crop DM (Table 2).

Interseeded cover crop DM varied during the fall of 2002, 2003, 2004 and 2005 growing seasons (Table 8). In 2002, cover crop emergence was delayed by a two-week period without rain after interseeding in July (Figure 1). Dry matter of interseeded red clover and AC Greenfix was numerically higher at the 37 500 plants ha⁻¹ except in 2003 for interseeded red clover. Dry matter of red clover was the lowest in 2003, ranging from 0.070 to 0.175 Mg ha⁻¹, with 65 000 plants ha⁻¹ producing the highest DM (Table 8). The low DM for red clover in 2003 was probably due to low germination (density) by the cover crop when compared with other growing seasons (Table 8). This might have been due to insufficient rainfall at the time of interseeding (Figure 1a). In 2005, hot and dry weather conditions (Figures 1 and 2) prevented red clover germination after the first and the second attempts to interseed in July and August, respectively. Shallow sowing combined with lack of moisture may have prevented red clover germination. During this growing season, AC Greenfix produced the lowest DM compared with 2002, 2003 and 2004 growing seasons (Table 8). The ability to survive in dry conditions of only 125-150 mm of rainfall (Biederbeck et al., 1993; Biederbeck and Bouman, 1994; Campbell, 1997; DFS, 2003) helped AC Greenfix survive hot and dry conditions in 2005. Results on clover DM are supported by Hively and Cox (2001) who found an average of 0.2 Mg ha⁻¹ DM in fall when red clover was interseeded into soybeans.

The three-year average DM for red clover ranged from 0.184 to 0.370 Mg ha⁻¹ and 0.228 to 0.567 Mg ha⁻¹ for AC Greenfix (Table 9). Red clover and AC Greenfix differed in DM, with AC Greenfix producing more DM than red clover

(Table 9). Corn density significantly affected cover crop growth and DM production. Overall interseeded cover crop DM decreased as corn density increased but significant differences were only observed at 37 500 plants ha⁻¹ compared to higher corn plant densities except for red clover at 55 000 plants ha⁻¹ (Table 9). Ross et al. (2003) also observed a decline in berseem clover (*Trifolium alexandrinum*) DM with increasing oat plant density. Cover crop DM was negatively correlated to corn plant density with a correlation coefficient of $r^2=-0.55$ (Table 9).

Cover crop (Red clover and AC Greenfix) DM was affected by other factors such as poor germination and soil moisture, which varied from year to year. There was an interaction between year and cover crop species on cover crop density (plants m⁻²) due to poor red clover germination in some of the growing seasons (Tables 2 and 8). No correlation was observed between cover crop DM and cover crop density. Singer et al. (2006) also reported no relationship between red clover DM and its density when red clover density was high.

No correlation was observed between cover crop height and cover crop DM (Table 2). The three-year average red clover and AC Greenfix height ranged from 9.1 to 13.0 cm and 91 to 102 cm, respectively. No significant difference was observed in height within species except in 2004 where AC Greenfix at 75 000 corn plants ha⁻¹ was significantly shorter in height than at lower corn plant densities (Table 9). Similarly, no significant difference was noticed in AC Greenfix density in all four growing seasons. However, in 2002 red clover density was

significantly lower at 75 000 plants ha⁻¹ compared with lower corn plant density. In 2003, red clover density was significantly higher at 65 000 plants ha⁻¹ compared with 55 000 and 75 000 plants ha⁻¹, but not different from 37 500 plants ha⁻¹.

Pure or monoculture cover crops in 2004 and 2005

Monoculture cover crop was planted at the time of interseeding and produced significant greater DM during the fall compared to interseeded cover crops (Table 8). Fall sampled pure red clover and AC Greenfix DM were respectively 1.4 and 2.2 Mg ha⁻¹ in 2004, and 0.46 and 1.6 Mg ha⁻¹ in 2005 (Table 8). Biomass production was highest in 2004 compared with 2005 for both cover crops. These differences could be attributed to above normal precipitation during the 2004 growing season (Figure 1a and 2). However, in 2005 hot and dry conditions resulted in poor cover crop germination (density) and hence low cover crop DM. In 2005, soil moisture content decreased with time, with little differences among treatments (Figure 3). In 2005 red clover density (256 m⁻² compared to 640 m⁻² in 2004) was very low, particularly for monoculture. The ability of AC Greenfix to perform well in low rainfall conditions allowed it to survive. No difference in cover crop density was observed between monoculture and interseeding. In both years, no difference was detected in AC Greenfix plant height between monoculture and interseeding. However, red clover plant height in monoculture was only similar to clover planted at 37 500 plants ha⁻¹. The height of both cover crops was lower in 2005 than in 2004 (data not shown).

AC Greenfix DM was similar to that reported by DFS (2003) who suggested that it produces DM of 2242 to 4483 kg ha⁻¹ and Biederbeck and Bouman (1994) who reported DM of 2130 to 4080 kg ha⁻¹ during five growing seasons. Monoculture red clover and AC Greenfix results are similar to those of Jeranyama et al. (1998) who obtained greater DM with clear seeded medics (up to 3.0 Mg ha⁻¹) compared with interseeded medics. AC Greenfix and red clover DM was significantly reduced in interseeding compared with the monoculture system. In 2004, interseeded red clover DM was 19 to 33 % of monoculture red clover DM, whereas interseeded AC Greenfix DM ranged from 12 to 26% of monoculture AC Greenfix DM. In 2005, interseeded AC Greenfix DM ranged from 8 to 15% of monoculture AC Greenfix DM. If moisture is not limiting, red clover can perform well compared with AC Greenfix in an interseeding system. When soil moisture was not limiting interseeded AC Greenfix performed well during a growing season with low temperature such as the 2004 growing season (Table 8 and Figure 1a).

Red clover dry matter the subsequent spring

AC Greenfix was hand-clipped a few days after collecting interseeded cover crop DM to prevent podfill. Regrowth was expected from AC Greenfix, however none occurred. In 2004, only few AC Greenfix plants produced regrowth, we think because of adequate rainfall. AC Greenfix is an annual cover crop and did not survive the winter. Corn stalks were mowed after corn harvest and plots left undisturbed until the subsequent spring. There was a seasonal

effect on red clover DM in the subsequent spring. In subsequent spring red clover accumulated DM ranging from 3.10 to 4.12 Mg ha⁻¹ in 2003, 2.34 to 3.38 Mg ha⁻¹ in 2004 and 5.36 to 6.05 Mg ha⁻¹ in 2005 (Table 10). In the subsequent spring of every growing season, red clover DM was similar regardless of the corn density into which it was seeded in the previous fall (Table 10). High DM of interseeded red clover in the subsequent spring of 2005 was due to higher and well-distributed rainfall during the fall of 2004 growing season. Dry matter accumulation increased significantly from the first sampling in the fall to the second in the spring regardless of corn plant density. Red clover DM accumulation was comparable to results of other studies. Blaser et al. (2006) found that DM of red clover intercropped with winter wheat was not affected by wheat seeding rates when harvested after cereal harvest, and that red clover produced up to 3.68 Mg ha⁻¹ of DM 80 days after wheat harvest. Blackshaw et al. (2001) found that sweet clover undersown in field pea (*Pisum sativum* L.), flax [*Brassica juncea* L.) produced biomass yields of 3110 to 5370 kg ha⁻¹ in June of the subsequent year depending on the year and companion crops. In New York, red clover interseeded into soybean in fall produced 0.8 Mg ha⁻¹ the subsequent spring (Hively and Cox, 2001). Hively and Cox (2001) obtained low DM because of the time of sampling (early may), as our samples were taken in the first week of June. In comparing the growth and DM of various cover crops, Odhiambo and Bomke (2001) found that late (May) sampling of cover crop during the spring provided a significant cover crop DM increase compared to earlier (March) sampling. Similar results to our findings were obtained by Scott et al. (1987) who

reported an average red clover DM of 2.9 Mg ha⁻¹ (roots and above ground DM) in the subsequent spring after interseeded red clover into corn, at Aurora in New York. Also, Cogger et al. (2006) reported that mid-may red clover sampling had the greatest DM, averaging 2.22 Mg ha⁻¹ DM. Interseeded red clover in high corn density does not affect red clover DM production the subsequent spring if allowed to grow during the spring. Delayed cover crop cultivation, presents an obstacle to some cropping systems where there might be a need to establish a crop early in the spring. For maximizing DM from red clover, it is better to allow the cover crop to grow as long as possible. However, there is a risk of soil moisture depletion with delayed cover crop plow down.

Conclusion

Under the conditions of this study, available N from red clover established in the summer after wheat harvest was sufficient to produce corn yield that exceeded the corn yield supplied with mineral N fertilizer in a dry year. When precipitation was adequate, corn supplied with mineral N fertilizer produced similar or higher yield than corn supplied with red clover derived-N. Interseeding red clover or AC Greenfix did not reduce corn yield at higher corn density. Corn density influenced red clover growth and DM in fall with a trend of higher corn density producing lower red clover DM. However the subsequent spring, red clover DM was similar regardless of plant density in the previous fall.

AC Greenfix had good establishment when interseeded, but produced less biomass compared with monoculture AC Greenfix. Although there are many

management questions to be investigated, this study suggests that red clover derived-N can produce corn yields comparable to those produced by N fertilizer. This research also suggests that interseeding red clover into corn densities up to 75 000 plants ha⁻¹ could produce enough DM to provide N to a subsequent crop. This type of management practice could be used in low-input farming systems to reduce N fertilizer costs, especially in developing countries and organic farming systems. Additional research is needed to investigate companion crop yield quality and N contribution to a subsequent crop. As new hybrids are developed that could withstand increased plant density, it would be interesting to investigate the effect of increased corn density up to 100 000 plants ha⁻¹ on cover crop DM. Since AC Greenfix did not perform well in the corn grain interseeding system compared to monoculture cropping, we recommend that research be done on early establishment in the spring before planting short cycle vegetables or relay establishment after winter wheat harvest or before soybean harvest.

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Table 1. Dry matter and N content of red clover (*Trifolium pratense* L.), established the previous year in strips and sampled before planting corn (*Zea mays* L.), in spring of 2002, 2003, 2004 and 2005 at Kellogg Biological Station, Hickory Corners, MI.

	DM	N content
	-----Kg ha ⁻¹ -----	
2002	6531	221
2003	7780	264
2004	5520	161
2005	3444	132

Table 2: Significance of the effect of plant density (PD) and management practices (MP) across four years (Y) on corn (*Zea mays* L.) yield (CY), days to flowering (DF), plant height (PH), ears per plant (EP), grain moisture (GM) and test weight (TW), and on cover crop fall dry matter (FDM), fall cover crop density (FCD), fall cover crop height (FCH) and spring red clover (*Trifolium pratense* L.) dry matter (SRDM) at Kellogg Biological Station, Hickory Corners, MI.

	Corn						Cover crop			
	CY	DF	PH	EP	GM	TW	FDM	FCD	FCH	SRDM
Y [†]	***	***	**	***	***	***	NS	*	***	***
PD	***	**	NS	***	NS	NS	***	NS	NS	NS
Y x PD	*	NS	NS	***	NS	NS	NS	NS	NS	NS
MP [‡]	NS	***	**	*	NS	NS	**	***	***	---
Y x MP	***	***	**	NS	***	*	*	***	***	---
PD x MP	NS	NS	NS	NS	NS	NS	NS	NS	NS	---
Y x PD x MP	NS	NS	NS	NS	NS	NS	NS	NS	NS	---

*Significant at the 0.05 level

**Significant at the 0.01 level

***Significant at the 0.001 level

[†]for SRDM is three years.

[‡]for FDM, FPM and FCH, MP is cover crops species

Table 3. Mean corn (*Zea mays* L.) yield (Mg ha⁻¹) of management practices across corn plant density during the 2002, 2003, 2004 and 2005 growing seasons at Kellogg Biological Station, Hickory Corners, MI.

Management practices	Corn density (plants ha ⁻¹)			
	37 500	55 000	65 000	75 000
	Mg ha ⁻¹			
	2002			
PRIR	7.85a* C**	10.35a A	9.41a AB	9.05a B
PRIA	8.10a B	9.21b A	10.01a A	9.02a AB
PRNI	7.58a B	10.05ab A	9.60a A	9.34a A
CMNF	6.08b A	5.99c A	6.93b A	6.36b A
CV (%)	12			
	2003			
PRIR	6.33a A	7.26a A	7.08a A	7.68a A
PRIA	6.36a B	6.86a B	5.88a B	7.98a A
PRNI	5.78a A	6.72a A	6.82a A	7.43a A
CMNF	6.43a A	7.26a A	6.91a A	7.63a A
CV (%)	12			
	2004			
PRIR	7.58a B	8.37a AB	8.56b AB	9.01b A
PRIA	7.85a B	8.17a B	8.61b AB	9.53b A
PRNI	7.79a B	8.13a AB	8.84b AB	9.14b A
CMNF	7.66a C	8.87a B	9.96a B	11.24a A
CV (%)	10			
	2005			
PRIR	9.11a B	9.82b AB	10.30ab A	9.7c AB
PRIA	8.53a C	9.72b B	10.71ab AB	10.98b A
PRNI	8.74a B	10.24ab A	9.64b AB	10.11bc A
CMNF	9.42a C	11.35a AB	11.04a B	12.32a A
CV (%)	8			

*Means within columns in the same year followed by the same lower case letter are not significantly different at P=0.05.

** Means within rows in the same year followed by the same upper case letter are not significantly different at P=0.05.

PRIR: Plowed red clover interseeded with red clover;

PRIA: Plowed red clover interseeded with AC Greenfix;

PRNI: Plowed red clover no interseeding;

CMNF: Conventional management, supplied with N fertilizer.

CV: coefficient of variation.

Table 4. Mean corn (*Zea mays* L.) yield (Mg ha^{-1}) of combined four-year data across management practices and corn plant density at Kellogg Biological Station, Hickory Corners, MI.

Management practices	Corn density (plants ha^{-1})			
	37 500	55 000	65 000	75 000
	Mg ha^{-1}			
PRIR	7.72a* B**	8.95a A	8.84a A	8.86a A
PRIA	7.71a C	8.49a B	8.80a AB	9.38a A
PRNI	7.47a B	8.78a A	8.73a A	9.00a A
CMNF	7.40a C	8.36a B	8.71a AB	9.39a A
CV (%)	10			

* Means within columns followed by the same lower case letter are not significantly different at $P=0.05$.

** Means within rows followed by the same upper case letter are not significantly different at $P=0.05$.

PRIR: Plowed red clover interseeded with red clover;

PRIA: Plowed red clover interseeded with AC Greenfix;

PRNI: Plowed red clover no interseeding;

CMNF: Conventional management, supplied with N fertilizer.

CV: coefficient of variation

Table 5. Mean corn (*Zea mays* L.) yield (Mg ha^{-1}) in management practices across plant density during the 2002, 2003, 2004, 2005 and four-year average at Kellogg Biological Station, Hickory Corners, MI.

Management practices	Corn yield				
	2002	2003	2004	2005	Average
	Mg ha^{-1}				
PRIR	9.16a*	7.09a	8.38b	9.73b	8.59a
PRIA	9.09a	6.77a	8.54b	10.0b	8.59a
PRNI	9.14a	6.69a	8.47b	9.68b	8.50a
CMNF	6.34b	7.05a	9.43a	11.03a	8.46a
CV (%)	12	12	10	8	18

*Means within columns followed by the same letter are not significantly different at $P=0.05$.

PRIR: Plowed red clover interseeded with red clover;

PRIA: Plowed red clover interseeded with AC Greenfix;

PRNI: Plowed red clover no interseeding;

CMNF: Conventional management, supplied with N fertilizer.

CV: Coefficient of Variation.

Table 6. Days to flowering, plant height, ears per plant, grain moisture and test weight of corn (*Zea mays* L.) in management practices across plant density during the 2002, 2003, 2004 and 2005 growing seasons at Kellogg Biological Station, Hickory Corners, MI.

Management practices	Days to flowering	Height (m)	Ears plant ⁻¹	Grain Moisture (g kg ⁻¹)	Test weight
2002					
PRIR	66.50a*	1.81a	1.02a	253.6a	52.56a
PRIA	66.63a	1.80a	1.02a	246.8a	52.53a
PRNI	66.56a	1.79a	1.02a	249.9a	50.81ab
CMNF	68.94b	1.63b	1.00a	243.5a	47.22b
CV (%)	1	7	2	16	11
2003					
PRIR	66.00a	1.97a	1.02a	294.9b	48.58a
PRIA	66.00a	1.94a	1.03a	301.8b	48.53a
PRNI	66.00a	1.94a	1.02a	300.9b	48.50a
CMNF	68.63b	1.93a	1.05a	328.8a	47.38b
CV (%)	1	4	3	5	1
2004					
PRIR	66.13a	1.75a	1.10b	209.1a	55.15b
PRIA	66.19a	1.76a	1.11ab	211.4a	55.19b
PRNI	66.00a	1.78a	1.11ab	210.8a	55.20b
CMNF	67.06b	1.74a	1.15a	199.9b	55.56a
CV (%)	1	7	6	5	1
2005					
PRIR	54a	2.00a	1.24b	179.8b	57.76a
PRIA	54a	2.00a	1.23b	180.8ab	57.70a
PRNI	54a	2.00a	1.21b	180.1b	57.70a
CMNF	54a	1.97a	1.49a	186.0a	57.75a
CV (%)	—	7	34	7	1

* Means within columns in the same year followed by the same letter are not significantly different at P=0.05.

PRIR: Plowed red clover interseeded with red clover;

PRIA: Plowed red clover interseeded with AC Greenfix;

PRNI: Plowed red clover no interseeding;

CMNF: Conventional management, supplied with N fertilizer.

CV: coefficient of variation.

Table 7. Days to flowering, plant height, ears per plant, grain moisture and test weight of corn (*Zea mays* L.) at four plant densities across management practices during the 2002, 2003, 2004 and 2005 growing seasons at Kellogg Biological Station, Hickory Corners, MI.

Corn density (plants ha ⁻¹)	Days to flowering	Height (m)	Ears plant ⁻¹	Grain moisture (g kg ⁻¹)	Test weight
2002					
37 500	66.75a*	1.75a	1.04a	233.8a	51.19a
55 000	66.81a	1.79a	1.00a	262.7a	49.43a
65 000	67.50b	1.75a	1.01a	248.1a	51.97a
75 000	67.56b	1.75a	1.00a	249.1a	50.53a
CV (%)	1	7	2	16	11
2003					
37 500	66.63a	1.94a	1.08a	303.8a	48.47a
55 000	66.63a	1.93a	1.02ab	303.1a	48.28a
65 000	66.63a	1.95a	1.01b	305.5a	47.86b
75 000	66.75a	1.96a	1.01b	313.9a	48.38a
CV (%)	1	4	3	5	1
2004					
37 500	66.00a	1.73b	1.29a	204.1a	55.54a
55 000	66.25a	1.72b	1.07b	208.7a	55.00b
65 000	66.50a	1.76ab	1.05b	207.9a	55.27ab
75 000	66.63a	1.83a	1.07b	210.6a	55.27ab
CV (%)	1	7	6	5	1
2005					
37 500	54a	1.94b	1.63a	179.2a	57.75a
55 000	54a	2.04a	1.17b	188.5a	57.85a
65 000	54a	2.01ab	1.10c	179.6a	57.76a
75 000	54a	1.99ab	1.07c	179.4a	57.56a
CV (%)	---	7	34	7	1

* Means within columns in the same year followed by the same letter are not significantly different at P=0.05.

CV: coefficient of variation

Table 8. Effect of monoculture and interseeding at four corn (*Zea mays* L.) densities on red clover (*Trifolium pratense* L.) or AC Greenfix (*Lathyrus sativum* L.) DM (Mg ha⁻¹), density (plants m⁻²) and height (cm) in fall during the 2002, 2003, 2004 and 2005 growing seasons at Kellogg Biological Station, Hickory Corners, MI.

