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Evaluation of a cool-season grass-white clover mixture for low-nitrogen input lawns

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EVALUATION OF A COOL-SEASON GRASS-WHITE CLOVER MIXTURE FOR LOW-NITROGEN INPUT LAWNS

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Submitted to the Faculty
of
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by
Gabriel A. Macke

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of
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ABSTRACT

Macke, Gabriel A. M.S. Purdue University, December 2016. Evaluation of a Cool-Season Grass-White Clover Mixture for Low-Nitrogen Input Lawns. Major Professor: Cale A. Bigelow.

Turfgrass lawns require supplemental nitrogen (N) to maintain green color and seasonal shoot density. Improper lawn fertilization with excess N or phosphorus has the potential to contaminate both surface and groundwater. Thus, to reduce the reliance on supplemental N fertilization, alternative strategies or novel turf systems like grass-legume mixtures need explored. White clover (*Trifolium repens* L.) is a stoloniferous legume that biologically fixes N from the atmosphere and adds N into the soil via mineralization. The objective of this field study was to evaluate the persistence and feasibility of a cool-season grass-clover lawn mixture. A lawn grass mixture with and without a novel white clover 'Microclover' (MC) was grown at two annual N rates (0 and 98 kg N ha^{-1} yr^{-1}) for two growing seasons. Dry matter yield (DMY), yield component analysis (YCA), visual appearance, canopy greenness, clover populations, and flower production were measured. Total DMY ranged from 3815 to 15583 kg ha^{-1} and turf that received supplemental N produced the most DMY, 15583 and 13136 kg ha^{-1}, respectively, for turf with and without MC. By contrast, unfertilized turf with and without MC produced 8754 and 3815 kg ha^{-1}, respectively. The YCA in year two showed that MC contributed approximately

15% to DMY in unfertilized turf, and 3% in turf receiving supplemental N. All treatments except the unfertilized turf without MC demonstrated acceptable visual quality and where supplemental N was applied, the highest visual quality was observed. In year two, the unfertilized grass-only turf lacked vigor and was affected by two leaf blighting diseases, red thread and dollar spot, resulting in localized patches of brown, dead turf which negatively impacted visual appearance. Canopy greenness was highest in turf with MC receiving supplemental N, and lowest in unfertilized turf without MC, while unfertilized turf with MC and turf without MC receiving supplemental N were identical. Clover populations decreased over the two years regardless of supplemental N. Clover in the turf receiving supplemental N decreased substantially (17 to 1%), while slightly less in the unfertilized turf (14 to 5%), which also affected subsequent flower numbers measured in year two.

In a second study, the effect of annual N-rate (0, 98, 146, 195 kg N ha^{-1} yr^{-1}) on MC population changes was assessed using a poultry manure fertilizer. Although the MC populations again decreased over time, roughly 25 to 11 % across all treatments, there was surprisingly no difference due to any N-rate. This observation, demonstrates that in the future, various N-sources deserve further exploration for their compatibility with grass-legume systems.

Overall, these results highlight the influence of traditional N fertilization practices on DMY, visual quality, canopy greenness, and MC persistence in a cool-season lawn grass mixture with and without MC. Further, this study demonstrated that a grass-MC lawn can persist and provide reasonable visual lawn quality and is a potentially feasible option for lawns in the cool-humid region where minimal supplemental N is the goal.

CHAPTER ONE - LITERATURE REVIEW

Turfgrass covers 1.9% of the total U.S. surface area (Milesi et al., 2005) or approximately 10 to 16 million hectares (Robbins and Birkenholtz, 2003). Compared to other vegetation, turfgrasses survive because they possess the ability to persist as ground cover under regular mowing and traffic (Turgeon, 2008). Turf use can be divided into three major categories: functional, ornamental, and recreational. The specific turf use affects the maintenance intensity for an area and in general lower inputs are desired for all uses. Further, each of these uses provides numerous environmental and other benefits (Beard and Green, 1994).

Benefits of Turfgrass

Turfgrasses provide humans with aesthetic, functional, and recreational benefits and have been used in lawns and gardens for centuries (Beard, 1973). Functional benefits provided by turfgrasses include soil erosion control, groundwater protection, carbon sequestration, soil remediation, heat dissipation, and noise abatement (Beard and Green, 1994). The turfgrass plant provides effective soil erosion control with its extensive fibrous root system and high shoot density that holds together the upper layer of soil and reduces lateral water movement (Beard, 1973). Furthermore, the plant's morphology enables it to trap and hold surface runoff, protect groundwater by improving water infiltration and percolation through the soil profile, and simultaneously filters sediment such as chemical precipitates and pollutants. One of the most beneficial functions of turfgrasses is the ability to remediate soil. Over time, roots and plant tissue decompose and turnover into organic matter increasing the soils fertility. The use of turfgrasses as a vegetative cover to increase soil fertility is a practice that has been adapted around the world (Gould, 1968). Turfgrasses also have an important impact in urban communities with their ability to dissipate heat and abate noise. Beard and Green (1994) report that on average urban areas can be as much as 5 to 7 °C warmer than neighboring rural areas. Using transpiration as a cooling process, turfgrasses are able to dissipate high levels of radiant heat and have been found to be 21 °C cooler than brown dormant turf, and 39 °C cooler than a synthetic surface (Johns and Beard, 1985). Lastly, turfgrass surfaces have the ability to abate noise or absorb sound better than hard surfaces such as pavement or bare ground (Cook and Haverbake, 1971; Robinette, 1972). Turfgrasses perform these processes best when they are taking up adequate nutrients and actively growing. However, native and disturbed urban soils often do not supply adequate N to the turfgrass plant to satisfy needs or provide acceptable landscaping appearance (Carey et. al., 2012). Therefore, as a means to supply the plant with adequate nutrients to meet aesthetic standards and expectations, the practice of providing supplemental fertilization, primarily N, by humans is a necessity

Turfgrass Fertilization

The nutrient that a turfgrass plant requires in the greatest amount is nitrogen (N) (Marschner, 2012), and is often the limiting factor in growth and quality (Easton and Petrovic, 2004) followed by phosphorus (P) and potassium (K). N uptake is directly correlated with vertical top growth, leaf color, and shoot density (Beard, 1973). A mature established cool-season lawn in the Midwest region of the U.S.A. requires approximately 49 to 245 kg N ha^{-1} yr^{-1} depending on the desired level of maintenance and aesthetic expectations (Bigelow et al., 2013). Several studies point to the importance of supplying adequate N to achieve and maintain a high shoot density and gain maximum benefits from the turfgrass plant and promote environmental stewardship (Bierman et al., 2010). Porshè et al. (2012) demonstrated that a highly maintained dense uniform tall fescue (*Festuca arundinaceae*) lawn receiving 105 kg N ha^{-1} yr^{-1} reduced frequency of runoff, total runoff volume, and nutrient losses during natural rain fall when compared to a lower maintained tall fescue lawn receiving 86 kg N/ ha^{-1}/yr^{-1}, slightly lower than the recommended rate (122 to 147 kg N ha^{-1}/yr^{-1}) for tall fescue in that region. Furthermore, Bierman et al. (2010) reported Kentucky bluegrass (*Poa pratensis*) receiving annual N and K rates of 146 and 56 kg ha^{-1} respectively, reduced total annual P runoff compared to fertilizer programs applying identical rates of N and K, but with high and low rates of P as well as unfertilized turf.

Concerns with Fertilization

In recent years, supplemental lawn fertilization has been viewed negatively for its possible contribution to non-point source (NPS) pollution and eutrophication of recreational and drinking water supplies. NPS pollution often results from surface runoff and consists of rainfall or snowmelt moving over or through the soil picking up organic and synthetic pollutants and depositing them in lakes, rivers, wetlands, coastal waters, and watersheds (Pollution Runoff, 2016). Significant runoff can occur in both traditional agriculture and urban settings. Agricultural land has been identified as a major contributor to NPS pollution (Daniel et al., 1998). Beard and Green (1994) report that runoff water from agricultural and urban areas account for 64 and 5%, respectively, of the NPS surface water pollution of rivers in the USA; and 57 and 12%, respectively, of the NPS surface water pollution of lakes in the USA. The United States Environmental Protection Agency states that agricultural land is the main source of lake and river pollution, and the primary reason the Clean Water Act is unable to meet water quality goals (USEPA, 1988).

Application of fertilizer to turfgrass is also a potential source of both surface and ground water contamination (Petrovic, 1990). Both N and P can effect ground and surface water at low levels (Sharpley et al., 1994; Parry, 1998). N in the form of nitrate (NO_3^-), is the most mobile nutrient applied to turfgrass (Watschke et al., 2000), and is capable of leaching through the soil profile and contaminating ground water. Through surface runoff, excess P is known to cause algal blooms (Bush and Austin, 2001), and eutrophication at levels as low as 0.01 to 0.035 mg L^{-1}(Mallin and Wheeler, 2000), the exact amount that results from supplemental lawn fertilization is uncertain. As a result, this has resulted in some states implementing laws that restrict or prohibit supplemental P application without a laboratory soil test (e.g. MN, WI, MI, NJ, VA, PA, DE). Further, industry leading companies have frequently removed P from commercial fertilizer products.