Plant density Plants ha ⁻¹	Red clover			AC Greenfix		
	DM Mg ha ⁻¹	Density plants m ⁻²	Height cm	DM Mg ha ⁻¹	Density plants m ⁻²	Height cm
2002						
37 500	0.480a*	839a	12.3a	0.455a	86a	71.0a
55 000	0.308b	636a	9.2a	0.360ab	79a	74.2a
65 000	0.263b	689a	13.1a	0.370ab	101a	79.3a
75 000	0.183b	571b	8.1a	0.203b	71a	78.5a
CV (%)	36	39	28	36	39	28
2003						
37 500	0.155a	345ab	9.7a	0.685a	127a	67.3a
55 000	0.100a	307b	8.5a	0.215b	117a	60.7a
65 000	0.175a	512a	7.9a	0.255b	113a	60.6a
75 000	0.070a	249b	6.7a	0.220b	137a	55.6a
CV (%)	33	31	22	33	31	22
2004						
0**	1.440a	640a	24.9a	2.195a	94a	94.6a
37 500	0.475b	737a	17.0ab	0.563b	93a	103.9a
55 000	0.375bc	629a	14.2b	0.340c	77a	103.6a
65 000	0.273c	646a	13.2b	0.383c	90a	98.5a
75 000	0.300c	753a	12.4b	0.263c	79a	87.8b
CV (%)	35	37	13	35	37	13
2005						
0	0.456	258	12.9	1.599a	80a	60.6a
37 500	-----	-----	-----	0.242b	76a	58.5a
55 000	-----	-----	-----	0.188bc	86a	61.3a
65 000	-----	-----	-----	0.216b	90a	63.5a
75 000	-----	-----	-----	0.127c	72a	63.3a
CV (%)				27	23	18

*Means within columns in the same year followed by the same letter are not significantly different at P=0.05.

**Monoculture cover crops.

CV: coefficient of variation.

Table 9. Mean interseeded red clover (*Trifolium pratense* L.) and AC Greenfix (*Lathyrus sativum* L.) DM (Mg ha⁻¹), density (plant m⁻²) and height (cm) at four corn (*Zea mays* L.) plant densities in fall 2002, 2003 and 2004 at Kellogg Biological Station, Hickory Corners, MI.

Plant density Plants ha ⁻¹	Red clover			AC Greenfix		
	DM Mg ha ⁻¹	Density plants m ⁻²	Height cm	DM Mg ha ⁻¹	Density plants m ⁻²	Height cm
37 500	0.370a*	640a	13.0a	0.567a	102a	80.7a
55 000	0.260ab	524a	10.6a	0.305b	91a	79.5a
65 000	0.237b	616a	11.4a	0.336b	101a	79.5a
75 000	0.184b	524a	9.1a	0.228b	95a	74.0a
CV (%)	38	39	21	38	39	21

Correlation coefficient between cover crop DM and corn plant density = -0.55 (p < 0.0001)

*Means within columns followed by the same letter are not significantly different at P=0.05.

CV: coefficient of variation.

Table 10. Effect of interseeding corn (*Zea mays* L.), at four plant densities, on red clover (*Trifolium pratense* L.) DM (Mg ha⁻¹) the subsequent spring in 2003, 2004 and 2005 at Kellogg Biological Station, Hickory Corners, MI.

Plant density (Plants ha ⁻¹)	Red clover DM			
	2003	2004	2005	Average
	-----Mg ha ⁻¹ -----			
0*	-----	-----	8.17a	-----
37 500	3.92a**	2.78a	6.05b	4.25a
55 000	3.62a	3.38a	5.48b	4.16a
65 000	4.12a	2.87a	5.88b	4.29a
75 000	3.10a	2.34a	5.36b	3.60a
CV (%)	20	32	14	19

*Monoculture cover crops.

**Means within column followed by the same letter are not significantly different at P=0.05.

CV: coefficient of variation

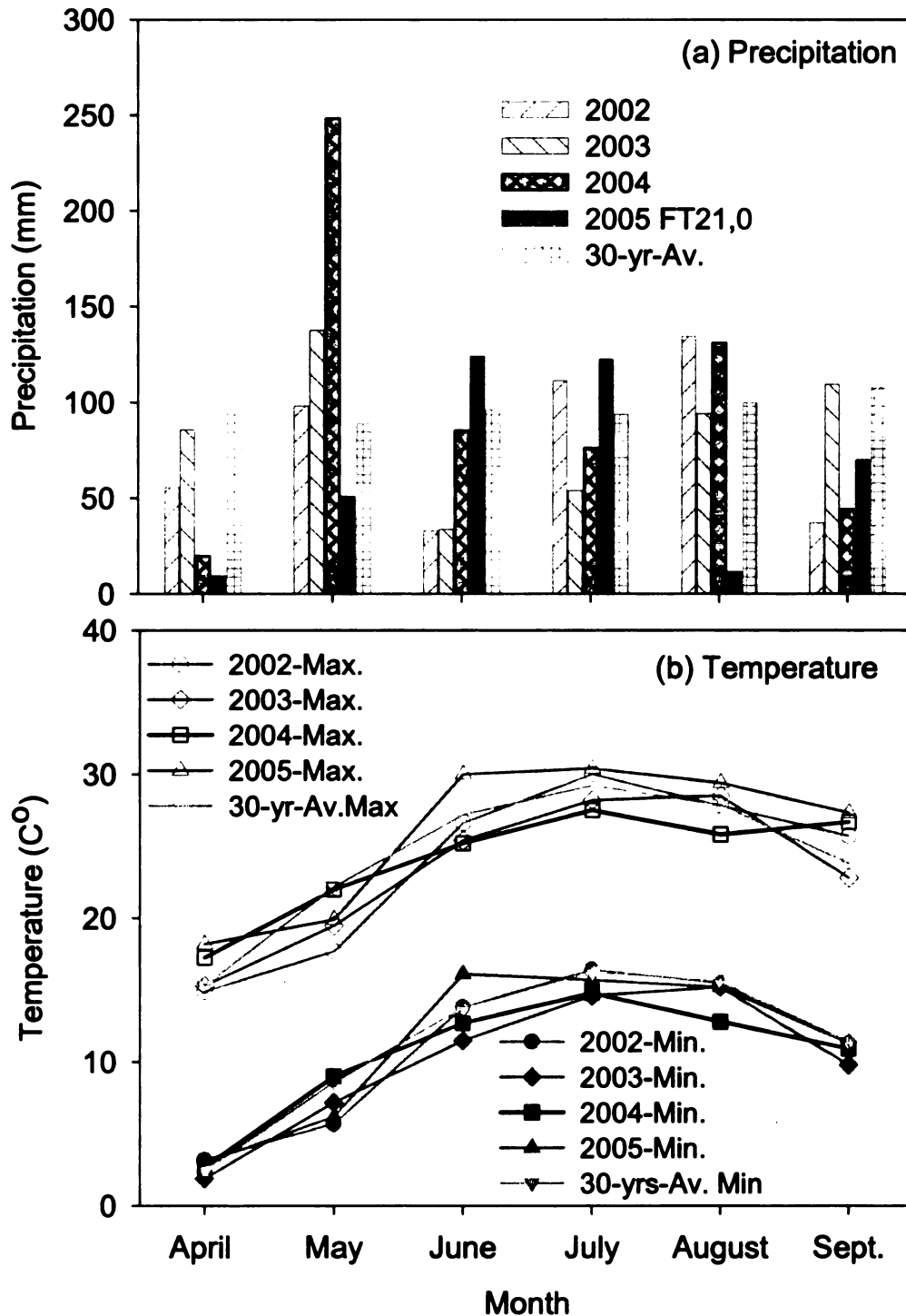


Figure 1. Monthly average minimum and maximum temperature, and total monthly precipitation during the 2002, 2003, 2004 and 2005 growing seasons compared with the 30-year monthly average at Kellogg Biological Station, Hickory Corners, MI.

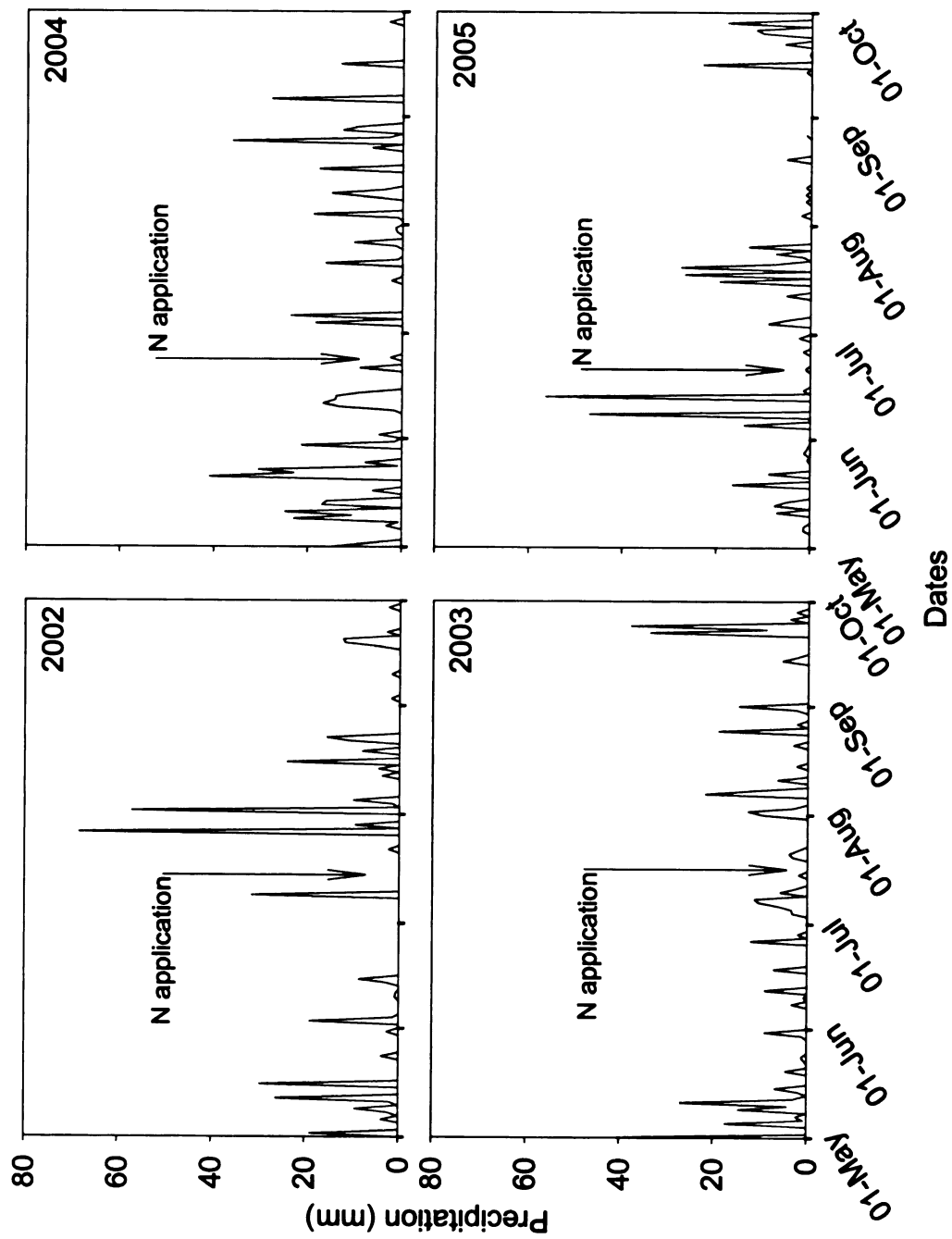


Figure 2. Total daily precipitation during the 2002, 2003, 2004 and 2005 growing seasons at Kellogg Biological Station, Hickory Corners, MI.

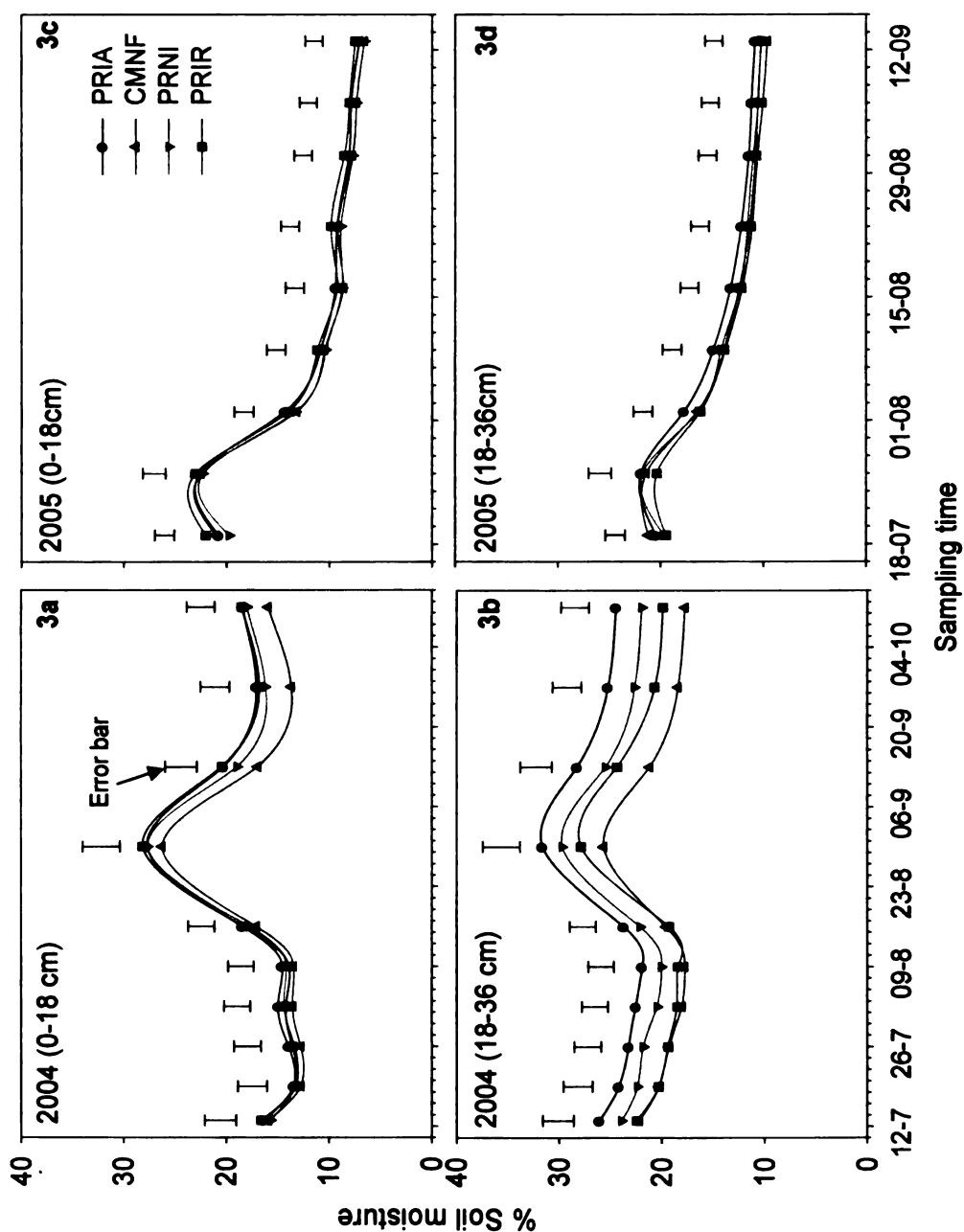


Figure 3. Weekly percent volumetric soil moisture at 0 to 18 and 18 to 36 cm depths in management practices across corn density during the 2004 (3a and b) and 2005 (3c and d) growing seasons at Kellogg Biological Station, Hickory Corners, MI. Within week error bars are similar for all treatments.

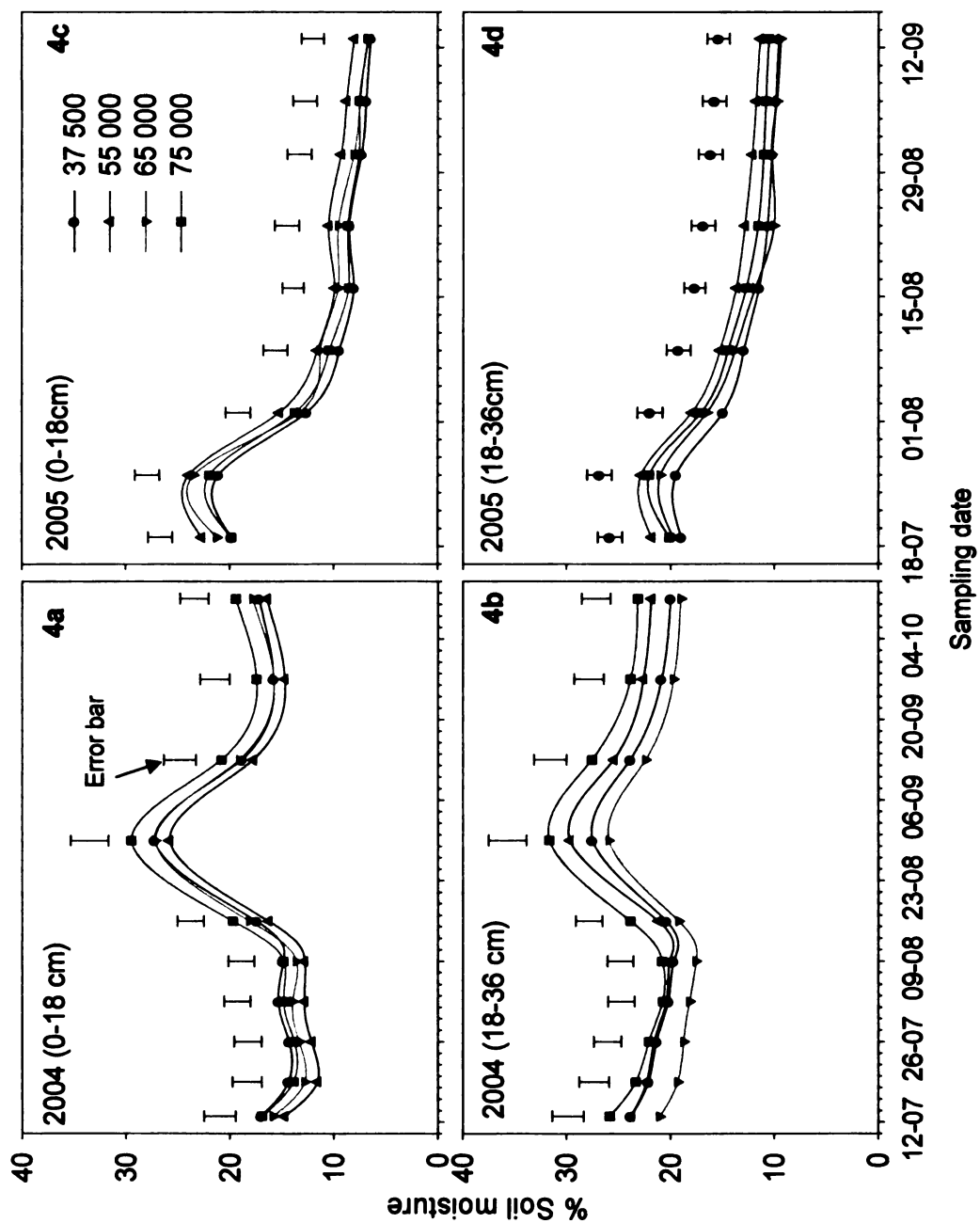


Figure 4. Weekly percent volumetric soil moisture at 0 to 18 and 18 to 36 cm depths at four corn densities across management practices during the 2004 (4a and b) and 2005 (4c and d) growing seasons at Kellogg Biological Station, Hickory Corners, MI. Within week error bars are similar for all treatments.

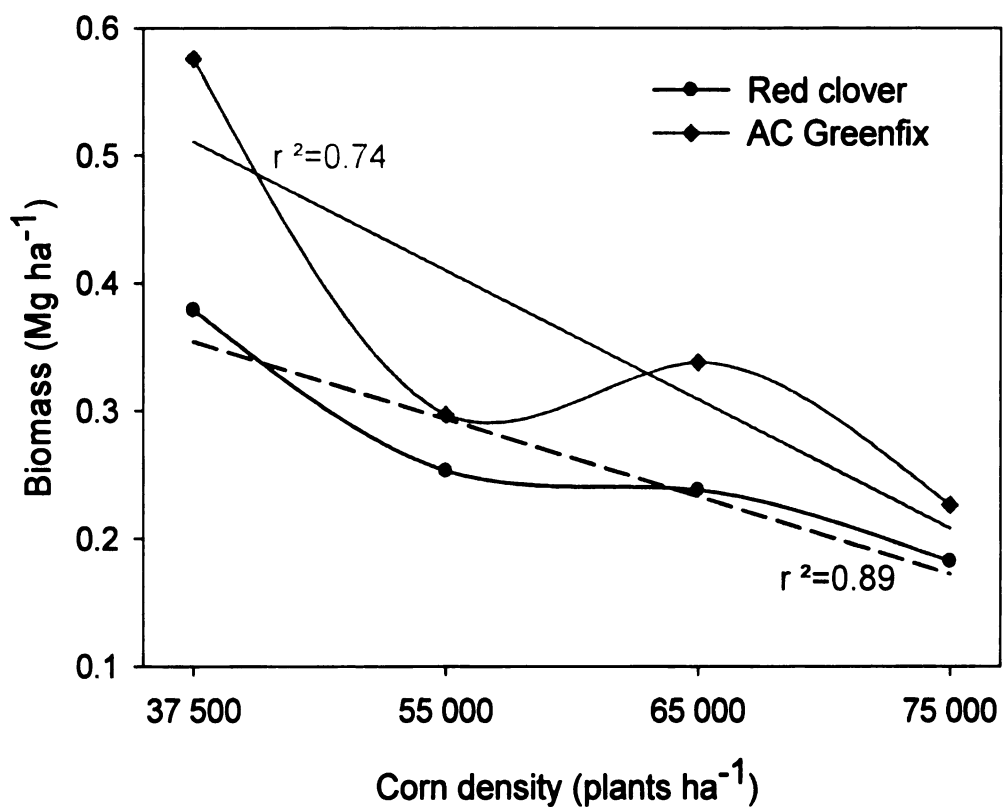


Figure 5. Relationship between interseeded cover crop dry matter and corn plant density from 2002 to 2004. Data are averaged across years. Each point is the mean of 10 samples.

Chapter two

Effect of Corn Density on Corn and Cover Crop Nitrogen in an Interseeding System

Abstract

Little is known about the effect of an interseeding system at various corn (*Zea mays* L.) densities on nitrogen (N) concentration and content of corn or cover crops. Field assessment and laboratory analysis of plant tissues can help to evaluate the effect of crop management on crop N. A study was conducted to evaluate (1) the effect of corn density (37 500 to 75 000 plants ha⁻¹) and plowed red clover (*Trifolium pratense* L.) versus mineral N fertilizer on corn N concentration and content during and at the end of the growing season; and (2) the impact of corn density on N concentration and accumulation of interseeded red clover or AC Greenfix (*Lathyrus sativus* L.). Both chlorophyll content and ear leaf N concentration were used to assess corn N status during the growing season. At the end of the growing season, N was measured in corn grain, leaves and stalks. Interseeded cover crop N was measured in fall of the growing season and the subsequent spring. Grain N concentration was higher at low corn density, with a decreasing trend as corn plant density increased. Grain N concentration was the highest in corn supplied with mineral N fertilizer. In three of the four years when rainfall was optimal, grain N content of corn supplied with mineral N fertilizer was the highest and accumulated up to 140 kg ha⁻¹.

Chlorophyll content and ear leaf N concentration were the highest in the lowest corn density and in corn supplied with N fertilizer. Chlorophyll content and ear leaf N at silking were good indicators of corn grain N concentration at the end of the growing season.

In the fall, N concentration of interseeded red clover and AC Greenfix was not affected by corn plant density. Nitrogen concentration of monoculture cover crops was similar to N concentration of interseeded cover crops, except for AC Greenfix in 2005. In the fall, N accumulation of interseeded cover crops at low corn density was significantly higher compared to higher plant densities, ranging from 2.18 to 15.58 and 5.0 to 20.25 kg ha⁻¹ for red clover and AC Greenfix, respectively. Monoculture red clover (20.62 to 48.15 kg ha⁻¹) and AC Greenfix (75.41 to 81.80 kg ha⁻¹) accumulated more N than interseeded red clover and AC Greenfix respectively.

There was a seasonal effect on cover crop dry matter in the subsequent spring. The subsequent spring, in two of the three years, interseeded red clover N concentration was highest at 37 500 plants ha⁻¹. Monoculture red clover accumulated more N (234.41 kg ha⁻¹) by the subsequent spring compared with interseeded red clover (58 to 162.3 kg ha⁻¹). Red clover can be interseeded at high corn plant densities and accumulate significant N the subsequent spring, sufficient to meet N demand of the following crop.

Introduction

Crop production and quality can be influenced by management practices. The use of management practices such as cover crops, nitrogen (N) fertilizer and interseeding can affect crop N concentration and uptake (Jeranyama et al., 1998; Eghball and Power, 1999; Sainju and Singh, 2001; Sweeney and Moyer, 2004). Legume cover crops provide a potential to meet the N demand of crops and to reduce the reliance on N fertilizer in agricultural production. Nitrogen source, such as the use of mineral or organic N can also affect crop yield and other quality attributes such as taste of tomatoes (*Lycopersicon esculentum* L.) (Heeb et al., 2005). Several studies evaluated the effect of N source on N concentration and content of various crops. Total N uptake of corn (*Zea mays* L.) fertilized with synthetic N was greater than in corn supplied with manure and compost (Eghball and Power, 1999; Eghball et al., 2004). Corn grain N concentration and uptake were influenced by varying N rates (Katsvairo et al., 2003). Marino et al. (2004) showed that N concentration and uptake of forage increased with increased N fertilizer rates. Similarly, Sweeney and Moyer (2004) found that N uptake by sorghum (*Sorghum bicolor* L.) increased with N fertilizer rates. In addition to increased N concentration with increased N fertilizer rates, Fan et al. (2004) observed a significant variation in N concentration of wheat (*Triticum aestivum*) in relation to N fertilizer source (Urea versus coated urea). Grain N uptake of wheat was higher with coated urea compared with common urea. Cover crop use can affect N concentration and uptake of crops. In comparing crop N uptake in various management systems, Sweeney and Moyer (2004) found that sorghum

following red clover (*Trifolium pratense*) accumulated more N than continuous sorghum with no previous cover crop. Balkcom and Reeves (2005) observed that corn grain N content was higher following sunn- hemp (*Crotalaria juncea* L.) than when corn followed fallow. Similarly, Jeranyama et al. (1998) found that N content of corn following interseeded medics (*Medicago polymorpha* and *M. scutellata* L.) was higher than in corn without medic. Vyn et al. (1999) found that whole plant N content of corn at anthesis was strongly affected by cover crop species, with corn following annual ryegrass (*Lolium multiflorum*) averaging one-half of the total N content of that observed after red clover. Monoculture or interseeding systems can also affect N concentration and content/accumulation of companion crops. Some studies have suggested that N accumulation of crops can increase with interseeding, whereas others have suggested no increase in total N yield (Carr et al., 1998; Hauggaard-Nielsen et al., 2001; Villar-Mir et al., 2002). Crude protein yield of berseem clover was lower in monoculture compared with interseeded berseem clover into oat (*Avena sativa* L.) (Ross et al., 2005). Yield and N content of corn and cowpea (*Vigna unguiculata*) were reduced when intercropped compared with monocropping, however N concentration was not affected (Ofori and Stern, 1986). When grown in monoculture or intercropped, barley (*Hordeum vulgare* L.) accumulated similar amount of aboveground N, however total N accumulation by field pea (*Pisum sativum* L.) was less when intercropped than as a monoculture crop (Hauggaard-Nielsen et al., 2001). In contrast, Szumigalski and Van Acker (2006) suggested that greater N concentrations were seen in wheat and canola when intercropped

with field pea. In comparing two cover crops in an interseeding system, Abdin et al. (1998) observed a lower concentration of grain protein when corn was interseeded with hairy vetch (*Vicia villosa* Roth) in comparison with subterranean clover (*Trifolium subterraneum*), red clover-rye mixture or a control. Increased plant density of intercrops can also affect N concentration and uptake of companion crops. Ross et al. (2005) showed that when oat was interseeded with berseem clover, crude protein was reduced with increased oat density compared with monoculture crops. Similar findings were obtained by Carr et al. (1998) who suggested that forage crude protein decreased as barley (*Hordeum vulgare*) density increased. Thorsted et al. (2006) found that increased wheat density reduced grain N concentration. Corn forage crude protein decreased with increased corn density (Cusicanqui and Lauer, 1999; Widdicombe and Thelen, 2002). Little is known about the effect of an interseeding system at various corn densities on N concentration and content of corn or cover crops.

These studies investigated the effect of monoculture or interseeded cover crop and/or various rates of fertilizer on corn N concentration and content/uptake. No study has looked at the effect of combination of N source versus corn density and interseeding versus corn density on N concentration and content of corn and cover crops. A study and comparison of red clover derived-N versus N fertilizer at various corn densities is needed to assess N concentration and content of corn. In addition, there is a need for an evaluation of the effect of interseeding system at various corn densities on N concentration and content of corn when interseeded with red clover or AC Greenfix.

Cover crop N accumulation and availability to a succeeding crop depend on cover crop species, environmental conditions, and management (Hesterman et al., 1992; Dekker et al., 1994; Stute and Posner, 1995). AC Greenfix (*Lathyrus sativum* L.), a variety of chickling vetch, has the potential to produce 90-112 kg ha⁻¹ of N in 8-10 weeks after planting (DFS, 2003). Rao et al. (2005) showed that at full bloom, AC Greenfix N concentration was 26.2 g kg⁻¹ and produced 168 kg ha⁻¹ of total N. Conversely, lentil contained 26.3 g kg⁻¹ N and accumulated only 53 kg ha⁻¹ of N. Red clover has the potential of accumulating 79-168 kg ha⁻¹ of N in a growing season (Bowman et al., 1998). Shrestha et al. (1998) showed a variation in crude protein concentration of annual medics (*Medicago spp.*), berseem clover (*Trifolium alexandrinum* L.), and alfalfa (*Medicago sativa* L.). Similarly, Alford et al. (2003) observed differences in crude protein of forages including alfalfa, sweet clover (*Melilotus officinalis* Lam) and various cultivars of medics. Sampling time can also affect N concentration in companion crops. Sainju and Singh (2001) found that biomass, N concentration and N accumulation of hairy vetch increased with late sampling compared to early sampling. In contrast, Marino et al. (2004) showed that annual grass N concentration decreased with time. Alford et al. (2003) observed a decrease in crude protein from 45 to 40 % of annual legumes from a July to November sampling time. There is a need to assess whether interseeded cover crops can perform similarly to monoculture cover crops and accumulate sufficient N to meet the needs of a subsequent crop. Supplying N through interseeded cover crops is an alternative to monoculture cover crops and provides the advantage of

producing a cash crop. An assessment of cover crop N accumulation, when in monoculture or interseeded into various plant densities, in the fall and the subsequent spring can help estimate its potential for N contribution to a subsequent crop.

Measurements of N status in corn

Various methods are used to evaluate corn N status in situ and at the end of the growing season. Methods include use of chlorophyll meter [(Minolta SPAD-502 meter), Spectrum Technologies, Inc. Plainfield, Illinois] and ear leaf analysis. These methods can help to assess whether N availability may have contributed to observed differences in grain N uptake or yield. The chlorophyll meter assesses the degree of greenness, which is an indication of chlorophyll content and leaf N concentration during the growing season (Varvel et al., 1997). A correlation of $R=0.78$ ($p=0.001$) has been reported between measured SPAD-502 meter values and leaf N concentration in corn (Bullock and Anderson, 1998). Chlorophyll content also can be used as an indicator of N uptake and corn yield (Eghball and Power, 1999). Chlorophyll content can be affected by N management and weather conditions such as wet or dry growing conditions (Hussain et al., 2000). Scharf et al. (2002) showed that relatively wet conditions during the growing season led to greater apparent N stress and lower chlorophyll readings. Ear leaf N has been used as a tool to assess in-season corn N status. Scott et al. (1987) found no significant differences in ear leaf N concentration of corn following legume cover crops compared with corn supplied with N fertilizer.

The above tools have been used in comparing various rates of fertilizer or cover crops on N content of corn. No study has looked at the use of these tools in assessing N status of corn in an interseeding system at various corn densities. There is a need to evaluate the effect of N source, interseeding and corn density on corn N status using a chlorophyll meter and ear leaf N analysis.

Research in Michigan has investigated the effect of interseeding cover crop on yield and N content of companion crops (Hesterman et al., 1992; Jeranyama et al., 1998). However, no study has looked at the impact of corn density on N concentration and content of corn and cover crops in an interseeding system. The objectives of this study were to: (1) compare the effect of mineral N fertilizer and monoculture red clover derived-N on corn N at various corn densities; (2) assess the effect of corn density in an interseeding system on red clover-N and AC Greenfix-N in fall and the subsequent spring; (3) assess the effect of interseeding and corn density on corn-N using the in- season test (ear leaf and chlorophyll content) versus end-season N test (plant analysis).

Materials and Methods

Site description

Field studies were conducted from 2002 to 2005 at the Kellogg Biological Station (KBS) in Hickory Corners, Michigan. The soil types at KBS were the Kalamazoo (fine-loamy, mixed, mesic Typic Hapludalfs) and Oshtemo (coarse-loamy, mixed, mesic Typic Hapludalfs) series (Crum and Collins, 2004).

The research was conducted on a different field each year to permit planting on site following red clover plow down. Corn research plots were established in 2002 (Field 1), 2003 (Field 2), 2004 (Field 3) and 2005 (Field 4). In the year prior to corn establishment, red clover was planted in each field into wheat stubble in July-August except in 2001, when red clover was established in corn stubble. Red clover was chisel-plowed the subsequent spring before corn planting in order to serve as a N source for the non-conventional treatments.

Experimental design

Each year the experiment was replicated four times except in 2003 where only two replications were used due to poor corn stand caused by wildlife damage. The experimental design was a split-plot with four corn densities and four management practices. The main-plots were four corn densities (37 500, 55 000, 65 000 and 75 000 plants ha⁻¹). Sub-plots were four management practices: (1) Conventional management, corn seeded into wheat stubble with N fertilizer applied (CMNF); (2) Corn seeded into plowed red clover, no N fertilizer, interseeded with AC Greenfix (PRIA); (3) Corn seeded into plowed red clover; no N fertilizer, interseeded with red clover (PRIR) ; (4) Corn seeded into plowed red clover, no N fertilizer, not interseeded with cover crop (PRNI). Based on the Preside-dress Nitrate Test (PSNT) results, N fertilizer was applied to the CMNF treatment up to a total of 140 kg ha⁻¹ every year.

Corn

The hybrid Great Lakes 4979 (Great Lakes Hybrids), relative maturity 99 days, was planted into 6-row plots of 4 by 4.5 m on 29 May 2002 and 06 June 2003. In 2004 and 2005, Pioneer Hybrid 38P05, relative maturity 93 days, was planted on 30 May and 27 May respectively, into 6-row plots of 5 by 4.5 m. The trials were planted at approximately 100,000 plants ha⁻¹. Two weeks after emergence, each plot was hand-thinned to the appropriate corn density. In season corn N status was measured only during the 2004 and 2005 growing seasons using chlorophyll meter and ear leaf N. Whole plant (grain, leaf and stalk) corn N was assessed at the end of the growing season from 2002 to 2005.

Chlorophyll content and ear leaf N

A chlorophyll meter (Minolta SPAD-502), was used to measure leaf chlorophyll content of corn during the 2004 and 2005 growing seasons. The procedure described by Piekielek et al. (1995) was used in collecting SPAD-502 meter readings. SPAD meter readings were taken at 1 to 2 cm from the edge of the leaf and two-thirds to three-quarters of the leaf length from the base. Damaged and diseased leaves were avoided.

Chlorophyll meter readings were taken six and five times in 2004 and 2005, respectively. In 2004, chlorophyll readings were taken on 10 July, 16 July, 23 July, 1 August, 6 August and 15 August. In 2005, chlorophyll readings were taken on 7 July, 15 July, 21 July, 29 July and 5 August. The average of ten chlorophyll meter readings (10 randomly selected plants per plot) was measured

starting at the V8-V9 growth stage. Measurements were taken on the uppermost top fully expanded leaf. Once corn reached the VT stage, measurements were taken on the ear leaf (leaf at the base of the primary ear).

The ear leaf was collected at corn silking stage for N analysis. Ten leaves were randomly collected from ten plants from each plot in the four center rows on 15 August 2004 and 05 August 2005. Ear leaf samples were dried in an oven at 60° C for 2 days, and then weighed to determine the dry matter (DM). Ear leaf N concentration was determined using Total Kjeldahl Nitrogen (TKN) analysis, described later.

Corn whole plant N

Corn was harvested from the four center rows of each plot using a combine on 17 October 2002, 29 October 2003, 10 November 2004 and 06 October 2005. Corn plants for N analysis were sampled 1-4 days prior to harvest, and separated into grain, leaves and stalks. In 2002 and 2003, two plants were collected from each plot. In 2004 and 2005 however, ten plants were collected from each plot. Grain yield was a summation of combine and hand harvested corn. Yield was adjusted to 155 g kg⁻¹ moisture content. The TKN procedure was used to measure grain, leaf and stalk N concentration.

Cover crop N

Red clover and AC Greenfix were interseeded when corn plants were between V5-V7 corn growth stages, the first two weeks of July each year.

In 2004 and 2005, monoculture red clover and AC Greenfix treatments were established at the time of interseeding to compare biomass production with interseeded cover crops. Red clover was broadcast with a hand-seeder at the seeding rate of 20.4 kg ha⁻¹ and AC Greenfix was hand-broadcast at the rate of 90 kg ha⁻¹. Red clover and AC Greenfix aboveground biomass (leaves and stem) were hand-clipped when AC Greenfix was at full bloom by removing plants from a random quadrat of 0.209 m² in each plot. Cover crop samples were dried in an oven at 60° C for 2 days, and then weighed to determine the DM. Dry matter of interseeded cover crops was determined in the fall during the year of establishment on 27 September 2002, 02 October 2003 and 23 August 2004. In 2005, only interseeded AC Greenfix was sampled on 08 August because interseeded red clover did not germinate due to dry, hot weather conditions. Monoculture cover crops were sampled on 23 August 2004 and 08 August 2005. The subsequent spring red clover was sampled on 02 June 2003, 02 June 2004 and 01 June 2005. The TKN was used to assess N concentration of red clover and AC Greenfix.

Total Kjeldahl nitrogen and N calculation

All corn (ear leaf, grain, leaf and stalk) and cover crop (aboveground biomass) tissue samples were ground in a Wiley mill to pass through 1-mm screen. All samples were digested using a 40-tube Tecator Model 1016 Digester (Tecator, Höganäs, Sweden). Cover crop and corn tissue samples of 0.1 g were digested in 4 ml of 18 M H₂SO₄ with 1.5 g K₂SO₄ and 0.015 g Se catalyst in 100

ml-constricted tubes. All samples were digested at 350° C for 4 hours. The tissue extracts were analyzed using a Lachat Flow Injection Analyzer (Hachat Co., Loveland, Colorado). Total N content or accumulation of corn grain or cover crop was calculated as the product of DM yield and N concentration.