By contrast, the growth habit and thatch forming capabilities of a well-cared for turf make it a very effective filter for reducing sediment and slowing runoff (Easton and Petrovic, 2004). For example, Ebdon et al., (1999) reported a dense stand of Kentucky bluegrass to be highly efficient at removing water from the soil, reducing soil moisture and in response decreasing runoff and leaching. Additionally, Vietor et al., (2002) reported Kentucky bluegrass to sequester up to 50% of applied N and 88% of applied P dependent on fertilizer application rate. Furthermore, Gross et al., (1990) found runoff losses of NO_3^- to be less than 1% of applied fertilizer.

Under responsible fertilization practices, NPS pollution can be mitigated in both rural and urban settings. As the public spotlight on urban fertilization and its potential to negatively impact water quality continues, there is also a strong desire to provide more sustainable or lower input turf areas. Thus, there is a need to explore alternative turfgrass nutrient management practices or species systems that supply the necessary nutrients with minimal environmental impact is desired.

Legumes Used in Pasture and Forage Systems

The use of legumes as a means for soil improvement and benefiting subsequent crops has been dated as far back as 37 B.C. during the Roman Empire (Fred et al., 1932). When considering land that is suitable for growing crops, forage legumes make up 20 $ha^{-1}x10^6$ across the world and produce 605 Mt $x10^6$ (Graham and Vance, 2003). They are an excellent source of protein, fiber, and energy that benefit animal health (Wattiaux and Howard, 2001). When compared to annual and perennial cool-season and warm-season grasses, legumes were reported to have the highest range of total digestible nutrients (Ellis and Lipke, 1976). As a result, forage legumes have played an important role in the diet of livestock responsible for meat and dairy production for centuries (Russelle, 2001).

Besides being an integral part of the diet of livestock and benefiting livestock production, legumes also have the unique ability to biologically fix their own N (BNF), from the atmosphere and generally do not require supplemental N. BNF is the natural phenomena of a leguminous plant species and a *Rhizobia* bacterial strain forming a symbiotic relationship. Rhizobia remove N_2 gas from the atmosphere producing ammonia (NH_3) which is used by the legume for plant growth. In return, the rhizobia infect the root hairs of the legume developing nodules that serve as a source of energy in the form of carbohydrates produced from photosynthesis (Evers, 2011). Further, BNF can improve soil N, replace N lost by crop removal, and reduce the dependency on supplemental N fertilization (Ledgard and Steele, 1992). This would therefore reduce leaching, volatilization, runoff, and denitrification that are potential byproducts of N fertilization (Peoples et al., 1995; Westhoff, 2009). For these reasons, the inclusion of legumes in grass systems appears to be both environmentally and economically responsible (Graham and Vance, 2003). Due to the growing dependency on N fertilization and potentially negative environmental impacts, there is an increasing interest in both Europe and the U.S.A. of the use of legumes in pastures. New Zealand and Australia have already adapted and extensively rely on the use of legumes in pastures to support low input sustainable agriculture and low cost farming systems (Ledgard and Steele, 1992).

BNF in legumes has been extensively studied. Russelle (2008) reports that BNF has a variable range from 0 to over 500 kg ha^{-1} due to a complex interaction of legume species, *rhizobia* strain, soil type, and climate. Among these factors, soil type and climate are the most influential on amount of N fixed. Legume species are more soil specific than grasses (Evers and Smith, 1998), and are more sensitive to soil pH and micronutrient deficiencies, especially molybdenum and boron (Evers, 2011).

The inclusion of legumes in grass systems to increase productivity has been well studied. Additionally, a pure legume stand will fix more N than a grass-legume mixture because of competition for water, nutrients, and light (Evers, 2011). Due to the popular use of grass-legume pastures being used in agricultural forage and pasture systems, the impact of BNF on associated grasses in grass legume mixtures has been researched extensively as well. Possible pathways of N transfer from legumes to associated grasses is the death or decay of legume herbage, roots, or nodules (Butler et al., 1959; Dubach and Russelle, 1994). Other pathways include N excretion from legume roots and nodules (Ta et al., 1986), hyphal links that directly transferred to non-legume roots via arbuscular mycorrhizal fungi (Haystead et al., 1988), and ammonia loss from legume herbage and reabsorption by grass herbage (Wedin and Russell, 2007). These pathways, however, have not been well studied in lawn systems that are regularly mowed (e.g. weekly) during the growing season.

Additionally, the desirable characteristics of a white clover cultivar would differ from pastures to lawn systems. While a variety with rapid top growth may be a desired growth habit in a forage system, it is not desired in a lawn system because it will likely lead to more frequent mowing or excess clipping production. Instead, a variety with a slower growth rate or more prostrate habit is desirable. For these reasons, a variety of white clover called ‘Microclover’ has been developed as a means to provide a clover that would be more compatible with lawn grasses and their mowing heights. Compared to the variety ‘Ladino’ commonly planted in forage systems, 'Microclover' possesses smaller leaves and a more prostrate growth habit that appears compatible with the growth and mowing requirements of turfgrasses found in cool-season lawns.

Grass-White Clover Mixtures and Nitrogen Transfer

A legume commonly mixed with grasses in temperate zones in pastures used for dairy farming around the world is *Trifolium repens* L., or white clover (WC), due to its feed quality and ability to fix nitrogen (Gibson and Cope, 1985; Ledgard and Steele, 1992). In grass-WC mixtures, WC can fix up to 400 kg N ha^{-1} yr, and productive systems, on average, fix 100 to 200 kg N ha^{-1} yr (Whitehead, 1995). Three primary factors affect BNF by legumes in mixed pastures. These include the present soil N status, legume persistence and production, and competition for light with the associated grass (Ledgard and Steele, 1992). For example, WC mixed with perennial ryegrass fixed 23, 187, and 177 kg N ha^{-1}during the seedling, first, and second production years. While WC in a pure stand fixed 28, 262, and 211 kg N ha^{-1} in the three years (Jorgensen et al., 1999). Apparent annual N transfer from WC to perennial ryegrass on a clay soil ranged from 57 to 104 kg N ha^{-1} (Elgersma and Scheplers 1997; Elgersma, Nassiri, and Scheplers 1998). Additionally, 33% of fixed nitrogen was transferred to the associated grass, reed canarygrass. In the second year of the four year study, Ladino WC mixed with reed canarygrass fixed 150 kg N ha^{-1} yr^{-1} and transferred 50 kg N ha^{-1} yr^{-1} (Heichel and Henjum, 1991). Nitrogen fixation decreases in soils with high levels of inorganic and mineral N commonly found in fertilized systems.

The benefits of including WC into a grass mixture as a solution to provide a more sustainable turf system requiring less N fertilization has been studied. Nitrogen fixation of WC in mixtures gradually decreases as nitrogen fertilization increases (Sincik and Acikgoz, 2007). When comparing low and high N rates, 20 and 400 kg N ha^{-1} yr, applied to WC-grass mixtures, N fixation is significantly reduced under high N fertilization. Under low fertilization WC fixed 118-

161 kg N ha^{-1} yr^{-1}, WC under high fertilization fixed 31-72 kg N ha^{-1} yr^{-1} (Hogh-Jensen and Schjoerring). N applications have been reported to decrease rhizobia activity and N fixation. Furthermore, soils with high levels of mineral N can inhibit root-hair infection and nodule development (Miller and Heichel, 1995).

Barriers and Obstacles to Adopting Grass-White Clover Lawns

While the potential benefits of a grass-legume system for reduced supplemental N are apparent, there are potential barriers and public acceptance of this system could be difficult. Currently, the presence of WC in urban lawns and grass seed mixtures in the United States is considered an impurity or weedy species (Robinson, 1947). Furthermore, current ideology or expectations for urban lawn systems entails a uniformly green, dense, monoculture of turfgrass species free of broadleaved “weeds,” or unwanted plant species. Like clover it is believed that changing homeowner and general public’s perception could be achieved through public outreach efforts. For the economic and environmentally conscious homeowner, the benefits of saving time and money by reducing the need for supplemental fertilization and simultaneously being more environmentally responsible by not applying excess nutrients may be an attractive incentive for adapting a grass-WC lawn mixture.