Statistical analysis

All data were analyzed using Proc Mixed in Statistical Software Package SAS version 8.2 (SAS, 2001). Plant density and cropping system were considered fixed effects. Two error terms were considered in the analysis of the data, one associated with the whole plot (plant density) and the other associated with the subplot (management practices) and the interaction (plant density x management practices). When interaction effects were found to be statistically significant, means separation was conducted for respective cell means. When main effects were significant while interactions were not, means separation was conducted for marginal means. Effects were considered statistically significant at $p = 0.05$. Data of weekly chlorophyll content of corn leaves was analyzed as repeated measurements.

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Results and Discussion

Weather patterns

Total monthly Precipitation and monthly average temperature (minimum and maximum) data from the 2002 to 2005 growing seasons were obtained from the Long-Term Ecological Research weather station (LTER-Weather, 2006). In 2002, there was a drought period in June and July, and the average rainfall in June was below the 30-year average (Figure 1a). Precipitation in June and July during the 2003 growing season was lower than the 30-year average (Figure 1a). Seasonal total precipitation in 2004 was above the 30-year average (Appendix A) and well distributed throughout the growing season (Figures 2). Precipitation in April, May and August during the 2005 growing season was lower than any other growing season and lower than the 30-year average (Figure 1a). Although total precipitation in the 2005 growing season was lower than the 30-year average, rainfall occurred at critical corn growth stages in June and July. The monthly average minimum temperature for April 2003 was lower than to the 30-year average (Figure 1b). Monthly average maximum temperatures during the 2005 growing season from June to September were higher than the 30-year average.

Corn grain N

Effect of corn density on corn grain N concentration

Plant density influenced N concentration in corn grain (Table 1). In 2002 and 2004, the lowest plant density, 37 500 plants ha⁻¹, had a significantly higher grain N concentration than all other plant densities. In 2003, grain N concentration at 37 500 plants ha⁻¹ was significantly higher than 65 000 and 75 000 plants ha⁻¹. In 2005, grain N concentration at 37 500 plants ha⁻¹ was significantly higher than other corn densities, and 55 000 plants ha⁻¹ was only significantly higher than 75 000 plants ha⁻¹. There was an interaction between plant density and year on corn grain N concentration (Table 2). This may have been due to climatic conditions. There was no corn yield increase at high plant density when moisture was limiting, suggesting a nutrient competition or low N uptake.

The four-year average showed that increased plant density decreased N concentration in grain with no significant differences between 65 000 and 75 000 plants ha⁻¹ (Table 1). These results corroborate the results by Thorsted et al. (2006) who also observed a decline in grain N concentration as wheat plant density increased. Similarly, Widdicombe and Thelen (2002), observed a decrease in crude protein of forage corn as corn density increased. In contrast, Shapiro and Wortmann (2006) observed no effect of corn plant density on grain N concentration. Grain N concentration was negatively, but significantly correlated to corn density with very low correlation coefficients of -0.37 (p=0.0027), -0.63 (p<0.0001), -0.34 (p=0.0057) and -0.41 (p=0.0008) for the

2002, 2003, 2004 and 2005 growing seasons respectively (Table 3). Across years, there was a significant negative correlation of -0.32 ($p < 0.0001$) (data not shown).

Effect of management practices on corn grain N concentration

Corn grain N concentration was influenced by management practices (Table 1). In 2002, 2004 and 2005 grain N concentration in CMNF was significantly higher than in other treatments. In 2003, grain N concentration differed only between CMNF and PRIA.

Corn grain N concentration ranged from 10.9 to 14.8 g kg⁻¹. These values are similar to those obtained by Brouder et al. (2000) who observed corn grain concentration ranging from 10.6 to 16.5 g kg⁻¹. Corn grain N concentration in 2002 and 2003 seemed to be higher than corn grain N concentration in 2004 and 2005. This may be related to the hybrid used during these two sets of years. Great Lakes Hybrid discontinued GL 4973 in 2003, so we switched to Pioneer 38P05 for the 2004 and 2005 growing seasons. Widdicombe and Thelen (2002) observed a variation in crude protein of forage by hybrid type, with dual-purpose hybrids containing higher crude protein than the full-season leafy hybrid. When averaged across four years, the data showed that corn supplied with N fertilizer had a significantly higher grain N concentration than corn planted into plowed red clover (Table 1).

Effect of management practices on corn grain N content

There was a seasonal (year) effect on corn grain N content (Table 4). There was an interaction between year and management practices on corn grain

N content (Table 2). This was due to dry weather conditions after N side dressing the CMNF treatment in 2002, resulting in reduced corn grain yield and hence reduced N content. In 2002, grain N content was lower in CMNF compared with PRIA, PRIR and PRNI (Table 4). In 2003, N content was higher in CMNF compared with PRIA. In 2004 and 2005, corn grain N content was higher in CMNF compared with PRIA, PRIR and PRNI. When rainfall was optimal, corn grain N content was the highest in corn supplied with N fertilizer compared with corn planted into plowed red clover (Table 4 and Figure 2). When averaged across four years, the data showed that corn supplied with N fertilizer had a significantly higher grain N content compared with corn planted into plowed red clover (Table 4). These findings are in accordance with results of Jeranyama et al. (1998) who showed low N content in corn grain obtaining N from cover crops rather than mineral N fertilizer.

Effect of interseeding system on grain N concentration and content

No significant difference was observed in grain N concentration and content when corn was grown in monoculture (PRNI) compared to corn interseeded with red clover (PRIR) or AC Greenfix (PRIA) (Tables 1 and 2). In contrast to our results, Sangakkara et al. (2003) found that the use of a green manure as an intercrop reduced corn grain N content. Similarly, Ofori and Stern (1986) found that corn grain N content was reduced by intercropping. However they found that corn grain N concentration was not reduced by intercropping.

Corn Leaf N

Both management practices and corn density had an interaction on N concentration in corn leaf (Table 2). This interaction may have been due to differences in climatic conditions during various growing seasons. In 2002 for instance, dry conditions resulted in no difference among treatments due to low N uptake by corn planted in CMNF.

Corn density affected N concentration in corn leaf at the end of the growing season (Table 5). In 2002, corn density at 75 000 plants ha⁻¹ had a significantly lower leaf N concentration than all other densities. No difference was observed in leaf N concentration in corn density during the 2003 growing season. In 2004, leaf N concentration was higher at 37 500 plants ha⁻¹ but was significantly different only from 65 000 plants ha⁻¹. In 2005, only leaf N concentration at 37 500 plants ha⁻¹ was significantly higher than 65 000 plants ha⁻¹. The four-year average shows that plant density at 37 500 plants ha⁻¹ was only significantly higher than 65 000 and 75 000 plants ha⁻¹.

Leaf N concentration varied from year to year in management practices (Table 5). In 2002, leaf N concentration was the same under all management practices. In 2003, across corn density, leaf N concentration was higher in CMNF and PRIR compared with PRIA and PRNI. In 2004, leaf N concentration was higher in CMNF but only significantly different from PRIR and PRNI. In 2005, leaf N concentration was significantly higher in CMNF compared with corn planted into plowed red clover. The four-year average for leaf N concentration was higher in CMNF compared with corn planted into plowed red clover. The four-year

average suggests that leaf N concentration was significantly higher in PRIR than in PRIA or PRNI. No clear pattern was observed in leaf N concentration in interseeding compared to no interseeding system.

Corn stalk N

There was a seasonal effect on stalk N concentration (Table 2). In 2002, 2004 and 2005 there was no significant difference in stalk N concentration among corn densities. In 2003, stalk N concentration at 65 000 plants ha⁻¹ had a lower N concentration compared to other plant densities (Table 6). The four-year average showed no difference in stalk N concentration among various corn densities. In 2002, stalk N concentration was significantly different in PRNI compared with CMNF (Table 6). In 2003 and 2004, no difference was detected in management practices with regard to stalk N concentration. In 2005, stalk N concentration was significantly higher in CMNF and PRIA treatments compared to PRNI (Table 6). The four-year average for stalk N concentration showed no significant differences within management practices.

Chlorophyll content

Effect of corn density on chlorophyll content

In 2004, there was a weekly variation in chlorophyll content of corn leaves in plant density (Table 7). Across management practices, chlorophyll content on 10 July was similar at all plant densities. On 16 July, the only difference was observed between 37 500 and 55 000 plants ha⁻¹. However, from the 23 July up

to 15 August, chlorophyll content was consistently higher in the lowest corn density (37500 plants ha⁻¹) compared with higher plant densities.

In 2005 similarly to 2004, 37 500 plants ha⁻¹ had the highest chlorophyll content compared to higher corn densities, except on 29 July where the three lowest plant densities were significantly higher than 75 000 plants ha⁻¹ (Table 8). On 7 July, 55 000 and 65 000 plants ha⁻¹ were higher than 75 000 plants ha⁻¹. On 15 July chlorophyll content decreased as plant density increased with significant difference at all four corn densities. On 21 and 29 July, and 5 August, chlorophyll content continued to be lower at higher corn densities with no significant difference between 65 000 and 75 000 plants ha⁻¹ on 21 July and 5 August.

Overall, chlorophyll content of ear leaf in 2005 was higher than in 2004 with a decreasing trend as corn plant density increased. There was also an interaction between corn density and sampling time on chlorophyll content in 2004. This may have been due to differences in plant stand as we did not achieve the targeted highest plant density in all plots in 2004. Chlorophyll content was negatively correlated to corn plant density in both years. In 2004, we observed low correlation coefficient values of -0.34 ($p<0.0062$), -0.50 ($p<0.0001$), -0.38 ($p=0.0021$), -0.35 ($p=0.0047$) for 23 July and 1, 6 and 15 August, respectively (Table 3). In 2005, we observed high correlation coefficient between chlorophyll content and plant density of -0.62 ($p<0.0001$), -0.83 ($p<0.0001$), -0.79 ($p<0.0001$), -0.64 ($p<0.0001$) and -0.57 ($p<0.0001$) for 7, 15, 21 and 29 July, and 5 August, respectively (Table 3).

Effect of management practices on chlorophyll content

In 2004, weekly chlorophyll content of corn leaves varied with management practices (Table 7). On 10 July, no clear pattern was observed between corn planted into plowed red clover and corn supplied with N fertilizer, whereas on 16 July, no significant difference was observed among treatments. On 23 July, chlorophyll content in CMNF was higher than corn planted into plowed red clover, except with PRIR. From the fourth sampling up to the last sampling, chlorophyll content of corn supplied with N fertilizer (CMNF) was consistently higher than chlorophyll content of corn planted into plowed red clover (PRIR, PRIA and PRNI) (Table 5). This differentiation among treatments may be due to increased N uptake overtime in CMNF.

In 2005, we had fewer sampling dates than in 2004 due to fast corn growth as monthly average temperatures were above the 30-year average during the months of June and July (Table 8 and Figure 1b). At the first sampling chlorophyll meter readings in PRIR, PRIA and PRNI were significantly higher than CMNF, but no clear pattern was observed between the interseeding and no interseeding systems, although PRNI was significantly higher than PRIR. On 15 and 21 July, chlorophyll content was not significantly different among treatments. On 29 July and 5 August, chlorophyll content in CMNF was significantly higher than all treatments planted into plowed red clover (Table 8).

There was an interaction between management practices and sampling time on chlorophyll content in both years. There were very high chlorophyll readings on 21 and 29 July and 05 August in 2005. This may be due to dry, high

temperatures that occurred in 2005 compared to wet and cool conditions in 2004. These results parallel the higher N concentration and content in the grain, and the higher leaf N concentration in the CMNF treatment. These findings corroborate results by Scharf et al. (2002), who observed higher chlorophyll meter readings on corn in dry years compared to wet growing seasons.

Similarly to corn grain N concentration, CMNF and 37 500 plants ha⁻¹ had higher chlorophyll content for ear leaf compared to corn planted into plowed red clover and higher plant densities. Chlorophyll content of ear leaf was a good indicator of corn grain N concentration with a positive correlation coefficient (Table 3). In 2004, correlation coefficients between chlorophyll content of ear leaf and corn grain N concentration were 0.66 ($p<0.0001$) and 0.62 ($p<0.0001$) for 6 and 15 August, respectively. In 2005, correlation coefficients between chlorophyll content of ear leaf and corn grain N concentration were 0.43 ($p=0.0004$) and 0.44 ($p=0.0003$) on 21 and 29 July, respectively (Table 3). When combined, the two-year data suggested that the two last samplings of chlorophyll content of ear leaf were good indicators of corn grain N content with positive correlation coefficients of 0.54 ($p<0.0001$) and 0.55 ($p<0.0001$) (data not shown). These findings are similar to those obtained by Eghball and Power (1999) who found that chlorophyll meter readings did provide a good indication of N uptake in a wet not in a dry growing season. In contrast to Eghball and Power (1999) our results showed that both wet and dry years provided a good indication of corn grain N concentration.

Ear leaf N

In both years as expected, ear leaf DM at 37 500 plants ha⁻¹ was higher than that at higher plant densities. In 2005, ear leaf DM at 55 000 and 65 000 plants ha⁻¹, were significantly higher than that at 75 000 plants ha⁻¹. Ear leaf DM at silking showed no significant difference due to management practices in 2004, but in 2005 DM in CMNF was significantly lower than PRIR and PRIA but not in PRNI (Table 9).

Ear leaf N concentration, in both years, was significantly higher in corn supplied with N fertilizer than in corn planted into plowed red clover. Conversely, Scott et al. (1987) found that ear leaf N concentration of corn following various legume cover crops was not different from controls that received between 56 and 112 kg ha⁻¹ of N fertilizer. In both years, ear leaf N concentration was the highest in plants grown at 37 500 plants ha⁻¹. In 2005, ear leaf N concentration was also higher in plants grown at 55 000 plants ha⁻¹ than in plants grown at 65 000 plants ha⁻¹.

Ear leaf N content varied with corn density and management practices (Table 9). There was an interaction between plant density and year on ear leaf N content (Table 2). This may have been due to differences in plant stand as we achieved the targeted high plant density in all plots in 2005 but not in 2004. In 2004, N content of corn supplied with N fertilizer was higher compared with corn planted into plowed red clover; however in 2005 significant differences were only detected between CMNF and PRNI. N content of corn at 37 500 plants ha⁻¹ in 2004 and 2005 was higher compared with N content at greater plant densities;

however in 2005 N content of corn grown at 55 000 plants ha⁻¹ was significantly higher than in plants grown at 65 000 and 75 000 plants ha⁻¹. There was a positive correlation coefficient of 0.51 (<0.0001) in both years between corn ear leaf and corn grain N concentration.

Effect of corn density on interseeded cover crop N

Nitrogen concentration and accumulation in interseeded cover crops varied between cover crops species (Table 10). We observed an interaction between cover crop species and year on fall N concentration and accumulation. This may be explained by variation in climatic conditions that occurred in 2005.

In 2002, N concentration ranged from 32.7 to 34.6 g kg⁻¹ for red clover and 31.5 to 33.9 g kg⁻¹ for AC Greenfix but differences were not significant in either cover crop (Table 10). In 2003, N concentration was similar at all plant densities within species ranging from 30.4 to 31.5 and from 29.0 to 34.3 g kg⁻¹ for interseeded red clover and AC Greenfix, respectively. In 2004, N concentration of interseeded red clover and AC Greenfix was similar within species at all plant densities. In 2005, interseeded red clover did not germinate due to dry, hot weather conditions. In 2005, no difference was observed in N concentration of interseeded AC Greenfix at any plant density. There was a trend of higher N concentration for interseeded AC Greenfix in 2004 and 2005 when compared to 2002 and 2003. However, interseeded red clover N concentration values seem to be similar across years.

There was a seasonal effect on cover crop N accumulation (Table 2). In 2002, N accumulation of interseeded red clover was highest at 37 500 plants ha⁻¹ but was only significantly different from N accumulation at 65 000 and 75 000 plants ha⁻¹ (Table 10). Similarly, N accumulation of interseeded AC Greenfix was highest at 37 500 plants ha⁻¹ but was only significantly different from N accumulation at 75 000 plants ha⁻¹. In 2003, N accumulation of interseeded red clover was lowest when compared to other growing seasons and no significant differences were noticed among plant densities (Table 10). However, N accumulation of interseeded AC Greenfix was significantly higher at 37 500 plants ha⁻¹ compared to other plant densities. The low N accumulation of interseeded red clover in 2003 was due to poor germination and growth of red clover compared to other growing seasons. In 2004, N accumulation of interseeded red clover at 37 500 plants ha⁻¹ was higher than other corn plant densities, except at 55 000 plants ha⁻¹. In 2004, Interseeded AC Greenfix N accumulation was higher at 37 500 plants ha⁻¹ compared with other plant densities. In 2005, N accumulation of interseeded AC Greenfix was only significantly different between 37 500 plants ha⁻¹ and 75 000 plants ha⁻¹. Differences observed in N accumulation were mainly related to DM matter production. Results similar to our findings were obtained by Hively and Cox (2001) who observed low N accumulation of interseeded cover crops of 2 to 6 kg ha⁻¹. Interseeded red clover and AC Greenfix N accumulation are in the range of those obtained in corn interseeded with medics (2.1 to 32 kg ha⁻¹; Jeranyama et al., 1998), red clover and hairy vetch (8 to 29 kg ha⁻¹; Scott et al., 1987), and

similar to oat interseeded with Nitro alfalfa and mammoth red clover (10 to 14 kg ha⁻¹; Hesterman et al., 1992).

Monoculture cover crop N

In 2004 and 2005, monoculture cover crops were included in our experiment for comparing N concentration and accumulation of monoculture with interseeded cover crops (Table 10). In 2004, N concentration of monoculture red clover was only higher than interseeded red clover at 55 000 and 75 000 plants ha⁻¹. In 2004, N concentrations of monoculture and interseeded AC Greenfix were similar whereas in 2005, N concentration of monoculture AC Greenfix was higher than interseeded AC Greenfix at all plant densities. Nitrogen concentration of monoculture red clover was significantly lower in 2004 than in 2005. Similarly, N concentration of AC Greenfix was significantly lower in 2004 than in 2005. This may be related to dry, hot conditions that occurred in 2005.

In 2004 and 2005, monoculture cover crops accumulated more N than interseeded cover crops (Table 10). Our findings are similar to results obtained by Jeranyama et al. (1998) who obtained N accumulation of up to 75 kg ha⁻¹ for clear-seeded medics. Although higher N concentrations were observed in 2005 than in 2004, N accumulation for both cover crops was the highest in 2004 compared with 2005. This was due to high DM of both cover crops resulting from good and well distributed rainfall that occurred in 2004 (Figure 2).

N of interseeded cover crop the subsequent spring

Nitrogen concentration of interseeded red clover in the subsequent spring varied from year to year (Table 11). In spring 2003, N concentration of red clover previously interseeded at 37 500 plants ha⁻¹ was only significantly different compared with plants at 75 000 plants ha⁻¹, whereas in spring 2004 it was only significant from plants at 65 000 plants ha⁻¹. In 2005, no differences were observed, even with monoculture red clover. N concentration of interseeded red clover in the fall (year of establishment) was higher than N concentration of interseeded red clover the subsequent spring (Table 12). These results corroborate findings of Marino et al. (2004) and Alford et al. (2003) who observed a decrease in N concentration of cover crops with sampling time.

Interseeded red clover accumulated more N in the subsequent spring compared to the fall sampling. There was a seasonal effect on N accumulation of interseeded cover crop in the subsequent spring (Table 2). In spring 2003, N accumulation of red clover was only significantly different between 37 500 and 75 000 plants ha⁻¹. However in spring of 2004 and 2005, no significant differences were observed in interseeded red clover N accumulation. Red clover N accumulation was the highest in 2005 followed by 2003 and 2004. Spring red clover N accumulation increased in the range of 7-28 times of fall N accumulation (Tables 10 and 11). In the spring of 2005, N accumulation of monoculture red clover was significantly higher than previously interseeded red clover.