By contrast, there are several factors that pose a threat to the mainstream adoption of grass-WC lawn mixtures in urban environments. These factors include; an increase in pollinators such as bees, attaining the consistent balance of clover populations in a grass-WC lawn mixture, and the inability to control other weed species via broadcast application of broadleaf herbicides. For example, during the flowering period of WC, the flower serves as a resource to bees by providing it nectar and pollen. In return, the bees collect the pollen and transfer it other nearby plants in fertilizing the female reproductive organs and completing the pollination process. This natural phenomena is very important in production of fruit that both animals and humans eat, especially as the population of bees is decreasing, endangering the balance of the entire ecosystem. Unfortunately, homeowners with small children or melissaphobia, fear of bees or bee stings, may not be keen in the increased activity of bees or other insects in their lawn during the summer while their children and pets are playing outside or they are trying to relax and enjoy the great outdoors, thus hindering the adaption of grass-WC lawn mixtures in residential areas.

From the homeowner’s perspective, achieving a visually acceptable and beneficial clover populations in a grass-WC lawn may also be a challenge. Finding the ideal balance of inputs that mediates interspecific competition between grass and WC, and how much WC is actually needed in a grass-WC stand to provide a sustainable low-input lawn has not been identified.

Lastly, because the presence of WC disrupts the uniformity of a grass system it is considered a weed. Clover is susceptible to selective broadleaf herbicides like 2-4 D, dicamba, and mecoprop (MCPP), which are commonly used in urban turf systems to control broadleaf weeds such as dandelion, plantain, ivy, thistle, and clover. As a result, broadcast applications of these herbicides to control non-clover broadleaf weeds would not be possible in grass-clover lawn mixtures without severely damaging or eradicating the clover populations. Other management practices could still be used to maximize clover populations in lawns. The practice of “spot spraying,” or individually selecting and spraying a specific non-clover unwanted plant, is still a viable option. This practice would reduce the amount of herbicide used on the lawn, ultimately saving the applicator time, product and money, while maintaining the desired beneficial clover populations.

Project Goal and Research Objectives

The overall goal of this project was to explore alternative lawn systems to reduce reliance on supplemental N fertilization. Prior research with grass-clover lawn systems included studying the effects of supplemental N and WC inclusion on botanical composition and N cycling in a bermudagrass (*Cynodon dactylon*) lawn in the southeastern region of the U.S.A. (McCurdy et al., 2014). In addition, the carbon (C) and N release from the decomposition of WC in a bermudagrass lawn was also quantified (McCurdy et al., 2013). Other studies in Kentucky evaluated techniques to help establish WC into preexisting turfgrass stands like the impact of cultivation technique and planting date (Sparks, 2014). There has been very little research examining the performance and persistence of a cool-season grass-WC lawn mixture when supplemental N-fertilizer practices are varied.

Therefore, if the overall goal in the turf industry is to rely less on supplemental fertilizer inputs to maintain a dense aesthetically pleasing turf and mitigate the potential for environmental pollution and therefore protecting water quality, the feasibility of grass-WC lawn mixtures is justified. The specific objectives of this field study were to 1) evaluate the persistence and feasibility of a novel grass-clover lawn mixture and compare that system to a traditional cool-season lawn grass species mixture under a conventional lawn fertilizer regime 2) measure differences in seasonal growth and appearance characteristics 3) and document the persistence of a WC population over time in a cool-season lawn mixture as affected by supplemental N-fertilizer.

CHAPTER TWO - EVALUATION OF A COOL-SEASON LAWN GRASS SPECIES MIXTURE AS INFLUENCED BY ‘MICROCLOVER’ INCLUSION AND NITROGEN FERTILIZATION

Abstract

Lawns require nitrogen (N) more than any other nutrient to maintain green leaf color and seasonal shoot density. Excess N fertilization can lead to surface and groundwater contamination, suggesting a need for alternatives to reduce the reliance on frequent N fertilization. Legumes, such as white clover (*Trifolium repens* L.), biologically fix their own N and add N to the soil via mineralization. This two-year field study evaluated the growth, appearance characteristics and the persistence of a grass and grass-legume ‘Microclover’ mixture with and without supplemental N fertilization (0 vs. 98 kg N ha^{-1} yr^{-1}). Turf with ‘Microclover’ that received supplemental N produced the most (15583 kg ha^{-1}) dry matter yield (DMY) and the highest visual appearance ratings. By contrast, unfertilized turf without ‘Microclover’ produced the least DMY, (3815 kg ha^{-1},) and the lowest visual appearance which lacked vigor and was negatively affected by leaf blighting diseases. The unfertilized turf with ‘Microclover’ produced moderate growth and an acceptable visual appearance. Microclover populations decreased over time in both unfertilized and fertilized turf, but less where supplemental N was not applied. The results of this study suggest that a cool-season lawn mixture combined with ‘Microclover’ can provide a persistent, visually acceptable lawn turf that would require less reliance on supplemental N fertilization.

Introduction

The nutrient that a turfgrass plant requires in the greatest amount is nitrogen (N) (Marshner, 2012). Increasing N is correlated with more vertical top growth, darker green leaf color, and shoot density (Beard, 1973), and therefore is the limiting factor for optimal growth and visual quality (Easton and Petrovic, 2004). Depending on the desired level of maintenance, an established cool-season lawn in the Midwest region of the U.S.A. requires approximately 49 to 245 kg N ha^{-1} yr^{-1} (Bigelow et al., 2013). The practice of supplemental N fertilization on lawns has received a negative reputation for its potential role in non-point source pollution, potential to contaminate both surface and groundwater and contribute to eutrophication (Petrovic, 1990). In general, properly nourished lawn grasses possess the growth habit and thatch forming capabilities that make them an effective filter for reducing the movement of water along the surface and through the soil profile (Easton and Petrovic, 2004). Responsibly managed and fertilized lawn turf can mitigate sediment loss and minimize surface and groundwater contamination (Bierman, 2010). As the public spotlight on urban fertilization increases, the need to explore alternative turfgrass nutrient management practices or novel turf species that are more efficient nutrient users or supply the necessary nutrients with minimal negative environmental impact is needed.

Historically, legumes have been used as a means to improve soil and benefit subsequent crops (Fred et al., 1932). With their ability to biologically fix atmospheric N legumes can improve soil N levels, replace N lost by crop removal, and reduce dependency on supplemental N fertilization (Ledgard and Steele, 1992). By reducing N fertilization needs, nutrient losses through leaching, volatilization, runoff, and denitrification can all be mitigated (Peoples et al., 1995; Westhoff, 2009). For these reasons along with reducing time and money spent on N fertilization, the use of legumes is considered both environmentally and economically responsible (Graham and Vance, 2003).

One legume commonly mixed with pasture grasses in temperate zones around the world is *Trifolium repens* L., or white clover (WC), due to its feed quality and ability to fix N (Gibson and Cope, 1985; Ledgard and Steele, 1992). In grass-WC mixtures, WC can fix up to 400 kg N ha^{-1}, while on average 100 to 200 kg N ha^{-1}yr is fixed (Russelle, 1994).

The benefits of including WC into a grass mixture as a solution to provide a more sustainable turf system requiring less N fertilization has been previously studied. For example, apparent annual N transfer from WC to perennial ryegrass on a clay soil ranged from 57 to 104 kg N ha $^{-1}$ (Elgersma and Scheplers, 1997; Elgersma, Nassiri, and Scheplers, 1998). Furthermore, when 'Ladino' WC was mixed with reed canarygrass, 33% of fixed nitrogen was transferred to the associated grass. It was estimated that the legume fixed 150 kg N ha^{-1} and transferred 50 kg N ha^{-1} (Heichel and Henjum, 1991).

The potential to use legumes in lawn grass systems has received some recent study (McCurdy et al., 2014; Sparks, 2014) There is little research, however, examining the performance and persistence of grass-WC lawn mixtures when different supplemental N fertilization practices are varied on a cool-season lawn mixture. With the overall goal of decreasing the potential for environmental pollution from lawns and decreasing the reliance on N fertilization to provide a dense aesthetically pleasing turf, understanding the feasibility and persistence of grass-WC lawn mixtures is justifiable. Therefore, the specific objectives of this field study were to 1) evaluate the persistence of a novel grass-WC lawn mixture compared to a traditional cool-season lawn grass species mixture under two supplemental N fertilizer programs and 2) measure the growth and appearance characteristics of these potential lawn turf systems.

Materials and Methods

A field study was conducted from Aug. 2013 to Oct. 2015 at the William H. Daniel Turfgrass Research and Diagnostic Center, Purdue University, West Lafayette, IN on a Starks-Fincastle silt loam soil (fine-silty, mixed, mesic, Aeric Ochraqualf) with a pH of 7.2, 203 kg ha^{-1} P, 503 kg ha^{-1} K, and 1.8% organic matter.