Conclusion

Corn density affected grain N concentration with high grain N concentration at low corn density. Interseeded cover crops did not affect corn grain N concentration and accumulation. Grain N concentration was higher in corn supplied with N fertilizer than in corn planted into plowed red clover. Chlorophyll content of ear leaf and ear leaf TKN were good indicators of corn grain N concentration. Interseeded cover crop accumulated more N at low corn density than at higher plant densities, but less than monoculture cover crops. Red clover biomass increased significantly up to 28 fold from fall to spring, however N concentration significantly decreased, suggesting N dilution by biomass production. Biederbeck et al. (1996) observed a low N concentration for less productive green manure when compared with highly productive cover crops. In the subsequent spring, interseeded and monoculture red clover accumulated up to 162.3 kg ha⁻¹ and 234.4 kg ha⁻¹ of N, respectively. When interseeded at high corn plant densities, red clover can accumulate significant N the subsequent spring to use in meeting the N demand of the following crop.

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Table 1. Nitrogen concentration (g kg^{-1}) in corn (*Zea mays* L.) grain across management practices and corn plant density during the 2002, 2003, 2004 and 2005 growing seasons at Kellogg Biological Station, Hickory Corners, MI.

Management practices	Grain N concentration				Average
	2002	2003	2004	2005	
	g kg^{-1}				
PRIR	13.2b*	12.6ab	11.1b	11.4b	12.1b
PRIA	13.0b	12.0b	11.0b	11.4b	11.8b
PRNI	13.1b	13.0ab	11.3b	10.9b	12.1b
CMNF	14.2a	13.6a	13.1a	12.7a	13.4a
CV (%)	9	11	7	8	9
Corn density (plants ha^{-1})					
37 500	14.3a	14.8a	12.4a	12.3a	13.4a
55 000	13.2b	13.1ab	11.5b	11.78b	12.4b
65 000	12.9b	11.3b	11.2b	11.5bc	11.7c
75 000	13.1b	12.0b	11.2b	10.8c	11.8c
CV (%)	9	11	7	8	9

*Means within columns in the same treatment followed by the same letter are not significantly different at $P=0.05$.

PRIR: Plowed red clover interseeded with red clover;

PRIA: Plowed red clover interseeded with AC Greenfix;

PRNI: Plowed red clover no interseeding;

CMNF: Conventional management, supplied with N fertilizer.

CV: Coefficient of Variation.

Table 2. Significance of the effect of plant density (PD) and management practices (MP) across four years (Y) on nitrogen concentration of corn (*Zea mays* L.) grain (G), leaf (L), stalk (S), ear leaf (EL), cover crop sampled in fall (FC) and cover crop sampled in spring (SC); on N accumulation of corn grain (G), ear leaf (EL), cover crops sampled in fall (FC) and cover crop sampled in spring (SC); and on SPAD-502 readings in 2004 and 2005 at Kellogg Biological Station, Hickory Corners, MI.

	N concentration						N accumulation				SPAD readings [‡]	
	G	L	S	EL	FC	SC	G	EL	FC	SC	2004	2005
Y	***	***	***	*	NS	*	**	**	NS	***	***	***
PD	***	**	NS	***	NS	NS	NS	***	***	NS	*	***
Y x PD	*	NS	NS	NS	NS	NS	NS	**	NS	NS	***	NS
MP [†]	***	***	NS	***	***	---	***	***	***	---	***	NS
Y x MP	NS	***	NS	NS	***	---	***	NS	*	---	***	***
PD x MP	NS	NS	NS	NS	NS	---	NS	NS	NS	---	NS	NS
Y x PD x MP	NS	NS	NS	NS	NS	---	NS	NS	NS	---	NS	NS

*Significant at the 0.05 level

**Significant at the 0.01 level

***Significant at the 0.001 level

[†]MP for FC are cover crop species

[‡]SPAD readings were measured in weeks not years

Table 3. Pearson correlation coefficients for chlorophyll content, plant density, grain N concentration, ear leaf N concentration and yield of corn (*Zea mays* L.) during the 2004 and 2005 growing seasons at the Kellogg Biological Station, Hickory Corners, MI. (n=64).

	Density		Grain N conc.		Ear Leaf N	
	R ²	p-value	R ²	p-value	R ²	p-value
2002						
Grain N-TKN*	-0.37	0.0027	-----	-----	-----	-----
Corn yield	0.26	0.0391	-0.34	0.0054	-----	-----
2003						
Grain N-TKN	-0.63	<0.0001	-----	-----	-----	-----
Corn yield	0.53	0.0017	-0.23	0.21	-----	-----
2004						
CC**-10 July	-0.02	0.86	-0.17	0.19	-0.15	0.44
CC-16 July	-0.18	0.15	0.11	0.36	0.18	0.15
CC-23 July	-0.34	0.0062	0.48	<0.0001	0.29	0.0215
CC-1 August	-0.50	<0.0001	0.59	<0.0001	0.59	<0.0001
CC-6 August†	-0.38	0.0021	0.66	<0.0001	0.56	<0.0001
CC-15 August†	-0.35	0.0047	0.62	<0.0001	0.61	<0.0001
Grain N-TKN	-0.34	0.0057	-----	-----	0.51	<0.0001
Ear Leaf N-TKN	-0.27	0.0315	0.51	<0.0001	-----	-----
Corn yield	0.62	<0.0001	0.09	0.51	0.15	0.2462
2005						
CC-7 July	-0.62	<0.0001	0.05	0.68	0.21	0.09
CC-15 July	-0.83	<0.0001	0.43	0.0003	0.57	<0.0001
CC-21 July	-0.79	<0.0001	0.52	<0.0001	0.61	<0.0001
CC-29 July†	-0.64	<0.0001	0.43	0.0004	0.58	<0.0001
CC-5 August†	-0.57	<0.0001	0.44	0.0003	0.58	<0.0001
Grain N-TKN	-0.41	0.0008	-----	-----	0.51	<0.0001
Ear Leaf N-TKN	-0.55	<0.0001	0.51	<0.0001	-----	-----
Corn yield	0.55	<0.0001	-0.06	0.6561	-0.16	0.2042

*TKN: Total Kjeldahl Nitrogen analysis

**CC: Chlorophyll content

†: Chlorophyll content measured on ear leaf

Table 4. Corn (*Zea mays* L.) grain N content (kg ha⁻¹) in four management practices across corn plant density during the 2002, 2003, 2004 and 2005 growing seasons at Kellogg Biological Station, Hickory Corners, MI.

Management practices	Grain N content				Average
	2002	2003	2004	2005	
	kg ha ⁻¹				
PRIR	121.0a*	89.1ab	92.6b	110.9b	103.4b
PRIA	117.5a	81.0b	93.5b	112.9b	101.2b
PRNI	118.8a	86.4ab	95.5b	105.6b	101.6b
CMNF	90.4b	95.3a	122.3a	140.1a	112a
CV (%)	16	16	13	10	13

*Means within columns followed by the same letter are not significantly different at P=0.05.

PRIR: Plowed red clover interseeded with red clover;

PRIA: Plowed red clover interseeded with AC Greenfix;

PRNI: Plowed red clover no interseeding;

CMNF: Conventional management, supplied with N fertilizer.

CV: Coefficient of Variation.

Table 5. Corn (*Zea mays* L.) leaf N concentration (g kg⁻¹) across management practices and corn plant density during the 2002, 2003, 2004 and 2005 growing seasons at Kellogg Biological Station, Hickory Corners, MI.

Management practices	Leaf N concentration				Average
	2002	2003	2004	2005	
	g kg ⁻¹				
PRIR	11.1a*	11.9a	10.2b	7.0b	10.1b
PRIA	10.5a	8.0b	10.7ab	6.9b	9.0c
PRNI	10.6a	9.4b	10.2b	6.9b	9.3c
CMNF	10.2a	12.2a	11.6a	9.1a	10.8a
CV (%)	24	22	17	14	21
Corn density (plants ha ⁻¹)					
37 500	11.7a	11.7a	11.5a	8.7a	11.0a
55 000	10.8a	11.0a	10.9ab	7.6ab	10.0ab
65 000	11.4a	9.6a	9.6b	6.8b	9.3bc
75 000	8.6b	9.2a	10.6ab	7.0ab	8.8c
CV (%)	24	22	17	14	21

*Means within columns in the same treatment followed by the same letter are not significantly different at P=0.05.

PRIR: Plowed red clover interseeded with red clover;

PRIA: Plowed red clover interseeded with AC Greenfix;

PRNI: Plowed red clover no interseeding;

CMNF: Conventional management, supplied with N fertilizer.

CV: Coefficient of Variation.

Table 6. Corn (*Zea mays* L.) stalk N concentration (g kg⁻¹) across management practices and corn plant density during the 2002, 2003, 2004 and 2005 growing seasons at Kellogg Biological Station, Hickory Corners, MI.

Management practices	Stalk N concentration				Average
	2002	2003	2004	2005	
	g kg ⁻¹				
PRIR	5.0ab*	4.7a	2.7a	6.4ab	4.8a
PRIA	4.9ab	4.1a	3.3a	7.0a	4.8a
PRNI	4.8a	4.5a	3.1a	5.8b	4.5a
CMNF	5.7b	4.0a	3.6a	7.0a	5.1a
CV (%)	24	28	12	31	28
Corn density (plants ha ⁻¹)					
37 500	5.0a	4.8a	3.4a	6.6a	5.0a
55 000	5.2a	4.4a	3.5a	6.4a	4.9a
65 000	5.2a	3.6b	3.1a	6.8a	4.7a
75 000	5.0a	4.5a	3.0a	6.3a	4.7a
CV (%)	24	28	12	31	28

*Means within columns in the same treatment followed by the same letter are not significantly different at P=0.05.

PRIR: Plowed red clover interseeded with red clover;

PRIA: Plowed red clover interseeded with AC Greenfix;

PRNI: Plowed red clover no interseeding;

CMNF: Conventional management, supplied with N fertilizer.

CV: Coefficient of Variation.

Table 7. Leaf chlorophyll content (SPAD-502 meter readings) across management practices and corn (*Zea mays* L.) plant density during the 2004 growing season at Kellogg Biological Station, Hickory Corners, MI.

Management practices	Chlorophyll meter readings in 2004					
	Top leaf				Ear leaf	
	10-Jul	16-Jul	23-Jul	1-Aug	6-Aug	15-Aug
PRIR	54.3b*	57.7a	54.1ab	52.1b	54.2b	52.8b
PRIA	53.5ab	56.9a	53.2b	51.8b	54.3b	52.3b
PRNI	54.4a	57.3a	53.9b	52.4b	54.7b	52.5b
CMNF	52.8b	57.6a	55.3a	55.4a	58.1a	58.4a
CV (%)	5	3	3	3	3	3
Corn density (plants ha ⁻¹)						
37 500	53.5a	58.2a	55.8a	55.5a	57.1a	56.4a
55 000	54.3a	56.6b	53.2b	51.8b	54.7b	53.1b
65 000	54.0a	57.6ab	53.8b	52.5b	54.5b	53.2b
75 000	53.2a	57.1ab	53.8b	51.8b	55.0b	53.2b
CV (%)	5	3	3	3	2	3

*Means within columns in the same treatment followed by the same letter are not significantly different at P=0.05.

PRIR: Plowed red clover interseeded with red clover;

PRIA: Plowed red clover interseeded with AC Greenfix;

PRNI: Plowed red clover no interseeding;

CMNF: Conventional management, supplied with N fertilizer.

CV: Coefficient of Variation.

Table 8. Leaf chlorophyll content (SPAD-502 meter readings) across management practices and corn (*Zea mays* L.) plant density during the 2005 growing season at Kellogg Biological Station, Hickory Corners, MI.

Management practices	Chlorophyll meter readings in 2005				
	Top leaf			Ear leaf	
	7-Jul	15-Jul	21-Jul	29-Jul	5-Aug
PRIR	55.3b*	53.4a	60.5a	63.0b	63.7b
PRIA	56.8ab	53.7a	60.5a	63.1b	63.8b
PRNI	57.4a	53.5a	60.6a	63.0b	63.9b
CMNF	53.0c	54.0a	62.1a	65.3a	65.9a
CV (%)	3	3	3	4	3
Corn density (plants ha ⁻¹)					
37 500	58.4a	57.1a	64.1a	66.1a	66.6a
55 000	55.5b	54.7b	61.4b	64.7a	64.6b
65 000	55.2b	52.4c	60.1bc	62.8b	63.5bc
75 000	53.2c	50.4d	58.2c	60.7c	62.6c
CV (%)	3	3	3	4	3

*Means within columns in the same treatment followed by the same letter are not significantly different at P=0.05.

PRIR: Plowed red clover interseeded with red clover;

PRIA: Plowed red clover interseeded with AC Greenfix;

PRNI: Plowed red clover no interseeding;

CMNF: Conventional management, supplied with N fertilizer.

CV: Coefficient of Variation.

Table 9. Dry matter (g), N concentration (g kg⁻¹) and N content of 10 ear leaves per plot of corn (*Zea mays* L.) across management practices and corn plant density during the 2004 and 2005 growing seasons at Kellogg Biological Station, Hickory Corners, MI.

Management practices	DM		N concentration		N content	
	2004	2005	2004	2005	2004	2005
	-----g /leaf-----		-----g kg ⁻¹ -----		-----g/leaf-----	
PRIR	4.6a*	5.11a	20.3b	23.6b	0.094b	0.122ab
PRIA	4.6a	5.12a	21.6b	24.8b	0.099b	0.128ab
PRNI	4.7a	5.05ab	19.7b	23.5b	0.093b	0.120b
CMNF	4.7a	4.86b	25.2a	27.0a	0.119a	0.132a
CV (%)	7	6	14	10	15	14
Corn density (plants ha ⁻¹)						
37 500	5.1a	5.8a	24.0a	27.7a	0.121a	0.160a
55 000	4.5b	5.0b	20.7b	25.3b	0.094b	0.126b
65 000	4.6b	4.9b	20.5b	22.7c	0.093b	0.111c
75 000	4.5b	4.5c	21.6b	23.3bc	0.097b	0.106c
CV (%)	7	6	14	10	15	14

*Means within columns in the same treatment followed by the same letter are not significantly different at P=0.05.

PRIR: Plowed red clover interseeded with red clover;

PRIA: Plowed red clover interseeded with AC Greenfix;

PRNI: Plowed red clover no interseeding;

CMNF: Conventional management, supplied with N fertilizer.

CV: Coefficient of Variation.

Table 10. Nitrogen concentration (g kg^{-1}) and accumulation (kg ha^{-1}) of interseeded and monoculture red clover (*Trifolium pratense* L.) or AC Greenfix (*Lathyrus sativum* L.) at four corn (*Zea mays* L.) plant densities in fall 2002, 2003, 2004 and 2005 at Kellogg Biological Station, Hickory Corners, MI.

Corn density (plants ha^{-1})	Red clover				AC Greenfix			
	2002	2003	2004	2005	2002	2003	2004	2005
	N concentration g kg^{-1}							
0 [†]	---	---	33.8a	45.0	---	---	37.5a	47.1a
37 500	32.7a*	31.5a	30.4ab	---	32.1a	29.0a	36.5a	39.2b
55 000	33.2a	31.0a	28.5b	---	32.a	32.2a	38.7a	38.1b
65 000	34.6a	30.4a	31.8ab	---	31.5a	31.1a	36.0a	38.0b
75 000	34.1a	31.3a	29.0b	---	33.9a	34.3a	38.4a	38.8b
CV (%)	8	6	12	---	8	6	12	8
	N accumulation kg ha^{-1}							
0	---	---	48.2a	20.6	---	---	81.8a	75.4a
37 500	15.6a	4.8a	14.5b	---	14.6a	20.0a	20.3b	9.5b
55 000	10.3ab	3.0a	10.6bc	---	11.4ab	7.0b	13.1c	7.1bc
65 000	9.2b	5.4a	8.7c	---	11.7ab	7.9b	13.5c	8.1bc
75 000	5.9b	2.2a	8.5c	---	6.8b	7.6b	10.0c	5.0c
CV (%)	37	52	29	---	37	52	29	38

[†]Monoculture cover crop

*Means within columns followed by the same letter are not significantly different at $P=0.05$.
CV: Coefficient of Variation.

Table 11. Nitrogen concentration (g kg^{-1}) and accumulation (kg ha^{-1}) of red clover (*Trifolium pratense* L.) during the subsequent spring in 2003, 2004 and 2005 at Kellogg Biological Station, Hickory Corners, MI.

Corn density (plants ha^{-1})	N concentration			N accumulation		
	2003	2004	2005	2003	2004	2005
	----- g kg^{-1} -----			----- kg ha^{-1} -----		
0 [†]	-----	-----	28.6a	-----	-----	234.4a
37 500	27.9a*	26.2a	25.8a	108.6a	68.7a	157.1b
55 000	26.2ab	24.7ab	27.9a	94.5ab	83.3a	152.3b
65 000	25.7ab	21.4b	27.7a	105.2ab	63.6a	162.3b
75 000	24.4b	25.1ab	26.8a	75.5b	58.3a	143.5b
CV (%)	7	17	8	18	29	17

[†]Monoculture cover crop

*Means within columns followed by the same letter are not significantly different at $P=0.05$.

CV: Coefficient of Variation.

Table 12. Comparison of N concentration of interseeded red clover (*Trifolium pratense* L.) at the same corn (*Zea mays* L.) density from the first sampling in fall (the year of establishment) to the second sampling in spring (the subsequent spring) in 2002, 2003, 2004 and 2005 at Kellogg Biological Station, Hickory Corners, MI.

	Corn density (plants ha^{-1})			
	37 500	55 000	65 000	75 000
	Pr > F			
Fall 2002 x Spring 2003	0.012*	0.004	<0.0001	<0.0001
Fall 2003 x Spring 2004	0.049	0.019	0.001	0.022
Fall 2004 x Spring 2005	0.014	0.753	0.029	0.259
Across years Fall x Spring	0.004	0.002	<0.0001	<0.0001

*No significant difference if p value=0.05

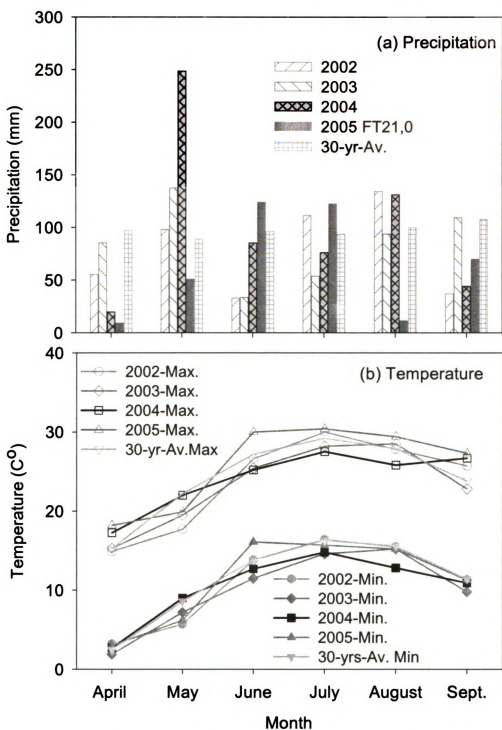


Figure 1. Monthly average minimum and maximum temperature, and total monthly precipitation during the 2002, 2003, 2004 and 2005 growing seasons compared with the 30-year monthly average at Kellogg Biological Station, Hickory Corners, MI.

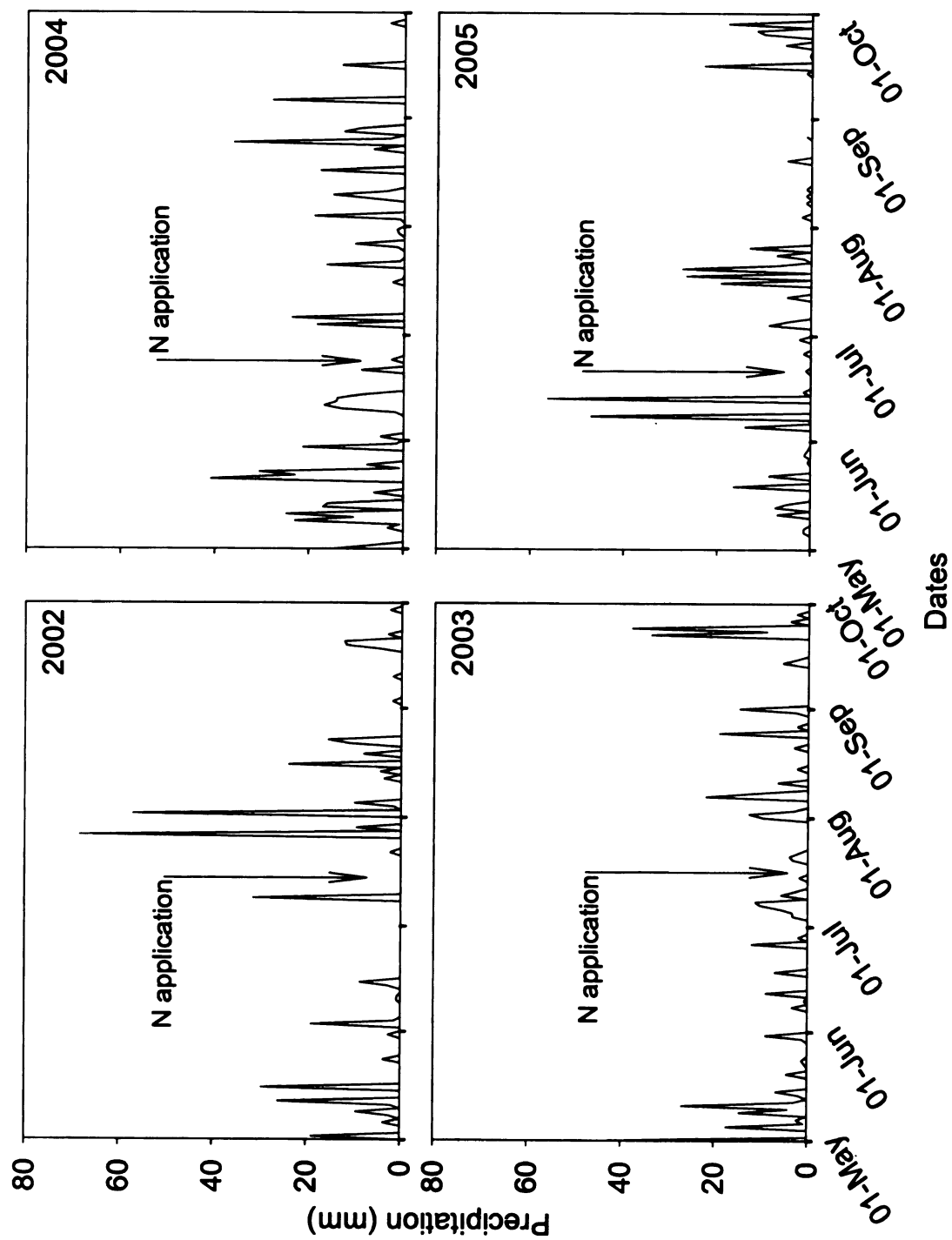


Figure 2. Total daily precipitation during the 2002, 2003, 2004 and 2005 growing seasons at Kellogg Biological Station, Hickory Corners, MI.

Chapter three

Effect of Interseeded Cover Crop on Soil Mineral N and Subsequent Dry Bean Yield and N Status

Abstract

Reliable cropping strategies are needed to enhance N contribution from legume cover crops to subsequent crops. Field studies were conducted at the Kellogg Biological Station, Hickory Corners, MI, to assess the effect of interseeded red clover (*Trifolium pratense* L.) or AC Greenfix (*Lathyrus sativum* L.) on soil mineral N (NO_3^- -N and NH_4^+ -N) and on subsequent dry bean (*Phaseolus vulgaris* L.) yield and N status. In 2003, 2004 and 2005, dry bean was planted at 232,500 plants ha^{-1} into previous corn (*Zea mays* L.) treatments that consisted of four corn densities ranging from 37 500 to 75 000 plants ha^{-1} and four management practices: conventional (no cover crop, N fertilizer applied) and three non-N fertilized treatments planted into plowed red clover (interseeded with AC Greenfix or red clover, and no interseeding). Bean planted into conventional treatments received 45 kg ha^{-1} of N. There was a seasonal effect on dry bean yield, N concentration and content, and soil mineral N. In 2003, no yield difference was observed between fertilized bean and bean following interseeded red clover or AC Greenfix plots. In 2004, dry bean yield from the conventional treatment was significantly higher than yield of bean following interseeded AC Greenfix and no interseeding, but similar to bean planted into plowed interseeded red clover. In 2005 however, bean yield supplied with N fertilizer was lower than bean yield

following interseeded red clover or AC Greenfix and no interseeding. In two of the three years, bean seed N concentration and content was the highest in beans supplied with N fertilizer. No effect of interseeded cover crops was seen on soil mineral N either in the fall or the subsequent spring. During optimal growing conditions, interseeded red clover contributed sufficient N and was able to produce dry bean yields comparable to bean supplied with 45 kg ha⁻¹ of N fertilizer. This system has the potential to help farmers reduce or eliminate N application in dry beans, resulting in positive environmental and economic impacts.

Introduction

In 2004, the state of Michigan was ranked second after North Dakota in dry bean (*Phaseolus vulgaris* L.) production in the US (USDA-NASS, 2005). Dry bean requires 45 kg ha⁻¹ of supplemental nitrogen (N) fertilizer to maintain optimum growth and yield. Alternative management practices for dry bean production are needed to help address issues related to increased environmental pollution and to reduce the cost of agricultural inputs. The ability to supply all or part of the plant N needs via cover crops is a potential alternative to conventional practices. Monoculture or interseeded cover crops have been used to reduce N fertilizer application (Hesterman et al., 1992; Jeranyama et al., 1998). Vyn et al. (1999) showed that corn (*Zea mays* L.) yield following red clover (*Trifolium pratense*, L.) was consistently higher compared with corn following oilseed radish (*Raphanus sativus* [L.] var *oleiferus* Metzg [Stokes]) or annual ryegrass (*Lolium multiflorum*). Also, Sogbedji et al. (2006) observed that planting mucuna [*Mucuna pruriens* (L.) D.C.] and pigeon pea (*Cajanus cajan* L.) after corn reduced N fertilizer needs of the subsequent corn crop. Similarly, Balkcom and Reeves (2005) observed that corn yield following sunn-hemp (*Crotalaria juncea* L.) with no additional N fertilizer was greater than corn yield supplied with 56 kg N ha⁻¹.

Nitrogen fertilization is important in dry bean production because excess N can delay leaf canopy growth which can result in disease incidence (Moraghan and Franzen, 1995). Legume cover crops can provide adequate N to meet the demand of a subsequent dry bean crop. Liebman and Gallandt (2002) found that dry bean yield following red clover was similar to dry bean yield supplied with 84

kg ha⁻¹ of ammonium nitrate fertilizer. Plots planted to snap bean following cover crops yielded higher than those without cover crop, particularly in dry seasons (Peet, 1995). Studies have been conducted to evaluate the effect of management practices such as plant density, row spacing, different tillage methods and herbicides on weed density and dry bean yield (Aleman, 2001; Amador-Ramirez et al., 2001; Amador-Ramirez et al., 2002; Blackshaw et al., 2000; Soltani et al., 2006; Wilson, 2005; Xu and Pierce, 1998). Liebman et al. (1995) recommended an investigation of the use of legume green manure as a N source in the temperate bean production systems after they observed growth, N status, and yield reduction of bean in a no-tillage-rye mulch system. Interseeding a legume cover crop into corn has the potential to provide N to a subsequent crop. Studies have assessed the effect of cover crops in a monoculture system on various crops including dry beans. Other studies have investigated the effect of an interseeded cover crop on subsequent corn crop. However, little or no research has examined the effect of interseeded cover crops on subsequent dry bean yield and quality.

Soil chemical properties are essential in assessing the soil's ability to supply nutrients (Campbell et al., 1991; Mikha et al., 2006). Researchers have attempted to develop yield response functions by regressing crop yield against late-spring NO₃⁻-N concentration (Katsvairo et al., 2003) and soil organic matter (Schmidt et al., 2002). Studies have shown that cover crops can influence soil NO₃⁻-N and that corn yield is correlated with soil NO₃⁻-N (Shahandeh et al., 2005; Vyn et al., 1999). However, Villar-Mir et al. (2002) showed that plant N uptake

and grain yield were not related to soil N availability. Management practices such as the use of monoculture and interseeded cover crops can also influence soil mineral N. Vaughan and Evanylo (1998) found that soil nitrate was higher following hairy vetch compared with rye. Hairy vetch leached more NO_3^- -N compared with a rye cover crop (McCracken et al., 1994). Ditsch and Alley (1991) found no significant difference in soil NO_3^- -N in spring at plowdown of various cover crops.

N fertilizer can also influence soil mineral N. Vyn et al. (1999) found that applying more fertilizer N to the previous year's small-grain crop rarely increased spring soil NO_3^- -N concentrations or corn yields. However, MacKown et al. (1999) showed that soil mineral N was related to the quantity of broadcast-applied N fertilizer. Doran et al. (1987) observed an increase in soil nitrate due to fertilizer application, red clover and hairy vetch (*Vicia villosa* Roth). Little is known about the effect of management practices such as the use of red clover as a N source combined with interseeding on soil mineral N. The objectives of this study were (1) to assess the effect of plowed red clover and interseeded red clover or AC Greenfix on soil mineral N in fall and the subsequent spring before dry bean establishment and (2) to assess the effect of interseeding red clover or AC Greenfix on subsequent dry bean yield and N status.

Materials and Methods

Site description

The research was conducted from 2002 to 2005 at the Kellogg Biological Station (KBS) in Hickory Corners, Michigan. The soil types at KBS were the Kalamazoo (fine-loamy, mixed, mesic Typic Hapludalfs) and Oshtemo (coarse-loamy, mixed, mesic Typic Hapludalfs) series (Crum and Collins, 2004). The experiment was conducted on a different field each year to permit planting on site following red clover plow down. Corn research plots were established in 2002 (Field 1), 2003 (Field 2), 2004 (Field 3) and 2005 (Field 4). In the year prior to corn establishment, red clover was planted in each field into wheat stubble in July-August except in 2001 when it was planted into corn stubble. Red clover was chisel-plowed the following spring before corn planting to serve as a N source for the non-conventional plots.

Experimental design

Each year the experiment was replicated four times except in 2003 where only two replications were used due to animal damage. The experiment was a split-plot in a completely randomized design. The main-plots were four corn densities (37 500, 55 000, 65 000 and 75 000 plants ha⁻¹). Subplots were four management practices: (1) Conventional management, corn seeded into wheat stubble with N fertilizer applied (CMNF); (2) Corn seeded into plowed red clover, no N fertilizer, interseeded with AC Greenfix (PRIA); (3) Corn seeded into plowed

red clover; no N fertilizer, interseeded with red clover (PRIR) ;(4) Corn seeded into plowed red clover, no N fertilizer, not interseeded with cover crop (PRNI). Based on Preside-dress Nitrate Test (PSNT) results, N fertilizer was applied to the conventional corn plots, up to a total of 140 kg ha⁻¹ every year. Corn was planted into 6-row plots of 4 by 4.5 m in 2002 and 2003 and of 5 by 4.5 m in 2004 and 2005.

Cover crops

Red clover and AC Greenfix were interseeded in the first two weeks of July when corn plants were between V5-V7 growth stages. Red clover was seeded at the rate of 20.4 kg ha⁻¹ and AC Greenfix at the rate of 90 kg ha⁻¹. Above ground biomass of red clover and AC Greenfix were hand-clipped at full bloom of AC Greenfix by removing plants from a random quadrat of 0.209 m² in each plot. AC Greenfix was cut to allow regrowth, but was winter-killed and could not be sampled the subsequent spring. After corn harvest, corn stalks were mowed and plots were left undisturbed until the following spring. Before bean establishment, the subsequent spring, red clover was again sampled to assess its biomass and N accumulation.

Soil mineral N

Soil samples were collected in each field (Field 1, 2, 3 and 4) after corn harvest from fall 2002 to fall 2005 and the subsequent spring of 2003 to 2005 to assess the effect of the four different management practices (PRIR, PRIA, PRNI

and CMNF) on soil mineral-N (NO_3^- -N and NH_4^+ -N). Soil nitrate was assessed from 2002 to 2005 whereas soil ammonium was measured only in spring and fall of 2004 and 2005. Depending on the weather, soil samples were collected a few weeks after corn harvest in the fall and a few weeks before bean establishment in the following spring. Soil samples were taken in the fall on 01 November 2002, 18 November 2003, 22 November 2004 and 05 November 2005; and the subsequent spring on 03 May 2003, 02 May 2004 and 23 May 2005. Eight 2-cm-diameter soil cores were randomly taken at the depth of 25 cm in the four center rows from each subplot, air-dried for 2 days and mixed thoroughly to obtain a composite sample. Soil NO_3^- -N and NH_4^+ -N were measured using Cadmium reduction and Salicylate method, respectively.

Dry bean

Planting

Three 2-year crop rotations were used in this study to assess the effect of interseeded cover crops on subsequent dry bean. Dry bean was established in 2003 (Field 1), 2004 (Field 2) and 2005 (Field 3). Dry bean planted into the conventional plots (CMNF) was supplied with N fertilizer and no N was applied to bean planted into plowed interseeded red clover or AC Greenfix and no interseeding (PRIR, PRIA and PRNI). Nitrogen fertilizer was applied at the rate of 45 kg ha^{-1} as urea in 2003 and 2004 and as liquid N fertilizer in 2005. Nitrogen was applied a few days after planting except in 2005 where the application was delayed due to dry conditions and high temperature. Phosphorus and K were

applied a few days before planting based on the soil test recommendation of the MSU Soil and Plant Nutrient Laboratory. Navy bean (cultivar Seahawk,) was planted at a seeding rate of 232,500 plants ha⁻¹ on 06 June in 2003, 24 June in 2004 and 16 June in 2005. Dry bean seed was inoculated with a Rhizobial inoculant (*Bacillus subtilis*, MBI 600).

Pest control

Herbicides were used on the whole field to control weeds, and cultivation was utilized when necessary. In 2003, both insecticides and herbicides were used. On 18 July 2003, Esfenvalerate was applied at the rate of 0.056 kg ai ha⁻¹ to control leafhoppers. On July 22, Fomesafen was applied at the rate of 0.28 kg ai ha⁻¹ for weed control. On 11 August, beans were cultivated because the herbicide did not provide good weed control. An additional hand pulling was done to remove weeds missed by cultivation. In 2004, herbicides were applied two times. On 27 June, Imazethapyr (0.035 kg ai ha⁻¹) and S-metolachlor (1.07 kg ai ha⁻¹) were broadcast on the entire field. On 21 July, Bentazon (1.121 kg ai ha⁻¹) and Quizalofop (0.049 kg ai ha⁻¹) were applied to the whole field. The same day, Esfenvalerate (0.056 kg ai ha⁻¹) was sprayed to control leafhoppers. In 2005 after bean planting hot, dry conditions followed which delayed herbicide application. To control weeds, on 6 July Quizalofop (0.049 kg ai ha⁻¹) and on 8 July Imazamox (0.036 kg ai ha⁻¹) and Bentazon (0.52 kg ai ha⁻¹) were broadcast on the entire field. On 29 July a second herbicide application was made using Bentazon and Clethodim at the rate of 1.121 kg ai ha⁻¹ and 0.140 kg ai ha⁻¹, respectively. Since herbicides did not provide very good weed control after the

second application, beans were cultivated on 5 August 2005. In 2005, beans were burned by the application of herbicide due to hot, dry conditions.

Harvest and N analysis

Only the four center-row of the six row-plots dry bean were harvested on 25 September 2003, 01 October 2004 and 06 October in 2005. After harvest, beans were dried at air temperature and threshed using a thresher (ALMACO, Nevada, Iowa). In 2003, a sub-sample of approximately half a kilogram of threshed bean seed was taken for estimating N concentration. The two following years, five plants were collected from each plot a few weeks before harvest to assess N concentration. At each sampling, leaf, stem and seed were separated and dried for two days at 60°C. Samples were digested using a 40-tube Tecator Model 1016 Digester (Tecator, Höganäs, Sweden). Bean tissue samples of 0.1 g were digested in 4 ml of 18 M H₂SO₄ with 1.5 g K₂SO₄ and 0.015 g Se catalyst in 100 ml-constricted tubes. All samples were digested at 350° C for 4 hours. To determine N concentration, tissues extract were analyzed using a Lachat Flow Injection Analyzer (Hachat Co., Loveland, Colorado).

Statistical analysis

All data were analyzed using Proc Mixed in SAS Statistical Software Package version 8.2 (SAS, 2001). Plant density and management practices were considered fixed effects. Two error terms were considered in the analysis of the data, one associated with the whole plot (plant density) and the other associated with the subplot (management practices) and the interaction (plant density x

management practices). When interaction effects were found to be statistically significant, means separation was conducted for respective cell means. When main effects were significant while interactions were not, means separation was conducted for marginal means. Effects were considered statistically significant at $p = 0.05$.

Results and Discussion

Weather conditions

Total monthly precipitation and monthly average temperature (minimum and maximum) data from the 2002 to 2005 growing seasons was obtained from the Long-Term Ecological Research weather station (LTER-Weather, 2006). Weather conditions during the 2003, 2004 and 2005 dry bean growing seasons from June to September were variable and affected treatments (Figures 1 and 2). Likewise, climatic conditions were variable from 2002 to 2005 during the assessment of soil mineral N (Figures 3 and 4). Total monthly precipitation in June and July during the 2003 growing season were lower than the 30-year average (Figure 1a). Monthly average maximum temperatures during the 2005 growing season from June to September were higher than the 30-year average (Figure 1b). Precipitation in August 2005 was lower than in any other growing season and than the 30-year average (Figure 1a). Rainfall during the 2004 growing season was well distributed in compared with 2003 and 2005 (Figure 2).

N accumulation by interseeded red clover or AC Greenfix

N accumulation by interseeded cover crops during the year of establishment (fall) was variable and very low compared with N accumulation the subsequent spring (Table 1). AC Greenfix was hand-clipped a few days after sampling cover crop DM to prevent podfill. Regrowth was expected from AC Greenfix, however none occurred. The subsequent spring, prior to dry bean

establishment, interseeded red clover accumulated substantial N (Table 1). In spring 2004 and 2005 red clover N accumulation was similar regardless of corn density, but differed in 2003. Spring red clover N accumulation was significantly higher in 2005, followed by 2003 and then 2004.

Dry bean yield

Dry bean yield varied across years and among treatments (Table 2). No interaction was observed between year and treatments (data not shown). Dry bean yield was comparable to results of Xu and Pierce (1998) who obtained dry bean yield ranging from 2.3 to 3.4 Mg ha⁻¹. In 2003, bean planted in CMNF had similar yield to bean planted after interseeded red clover or AC Greenfix (Table 2). Bean planted in CMNF and PRIR yielded more than bean following PRNI but were not significant different from PRIA. In 2004, dry bean yield in CMNF was significantly higher than PRIA, but similar to PRIR (Table 2). In both years, dry bean following interseeded red clover treatments produced yields comparable to bean supplied with 45 kg ha⁻¹ of N. Results support work of Skarphol et al. (1987) who found that bean yield following legume cover crops was similar to yield obtained with N fertilizer without a legume cover crop. Similarly, Liebman and Gallandt (2002) found comparable yield of dry bean following red clover and bean supplied with 84 kg ha⁻¹ N fertilizer. In 2003 and 2004, dry bean following interseeded red clover treatments produced greater yields than the no interseeding system. Peet (1995) found that planting snap bean following cover crops yielded higher than snap bean without cover crop. In 2005, however, bean

supplied with N fertilizer yielded lower than any other treatment. The difference among treatments may be due to the dry, hot conditions that occurred in 2005. These conditions may have reduced N fertilizer uptake by bean planted in CMNF. Poor weed control by herbicides may be an additional factor in explaining lower yields in 2005 compared with other growing seasons. In 2005, bean yield following no interseeding were comparable to yield of beans following interseeded red clover or AC Greenfix.