The research study area was planted on 10 Aug., 2013 at 440 kg ha^{-1} with a commercially available cool-season lawn species mixture (Scott's Midwest Mix containing 11.83% 'Park Bench' Creeping Red Fescue, 8.78% 'Jumpstart' Kentucky bluegrass, 6.87% 'Uno' Perennial ryegrass, 6.83% 'Midnight II' Kentucky bluegrass, 5.78% 'Defender' Perennial ryegrass, 4.83% 'Greenstar' Kentucky bluegrass, 3.82% 'Treazure II' Chewings fescue, and 50% Water Smart Coating, Marysville, OH). Prior to planting, the grass seed was mixed with 5% by weight white clover ('Microclover' DLF Pickseed USA Inc., Halsey, OR), hereafter referred to as Microclover (MC) based on the aforementioned seeding rate, starter fertilizer (Shaw's Turf Food 6-24-24 Knox Fertilizer Company Inc., Knox, IN) was surface applied to provide 24 kg N and 98 kg P_2O_5 ha^{-1}. After seeding, the entire area received a liquid application of a crop legume inoculant (Alfalfa/True Clover inoculant-N-DURE; INTX MICROBIALS LLC, Kentland, IN) to provide 0.34 kg of inoculant to 23 kg of seed. Inoculant was prepared with water in a five gallon bucket to ensure agitation and applied using a 4-gallon piston pump back pack sprayer (SOLO 425 SOLO USA, Newport News, VA). After all applications were made, the study area was covered with a geotextile cover to conserve moisture and promote uniform germination. The cover was removed after 28 days.

Once a uniform cover of both grass and MC had established (e.g. in late autumn), individual plots measuring (0.9 x 3.05 m) were defined to create the grass only and grass-MC turf areas. The grass only plots, and surrounding borders, were defined by chemically removing the clover with a selective broadleaf herbicide (T-Zone: 4.7 L ha^{-1}: containing triclopyr, sulfentrazone, 2, 4-D, and dicamba; PBI Gordon Inc., Kansas City, MO).

The supplemental N-program was initiated 21 May, 2014. Where the treatments specified supplemental inorganic N, the turf received five 19.6 kg N ha^{-1} applications evenly spaced across the growing season (e.g. mid-May, June, Aug., Sept., and October) to simulate a standard inorganic granular fertilizer program used by lawn care operators in the Midwest USA. Granular urea based N-fertilizer products (e.g. urea and/or sulfur coated urea (SCU)) were used. The specific urea N-source varied by application timing. A 50% SCU: 50% urea (w/w) mixture was applied in May and September, 100% SCU in the summer, June and Aug., and 100% urea in October. The fertilizers were watered into the turf within 12 hours of application via an overhead irrigation system.

Data Collection and Measurements

Turf responses during the study were measured using both visual and quantitative methods. The specific evaluations and measurements for this study included dry matter yield (DMY), yield component analysis of DMY, visual appearance or quality, temporal changes in percentage clover, visual evaluations of the presence of white clover flowers and canopy greenness as reflectance.

DMY was determined throughout the growing season by regularly harvesting fresh clippings from an entire plot at a 6.4 cm cutting height using a rotary lawn mower with a bagging attachment (Honda Quadracut System; Honda Motor Company Inc., Tokyo, Japan). Fresh clippings were oven dried at 82°C in a forced-air drying oven for a minimum of 72 h. Dry samples were weighed to the nearest gram, ground into fine pieces (e.g. < 12 mm) using a rotary blender (Ninja Kitchen, Euro-Pro Operating LLC, Newton, MA) and returned to their respective plots and lightly raked back into the turf canopy to avoid any excess DMY that might affect subsequent DMY measurements.

To better understand the contribution of clover to DMY, a yield component analysis (YCA) method was used. Samples were collected at four locations approximately 0.6 m apart on a transect down the center of each plot. The turf was allowed to grow to approximately 15 cm. A 15 cm diameter by 6.4 cm tall PVC ring was pressed into the turf canopy until it came in contact with the soil surface. All the vegetative tissue above the edge of the ring was manually harvested with scissors. The grass was separated from the clover in each fresh sample, oven dried at 82°C in a forced-air drying oven for a minimum of 72 h, weighed to the nearest 0.0001 and the percentage of each component calculated.

Visual turf quality ratings were recorded regularly during each growing season (e.g. 2-4 times monthly) using a 0-10 scale where 10=optimum density, uniformity, and greenness, and 0= brown, dead or dormant turf, ≥6= minimally acceptable lawn turf. Visual ratings were always recorded on freshly mowed turf following DMY harvest.

Changes in percentage clover was determined using two methods, visual ratings on a 0-100% linear scale where 100 = complete clover coverage with MC and line-intersect grid counts. Grid counts were recorded four times throughout the growing season (e.g. May, June, July, and October) using the line-intersect method (Tinney et. al., 1937). Grid dimensions were 0.9 X 1.8 m^2 with 11 vertical lines and 23 horizontal lines consisting of 253 total intersects spaced 0.03 m apart. Measurements were noted at individual intersects where presence of MC was recorded and divided over the total number of intersects to calculate the percentage of MC in stand of turf.