There was a seasonal effect on dry bean yield. Across years, dry bean yield was higher in 2004, followed by 2003 and then by 2005 (data not shown). The high yield in 2004 was due in part to well-distributed rainfall that occurred throughout the growing season (Figure 2). Poor herbicide efficacy due to low rainfall helps explain low yield in 2003 compared with 2004 (Table 2). These differences in yield during drought versus wet conditions may be explained by findings from Lodeiro et al. (2000) who concluded that common bean grown under conditions of N fixation were more drought tolerant than those provided with sufficient levels of N fertilizer. The 100 seed weight varied with treatments and year and no clear pattern was observed (Table 3).

Dry bean N concentration and content

Nitrogen concentration varied with management practices and among growing seasons (Table 4). In 2003, seed N concentration of bean planted in CMNF was significantly higher than bean seed N concentration in PRIA and PRNI. No significant difference was observed in seed N concentration of bean

following interseeded red clover and bean supplied with N fertilizer. In 2004, seed N concentration was higher in CMNF and significantly different from PRIR and PRNI, but not significant different from PRIA. The low N concentration in dry bean seed may be related to low N contribution by interseeded red clover in the subsequent spring of 2004, before dry bean establishment. This is supported by a positive correlation coefficient of 0.61 (<0.0001) between dry bean seed N concentration and red clover N accumulation before planting dry bean (data not shown). In 2005 however, no significant difference was observed in bean seed N concentration among management practices. Across years, N concentration of bean seed was significantly higher in 2005, followed by 2003 and then by 2004 (data not shown). There was a negative correlation coefficient of -0.28 (0.0003) between dry bean yield and seed N concentration (data not shown).

N concentration in beans stem and leaf showed no significant difference among treatments in either 2004 or 2005 (Table 4). Nitrogen concentration of bean leaves and stems were significantly higher in 2005 compared with 2004 (data not shown). This was probably due to hot, dry weather conditions that occurred in 2005.

Dry bean seed N content followed the yield trend (Table 2). In 2003, bean planted in both CMNF and PRIR accumulated more seed N than PRNI. In 2004 only bean planted in CMNF had a higher seed N content than PRNI. However in 2005, bean planted in CMNF accumulated less N than all other treatments (Table 2). Across years, there was a very high positive correlation coefficient of 0.91($p<0.0001$) between dry bean yield and seed N content. Results of this study

are similar to findings of Lopez-Bellido et al. (2003) who observed faba bean seed N content ranging from 50 to 127 kg N ha⁻¹.

Fall and subsequent spring soil mineral N

Soil mineral N was variable within sampling period (fall or spring) and from fall to the subsequent spring, due probably to weather conditions and time of sampling (Table 5, 6 and 7, and Figures 3 and 4).

Soil Nitrate and Ammonium in fall

In fall 2002, soil nitrate was the lowest in the treatment supplied with N fertilizer, but only significantly different from PRNI (Table 5). No difference was observed between interseeded cover crop and no interseeding. In fall 2003, no significant difference was observed among treatments; however soil nitrate was lower in all management practices. Low values in fall 2003 may be explained by leaching due to increased rainfall before soil sampling (Figure 3). In fall 2004, soil nitrate in CMNF was significantly higher than in PRIR, but not different from PRIA and PRNI. In fall 2005, soil nitrate in CMNF was significantly higher compared to PRIR, PRIA and PRNI. In fall 2004 and 2005, no significant difference was observed in soil ammonium among management practices. In fall 2004 and 2005, soil ammonium was significantly higher than nitrate in all management practices except in CMNF in 2004 (Table 7).

Soil Nitrate and Ammonium in the subsequent spring

In spring 2003, nitrate decreased significantly, no difference was observed among management practices (Table 5). Low values in spring 2003 may be due

to leaching as heavy rain occurred in spring before soil samples were collected (Figure 4). In spring 2004, soil NO_3^- -N was significantly higher in PRNI compared with CMNF, but not different from PRIA and PRIR. In spring 2005, soil nitrate in PRNI and PRIA were significantly higher than PRIR but not different from CMNF. In spring 2004 and 2005, no significant difference was observed in soil NH_4^+ -N among management practices (Table 5). No significant difference was observed between soil nitrate and ammonium in spring 2004 and 2005 (Table 7).

Soil NO_3^- -N varied significantly in all management practices from fall 2002 to spring 2003 (Table 6). However, no variation in soil NO_3^- -N occurred among management practices from fall 2003 to spring 2004 (Table 6). From fall 2004 to spring 2005, no difference was observed in soil NO_3^- -N among management practices, except in PRNI where soil NO_3^- -N in fall 2004 was significantly lower than the subsequent spring in 2005 (Table 6). From fall 2004 to spring 2005, there was a trend of increase in soil nitrate that was probably due to temperature and rainfall that may have contributed to more soil N mineralization. No significant difference was observed in soil NH_4^+ -N from fall 2004 to the subsequent spring in 2005 (data not shown).

Fall comparison across years showed that soil nitrate was significantly higher in 2002 compared with 2005, which was also significantly higher than 2003 and 2004 (data not shown). This may be explained by good cover crop stand in spring 2002 combined with high temperature and moderate rainfall early in the season and low rainfall toward the end of the growing season (Figure 3). The correlation coefficient between dry bean yield and soil mineral N was very

small and no significant difference was detected (data not shown). Overall, interseeded cover crops did not influence soil nitrate and ammonium. Soil mineral N was influenced by the time of sampling as also observed in a study conducted by Isse et al. (1999).

Conclusion

In two of the three years, dry bean yield following corn interseeded with red clover was similar to dry bean yield following corn and supplied with mineral N fertilizer. Dry bean yield following interseeded red clover could be better explained by N accumulation from interseeded cover crop than soil mineral N before bean establishment. There was a positive correlation between N accumulation of interseeded red clover the subsequent spring (before bean establishment) and dry bean yield. Dry bean seed N concentration was variable within management practices and years. Dry bean N concentration in seed, leaf and stem tended to be high during hot and dry conditions. In two of the three years, bean seed N content was the highest in beans supplied with N fertilizer and the lowest during dry conditions. Across years, there was a strong correlation between interseeded red clover N accumulation before bean establishment and dry bean seed N concentration. Soil nitrate and ammonium in the fall and the subsequent spring were not influenced by interseeded cover crops but rather by climatic conditions. No correlation was found between early spring soil mineral N with dry bean yield. The data suggest that interseeding red clover into corn can result in sufficient N accumulation to meet the demand of a

subsequent dry bean crop. This system appears to have the potential to help reduce N fertilizer use and hence could reduce the cost of inputs and environmental pollution. It could also be useful system for organic farmers and for low-resource farmers in developing nations.

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Table 1. Effect of corn (*Zea mays* L.) density on N accumulation (kg ha⁻¹) of interseeded red clover (*Trifolium pratense* L.) or AC Greenfix (*Lathyrus sativum* L.) in fall during 2002, 2003 and 2004 and the subsequent spring during the 2003, 2004 and 2005 at Kellogg Biological Station, Hickory Corners, MI.

Corn density (plants ha ⁻¹)	N accumulation					
	Red clover			AC Greenfix		
	kg ha ⁻¹					
	Fall (year of establishment)					
	2002	2003	2004	2002	2003	2004
37 500	15.6a*	4.8a	14.5a	14.6a	20.a	20.3a
55 000	10.3ab	3.0a	10.6ab	11.4ab	70.b	13.1b
65 000	9.2b	5.4a	8.7b	11.7ab	7.9b	13.5b
75 000	5.9b	2.2a	8.5b	6.8b	7.6b	10.0b
CV (%)	37	52	29	37	52	29
	Subsequent spring					
	2003	2004	2005			
37 500	109a	69a	157a	-----	-----	-----
55 000	95ab	83a	152a	-----	-----	-----
65 000	105ab	64a	162a	-----	-----	-----
75 000	76b	58a	144a	-----	-----	-----
CV (%)	18	29	17	-----	-----	-----

*Means within columns, cover crop and season followed by the same letter are not significantly different at P=0.05.

CV: Coefficient of variation

Table 2. Dry bean (*Phaseolus vulgaris* L.) yield (kg ha⁻¹) and seed N content (kg ha⁻¹) under various management practices during the 2003, 2004 and 2005 growing seasons at Kellogg Biological Station, Hickory Corners, MI.

	Yield			Seed N content		
	2003	2004	2005	2003	2004	2005
	kg ha ⁻¹					
PRIR	3424a*	3737ab	2964a	109.8a	102.6ab	103.3a
PRIA	3220ab	3453bc	2873a	100.3ab	99.0ab	99.7a
PRNI	3057b	3384c	2810a	95.0b	93.0b	92.6a
CMNF	3430a	3863a	2244b	112.7a	115.2a	77.8b
CV (%)	17	9	33	19	8	35

*Means within columns followed by the same letter are not significantly different at P=0.05.

PRIR: Plowed red clover interseeded with red clover;

PRIA: Plowed red clover interseeded with AC Greenfix;

PRNI: Plowed red clover no interseeding;

CMNF: Conventional management, supplied with N fertilizer.

CV: Coefficient of variation

Table 3. Seed moisture content (g kg^{-1}) and 100 seed weight (g) of dry bean (*Phaseolus vulgaris* L.) in various management practices after harvest during the 2003, 2004 and 2005 growing seasons at Kellogg Biological Station, Hickory Corners, MI.

	100 seeds weight			Seed moisture		
	2003	2004	2005	2003	2004	2005
	-----g-----			-----g kg ⁻¹ -----		
PRIR	18.8a*	19.8a	22.6a	118.9a	116.9a	108.3a
PRIA	17.9b	19.5ab	21.8a	118.6a	113.8a	110.3a
PRNI	18.2b	19.4b	21.9a	118.6a	117.9a	109.8a
CMNF	18.0b	19.9a	21.5a	118.5a	115.5a	109.4a
CV (%)	7	4	10	3	9	3

*Means within columns followed by the same letter are not significantly different at $P=0.05$.

PRIR: Plowed red clover interseeded with red clover;

PRIA: Plowed red clover interseeded with AC Greenfix;

PRNI: Plowed red clover no interseeding;

CMNF: Conventional management, supplied with N fertilizer.

CV: Coefficient of variation

Table 4. Effect of management practices on N concentration(g kg^{-1}) of seed, leaves and stem of dry bean (*Phaseolus vulgaris* L.) a few days before harvest during the 2003, 2004 and 2005 growing seasons at Kellogg Biological Station, Hickory Corners, MI.

Management practices	Seed N concentration			Leaves N concentration		Stem N concentration	
	2003	2004	2005	2004	2005	2004	2005
	-----g kg ⁻¹ -----						
PRIR	32ab*	27.5b	35.1a	20.9a	25.5a	6.2a	13.4a
PRIA	31.1bc	28.7ab	34.9a	22.4a	25.0a	6.3a	12.9a
PRNI	30.3c	27.8b	33.3a	21.3a	25.3a	6.7a	14.0a
CMNF	32.7a	29.8a	34.3a	20.5a	25.1a	6.3a	12.2a
CV (%)	7	7	10	12	20	9	27

*Means within columns followed by the same letter are not significantly different at $P=0.05$.

PRIR: Plowed red clover interseeded with red clover;

PRIA: Plowed red clover interseeded with AC Greenfix;

PRNI: Plowed red clover no interseeding;

CMNF: Conventional management, supplied with N fertilizer.

CV: Coefficient of variation

Table 5. Effect of management practices on soil nitrate and ammonium after corn (*Zea mays* L.) harvest in fall 2002, 2003, 2004 and 2005 and before planting dry bean (*Phaseolus vulgaris* L.) during the subsequent spring of 2003, 2004 and 2005 growing seasons at Kellogg Biological Station, Hickory Corners, MI. Each year, planting occurred in a different field.

	NO ₃ ⁻	NO ₃ ⁻	NH ₄ ⁺	NO ₃ ⁻	NH ₄ ⁺	NO ₃ ⁻	NH ₄ ⁺
	g kg ⁻¹						
	Fall						
	2002	2003		2004		2005	
PRIR	12.5ab*	2.2a	-----	1.3b	2.6a	6.1b	4.4a
PRIA	15.4ab	2.3a	-----	1.7ab	2.7a	5.8b	4.2a
PRNI	16.1a	2.5a	-----	1.6ab	2.9a	5.8b	3.7a
CMNF	11.6b	1.3a	-----	2.2a	2.8a	7.6a	3.7a
CV (%)	41	62	-----	54	26	23	39
	Subsequent spring						
	2003	2004		2005			
PRIR	1.7a	3.8ab	3.6a	2.4b	3.2a	-----	-----
PRIA	2.0a	4.0ab	3.3a	3.4a	2.7a	-----	-----
PRNI	1.5a	4.4a	3.5a	3.7a	3.1a	-----	-----
CMNF	2.0a	2.7b	3.5a	3.0ab	2.7a	-----	-----
CV (%)	68	33	22	36	26	-----	-----

*Means within columns and season followed by the same letter are not significantly different at P=0.05.

PRIR: Plowed red clover interseeded with red clover;

PRIA: Plowed red clover interseeded with AC Greenfix;

PRNI: Plowed red clover no interseeding;

CMNF: Conventional management, supplied with N fertilizer.

CV: Coefficient of variation

Table 6. Comparison of soil NO_3^- -N concentration in fall [the year of corn (*Zea mays* L.) establishment] to the second sampling under the subsequent spring (before planting beans) in the same management practices in 2002, 2003, 2004 and 2005 at Kellogg Biological Station, Hickory Corners, MI.

	Management practices			
	PRIR	PRIA	PRNI	CMNF
	Pr > F			
Fall 2002 x Spring 2003	<0.0001*	<0.0001	<0.0001	<0.0001
Fall 2003 x Spring 2004	0.256	0.214	0.177	0.325
Fall 2004 x Spring 2005	0.259	0.083	0.036	0.409

*No significant difference if p value=0.05

PRIR: Plowed red clover interseeded with red clover;

PRIA: Plowed red clover interseeded with AC Greenfix;

PRNI: Plowed red clover no interseeding;

CMNF: Conventional management, supplied with N fertilizer.

Table 7. Comparison of soil NO_3^- -N and soil NH_4^+ -N concentration in fall and subsequent spring under the same management practices in 2004 and 2005 at Kellogg Biological Station, Hickory Corners, MI.

	Management practices			
	PRIR	PRIA	PRNI	CMNF
	Pr > F			
Spring 2004 (NO_3^- -N x NH_4^+ -N)	0.7670*	0.2151	0.1118	0.2023
Fall 2004 (NO_3^- -N x NH_4^+ -N)	0.002	0.0159	0.0040	0.1271
Spring 2005 (NO_3^- -N x NH_4^+ -N)	0.0694	0.1015	0.1341	0.5146
Fall 2005 (NO_3^- -N x NH_4^+ -N)	<0.0001	0.0002	<0.0001	<0.0001

*No significant difference if p value=0.05

PRIR: Plowed red clover interseeded with red clover;

PRIA: Plowed red clover interseeded with AC Greenfix;

PRNI: Plowed red clover no interseeding;

CMNF: Conventional management, supplied with N fertilizer.

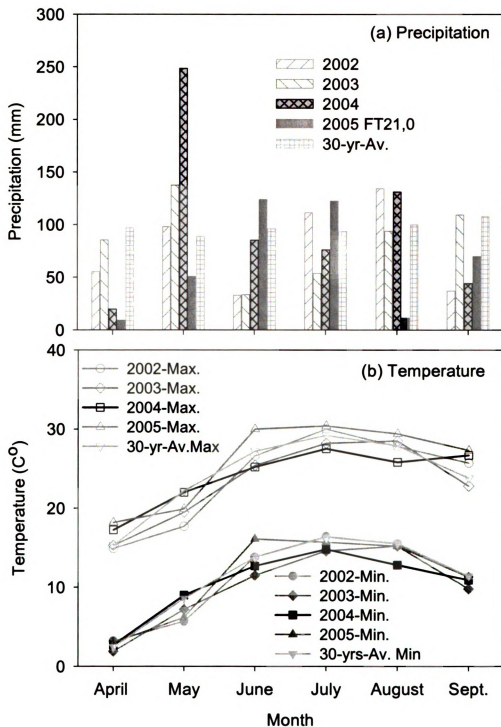


Figure 1. Monthly average minimum and maximum temperature, and total monthly precipitation during the 2002, 2003, 2004 and 2005 growing seasons compared with the 30-year monthly average at Kellogg Biological Station, Hickory Corners, MI.

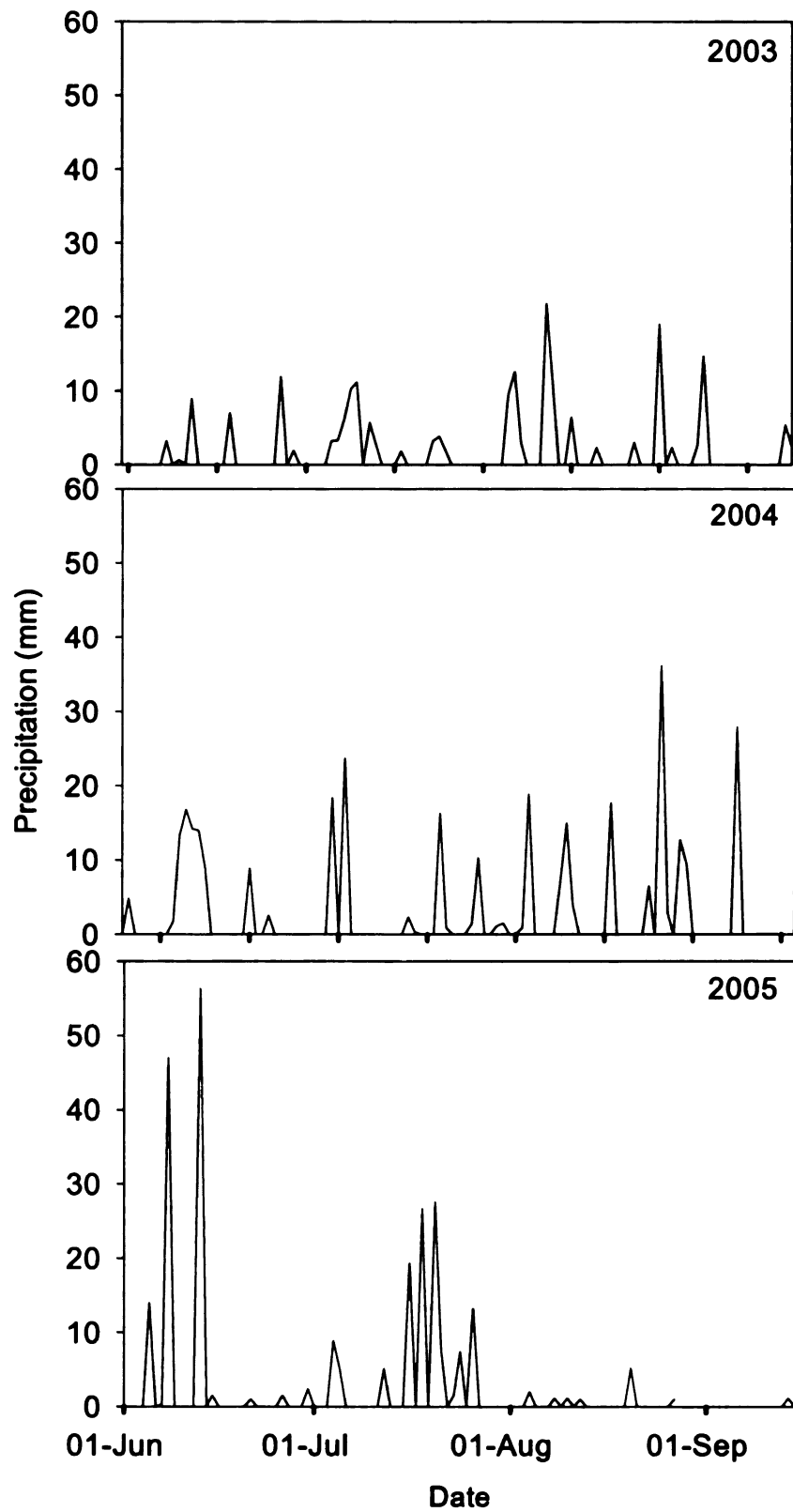


Figure 2. Total daily precipitation during the 2003, 2004 and 2005 dry bean growing seasons at Kellogg Biological Station, Hickory Corners, MI.

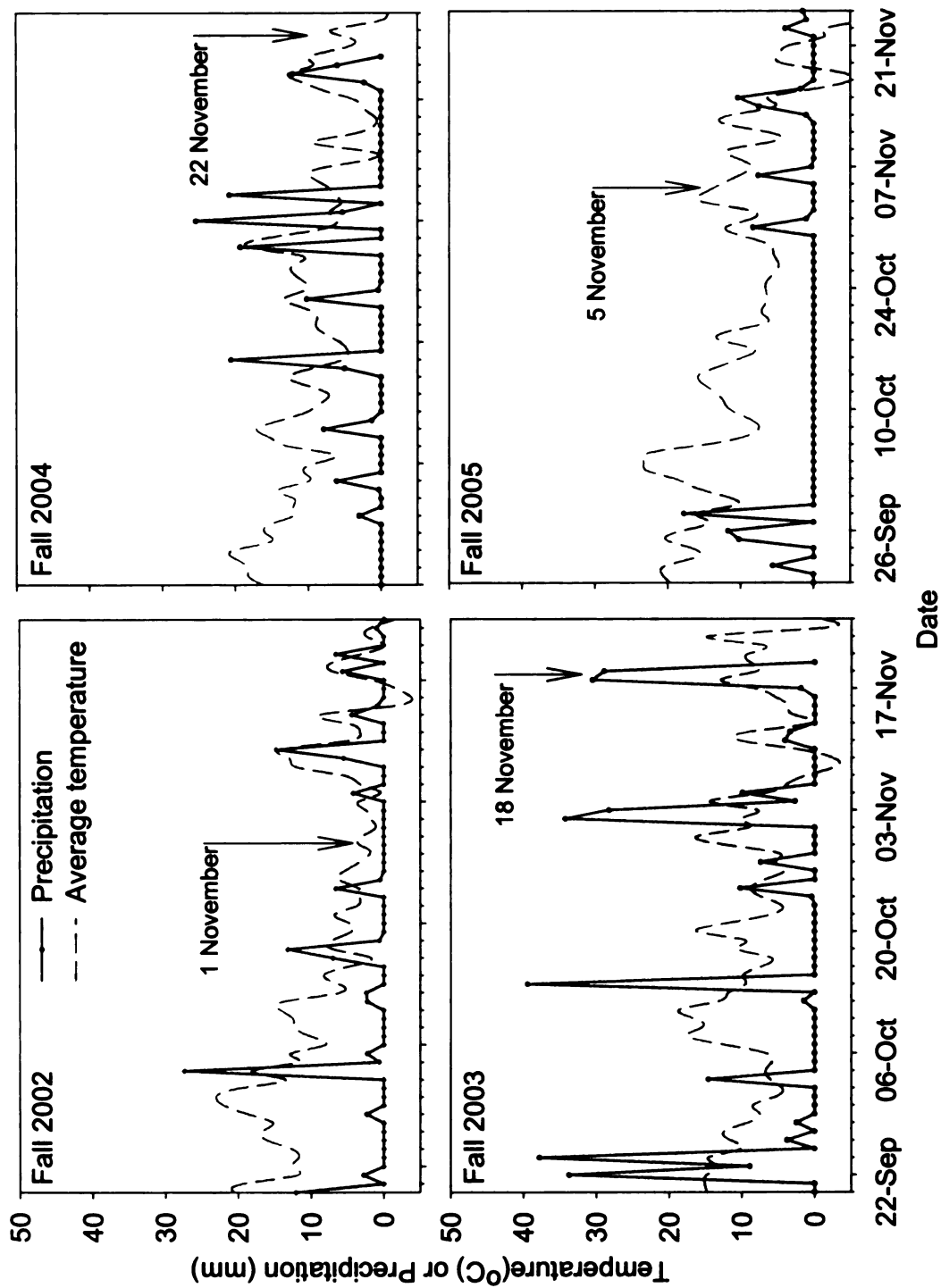


Figure 3. Total daily precipitation and average temperature from 20 September to 25 November during the 2003, 2004 and 2005 growing seasons at Kellogg Biological Station, Hickory Corners, MI.

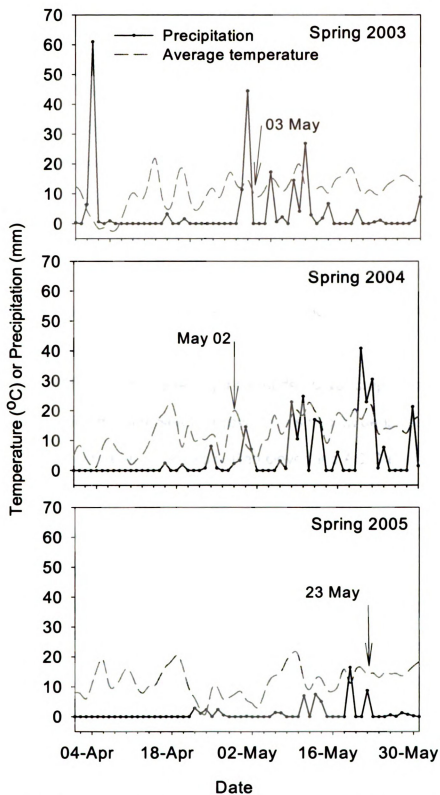


Figure 4. Total daily precipitation and average temperature from 1 April to 31 May during the 2003, 2004 and 2005 growing seasons at Kellogg Biological Station, Hickory corners, MI.

Summary and Conclusions

The objectives of this study were to evaluate (1) the effect of corn density (37 500, 55 000, 65 000 and 75 000 plants ha⁻¹) and cover crops in an interseeding system on corn yield and on red clover or AC Greenfix dry matter; (2) the effect of nitrogen fertilizer versus nitrogen provided by plowed red clover on corn yield and N status at various corn densities; (3) the effect of corn density and interseeding on nitrogen status in corn and cover crop; and (4) the effect of interseeded cover crops on soil mineral N (NO₃⁻ and NH₄⁺), and subsequent dry bean yield and N status.

Corn yield was not affected by interseeded cover crops at any plant density, suggesting that interseeding cover crops at corn densities up to 75 000 plants ha⁻¹ does not reduce corn yield. Red clover and AC Greenfix responded similarly to corn density with a decrease in DM as corn density increased. AC Greenfix established well in the interseeding system but produced on average only 10 to 20 % of its expected biomass in a monoculture system. AC Greenfix performed best during cool, wet seasons such as in 2004 where it produced considerable biomass in only 48 days. Red clover biomass increased significantly from fall to the subsequent spring as did N accumulation. However N concentration significantly decreased, suggesting a dilution factor with increased biomass production.

Corn density influenced corn grain and ear leaf N concentration, and chlorophyll content with significantly higher values at the lowest corn density,

suggesting competition for N as corn density increased. Corn grain and ear leaf N concentration, and chlorophyll content were significantly higher in corn supplied with N fertilizer regardless of dry or wet growing seasons. Ear leaf N concentration and chlorophyll content can be used as a good indicator of N concentration in corn grain. Corn grain N concentration was reduced by using cover crops as a N source and by increasing corn density regardless of N source. There was a trend for increased N concentration in corn ear leaf and chlorophyll content, and in dry bean grain, leaf and stem during dry conditions. Corn density did not influence N concentration of red clover and AC Greenfix. N accumulation for corn grain, red clover, AC Greenfix and dry bean paralleled dry matter production in every growing season. Across years, the correlation coefficient between DM and N content were $R^2=0.97$ ($p<0.0001$) for cover crops, $R^2=0.98$ ($p<0.0001$) for corn grain and $R^2=0.91$ ($p<0.0001$) for dry bean (data not shown).

Yield of dry bean planted following red clover interseeded into corn was associated with the amount of N accumulated by red clover before bean establishment and with climatic conditions. In dry conditions, yield of dry bean supplied with N fertilizer was lower than bean planted after interseeded red clover, whereas comparable yields were observed during a wet growing season. Red clover consistently achieved the objective of supplying sufficient N to produce dry bean yield similar to bean supplied with N fertilizer. Soil mineral N (NH_4^+ and NO_3^-) was not influenced by interseeding cover crops and varied with sampling time and growing season.

Under the conditions of this study, interseeded AC Greenfix did not accumulate significant amount of N in the fall to maintain subsequent dry bean yield. Similarly, AC Greenfix was not suitable for interseeding due to its low biomass production when compared with growth in monoculture. Alternative management practices should be explored such as planting after wheat harvest in July or August since the cover crop does well in cool temperature. Another alternative to consider is early establishment of AC Greenfix in April before a short cycle crop, since AC Greenfix can tolerate very low temperatures and can produce significant biomass in only 60 days. Although red clover did have slow growth in fall, it accumulated significant N in the subsequent spring. To obtain adequate N accumulation by red clover, it should be plowed under at the end of May or early June. To allow this, there should be a plan to plant a short cycle crop such as bean or vegetable crops which do not require a full growing season.

Results of this study are valuable to conventional, organic, low-input, and low-resource farmers. For conventional farmers, interseeding at corn densities up to 75 000 plants ha⁻¹ will allow maximization of corn yield, the benefit of cover crops during the winter (reduction of soil erosion) and reduce or eliminate fertilizer need for the subsequent crop. Organic farming systems and low-input farming systems of developing countries may use this system to serve as N source and to reduce soil erosion. In conclusion, this type of production system can be utilized to reduce costs in conventional, organic, and low-input farming systems, while maintaining crop yield.

Appendices

Appendix A. Total monthly precipitation (mm) during the 2002, 2003, 2004 and 2005 growing seasons, compared with 30 year monthly precipitation average at Kellogg Biological Station, Hickory Corners, MI.

Month	2002	2003	2004	2005	30-year Ave.
	-----mm-----				
April	55.4	85.4	19.8	9.3	96.9
May	98.0	137.4	248.4	50.8	88.8
June	32.8	33.5	85.2	123.9	96.0
July	111.3	53.7	76.1	122.4	93.6
Aug.	134.2	93.8	131	11.4	99.8
Sep.	37.0	109.3	44.1	69.9	107.7
Total	468.7	513.1	604.6	384.8	585.8
Ave.	78.1	85.5	100.7	64.1	97.6

Appendix B. Monthly average minimum and maximum temperatures (°C) during the 2002, 2003, 2004 and 2005 growing seasons, compared with 30-year average minimum and maximum temperatures at Kellogg Biological Station, Hickory Corners, MI.

Month	2002		2003		2004		2005		30-year Ave.	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
April	3.2	14.9	1.9	15.3	2.7	17.26	3.0	18.2	2.4	15.3
May	5.7	17.7	7.2	19.5	9.0	22.0	6.2	19.9	8.6	22.2
June	13.8	26.6	11.5	25.4	12.7	25.2	16.1	30.0	13.8	27.2
July	16.4	30.0	14.6	28.2	14.8	27.5	15.7	30.4	16.3	29.2
August	15.5	27.8	15.2	28.5	12.8	25.8	15.2	29.4	15.6	27.9
September	11.3	25.7	9.8	22.8	10.9	26.67	11.3	27.3	11.4	23.8
Average	11.0	23.8	10.0	23.3	10.5	24.1	11.3	25.8	11.4	24.2

Appendix C. Percent volumetric soil moisture at 0 to 18 and 18 to 36 cm depths in management practices across corn density during the 2004 growing season at Kellogg Biological Station, Hickory Corners, MI.

Sampling date	Soil depth									
	0-18 cm					18-36 cm				
	PRIA	CMNF	PRNI	PRIR	PRIA	CMNF	PRNI	PRIR	CV (%)	
	% volumetric soil moisture									
13 July	16.4a*	15.9a	15.7a	16.6a	26.1a	22.2b	23.9ab	22.4b	21.5	
19 July	13.4a	12.7a	13.3a	13.2a	24.2a	20.3b	22.4ab	20.3b	23.7	
26 July	13.9a	12.7a	13.3a	13.5a	23.2a	19.3b	21.8ab	19.4b	22.2	
2 Aug.	14.9a	13.5a	14.3a	14.1a	22.5a	18.0c	20.5ab	18.5bc	21.6	
9 Aug.	14.6a	13.5a	14.2a	13.9a	21.9a	17.8b	20.0ab	18.4b	22.2	
16 Aug.	18.4a	17.1a	17.8a	18.3a	23.7a	19.6b	22.1ab	19.3b	20.3	
30 Aug.	27.8a	26.3a	27.7a	28.2a	31.6a	25.7c	29.7ab	27.9bc	18.4	
13 Sept.	20.3a	16.9b	18.9ab	20.4a	28.2a	21.2c	25.5ab	24.4b	20.0	
27 Sept.	17.1a	13.7b	16.2ab	16.8a	25.2a	18.4c	22.6ab	20.7bc	21.2	
11 Oct.	18.4a	15.9a	18.0a	18.5a	24.4a	17.7c	21.9ab	19.9bc	20.0	

* Means within date (row) and depth followed by the same letter are not significantly different at P=0.05.

PRIR: Plowed red clover interseeded with red clover;

PRIA: Plowed red clover interseeded with AC Greenfix;

PRNI: Plowed red clover no interseeding;

CMNF: Conventional management, supplied with N fertilizer.

CV=Coefficient of variation

1

Appendix D. Percent volumetric soil moisture at 0 to 18 and 18 to 36 cm depths in management practices across corn density during the 2005 growing season at Kellogg Biological Station, Hickory Corners, MI.

Sampling date	Soil depth									
	0-18 cm					18-36 cm				
	PRIA	CMNF	PRNI	PRIR	PRIA	CMNF	PRNI	PRIR	CV (%)	
	-----% volumetric soil moisture-----									
19 July	20.8ab*	21.0ab	19.8b	22.0a	20.4a	21.2a	19.7a	19.5a	13.6	
26 July	22.6a	22.5a	22.3a	23.0a	21.9a	21.4a	21.8a	20.4a	16.8	
2 Aug.	14.3a	13.2a	13.3a	14.0a	17.7a	16.4a	16.3a	16.1a	20.8	
9 Aug.	10.9a	10.5a	10.3a	11.2a	14.9a	13.7a	14.1a	13.8a	24.2	
16 Aug.	9.4a	8.7a	8.7a	9.2a	13.2a	12.0a	12.2a	12.3a	28.2	
23 Aug.	9.2a	9.2a	8.9a	9.8a	12.2a	11.1a	11.6a	11.3a	30.5	
31 Aug.	8.1a	7.8a	7.7a	8.6a	11.4a	10.6a	11.0a	10.8a	34.1	
06 Sept.	7.8a	7.4a	7.4a	8.0a	11.2a	10.1a	10.5a	10.5a	36.0	
13 Sept.	7.1a	6.6a	6.6a	7.5a	10.8a	9.6a	10.3a	10.2a	37.6	

* Means within date (row) and depth followed by the same letter are not significantly different at P=0.05.

PRIR: Plowed red clover interseeded with red clover;

PRIA: Plowed red clover interseeded with AC Greenfix;

PRNI: Plowed red clover no interseeding;

CMNF: Conventional management, supplied with N fertilizer.

CV=Coefficient of variation

Appendix E. Percent volumetric soil moisture at 0 to 18 and 18 to 36 cm depths at four corn densities across management practices during the 2004 growing season at Kellogg Biological Station, Hickory Corners, MI.

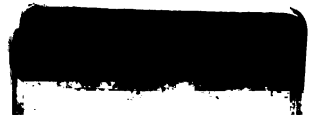
Sampling date	Soil depth									
	0-18 cm					18-36 cm				
	Corn density (plants ha ⁻¹)					Corn density (plants ha ⁻¹)				
	37500	55000	65000	75000		37500	55000	65000	75000	CV (%)
	% volumetric soil moisture									
13 July	17.0a*	14.8a	15.8a	16.9a		23.8ab	23.8ab	21.1b	25.9a	21.5
19 July	14.4a	11.6a	12.8a	13.9a		22.1ab	22.3ab	19.3b	23.4a	23.7
26 July	14.3a	12.1a	13.3a	13.8a		21.4ab	21.6ab	18.7b	22.1a	22.2
2 Aug.	15.3a	12.8a	14.0a	14.8a		20.2a	20.4a	18.2a	20.7a	21.6
9 Aug.	14.9a	12.8a	13.6a	14.8a		19.7ab	20.1ab	17.5b	20.8a	22.2
16 Aug.	17.5ab	16.3b	18.1ab	19.7a		20.4b	21.2ab	19.2b	23.9a	20.3
30 Aug.	27.3ab	25.8b	27.2ab	29.5a		27.5b	29.7ab	26.0c	31.7a	18.4
13 Sept.	18.9ab	17.8b	19.1ab	20.8a		23.9b	25.5ab	22.4c	27.6a	20.0
27 Sept.	15.8ab	14.7b	15.8ab	17.5a		20.9b	22.6ab	19.7b	23.8a	21.2
11 Oct.	17.2ab	16.4b	17.8ab	19.4a		20.0b	21.8ab	19.0b	23.2a	20.0

* Means within date (row) and depth followed by the same letter are not significantly different at P=0.05.

Appendix F. Percent volumetric soil moisture at 0 to 18 and 18 to 36 cm depths at four corn densities across management practices during the 2005 growing season at Kellogg Biological Station, Hickory Corners, MI.

Sampling date	Soil depth									
	0-18 cm					18-36 cm				
	Corn density (plants ha ⁻¹)					Corn density (plants ha ⁻¹)				
	37500	55000	65000	75000		37500	55000	65000	75000	CV (%)
	-----% volumetric soil moisture-----									
19 July	19.8b*	22.7a	21.2ab	19.8b	19.0b	21.9a	19.8ab	20.2ab		13.6
26 July	21.1b	24.0a	23.5a	22.0ab	19.5b	22.9a	21.0ab	22.1a		16.8
2 Aug.	12.6b	15.2a	13.3ab	13.7ab	15.0b	18.0a	16.5ab	17.1ab		20.8
9 Aug.	9.5a	11.6a	11.3a	10.4a	13.0a	15.3a	17.8a	14.5a		24.2
16 Aug.	8.0a	9.9a	9.5a	8.5a	11.5a	13.7a	11.9a	12.7a		28.2
23 Aug.	8.5a	10.5a	9.5a	8.6a	10.7a	12.9a	10.0a	11.6a		30.5
31 Aug.	7.3a	9.3a	8.0a	7.5a	10.2a	12.2a	10.3a	11.1a		34.1
06 Sept.	6.9a	8.7a	7.4a	7.5a	9.9a	11.8a	9.8a	10.8a		36.0
13 Sept.	6.4a	8.0a	6.7a	6.6a	9.6a	11.4a	9.4a	10.5a		37.6

* Means within date (row) and depth followed by the same letter are not significantly different at P=0.05.



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