Additionally, the presence of white clover flowers and turf disease were visually assessed. Flower prevalence was assessed by visually counting prior to DMY harvests. All flowers falling within the plot borders were recorded. Turf disease was rated visually when necrosis noticeably damaged the turf. Percentage turf blight was rated on 0-100% scale where 0 = no disease present 100% = entire plot damaged.

Canopy greenness was also measured following each DMY harvest using a hand-held reflectance meter (Field Scout TCM-500 Spectrum Technologies, Aurora, IL). Ten representative locations in each plot which were measured and averaged into a single plot value with canopy greenness expressed as a unitless color index.

In the absence of regular rainfall, the area received supplemental irrigation via an overhead irrigation system to prevent severe drought stress and promote active growth. In June and July 2015 two leaf blighting diseases; red thread *L. fuciformis* and dollar spot *S. homoeocarpa* began to damage some plots and two curative fungicide applications (chlorothalonil followed by boscalid on a 14-d interval) were made to arrest the progress of these diseases and minimize any negative influences of blighted turf on DMY or appearance measurements.

Weather data was recorded from April through November in both years, 2014 and 2015. High and low air temperatures were recorded each day and calculated to get a monthly average to compare to the historic twenty year average (Fig. 1). Precipitation, measured as rainfall, was also measured each day and calculated as cumulative monthly rainfall (cm) to compare both study years, 2014 and 2015. Lastly, seasons were defined by the astronomical seasons in the Northern hemisphere: spring (21 March to 19 June), summer: (20 June to 22) Sept., autumn: (23 Sept. to 20 December), and winter: (21 Dec. to 20 March).

This study was a 2 x 2 factorial with two factors ‘Microclover’ (yes vs. no) and supplemental annual fertilizer (0 vs. 98 kg N ha^{-1}). Treatments were replicated four times and arranged in a randomized complete block design. All data was subject to analysis of variance

(ANOVA) using the general linear model in SAS (SAS Institute v. 9.4, Cary, North Carolina, USA) and means separated using Fisher's protected least significant difference (LSD) t-test at ($P<0.05$).

Results and Discussion

Climate data for West Lafayette, IN shows that average monthly temperatures of April thru November of 2014 and 2015 varied at times from the 20 year average (Fig. 1). In April, 2014 and 2015 average temperatures were both similar to the 20 year average. In May and June of 2014, air temperatures were similar to the 20 year average, while May 2015 was slightly higher and June 2015 was slightly lower than the 20 year average. Average air temperatures in both July and August of 2014 and 2015 were lower than the 20 year average. In September and October of 2014, air temperatures were lower than the 20 year average, while in 2015 air temperatures in September and October were both higher. Lastly, in November, 2014 was much lower than the 20 year average, and by contrast 2015 was much higher.

Total monthly precipitation varied dramatically between the two the study years (Fig. 2). Rainfall totaled 81 and 67 cm yr^{-1} for 2014 and 2015 respectively. Differences in rainfall between 2014 and 2015 were greatest from June to October. June and July of 2015 received substantial amounts of rainfall (36 cm) compared to 2014 (22 cm) resulting in cooler air temperatures than the 20 year average. By contrast, in the late summer and fall months (August- October) 2015 received very little rainfall (14 cm) compared to 2014 (59 cm).

General Turf Responses

When the data are evaluated for the entire two years of this study, there were very highly significant effects ($P<0.001$) of MC and supplemental N on DMY, visual appearance and canopy greenness (Table 1). Total DMY values ranged from roughly 3800 to 15600 kg ha^{-1}, mean visual quality values ranged from 5.6 to 8.3 ($\geq$ 6.0 = acceptable lawn turf), and mean canopy greenness values ranged from 0.714 to 0.749.

Dry Matter Yield

When evaluating DMY for each individual year, DMY values ranged roughly from 2800 to 7200 kg ha^{-1} in year one (Table 2). The turf with MC and supplemental N resulted in the most DMY, and the unfertilized turf without MC resulted in the least DMY. The unfertilized turf with MC, and the fertilized grass-only turf produced roughly equal DMY amounts, 5312 and 5119 kg ha^{-1} respectively.

In year two, DMY values ranged from roughly 1000 to 8400 kg ha^{-1}. Again the fertilized turf with MC resulted in the most DMY, 8406 kg ha^{-1}, and the fertilized grass only turf was slightly less, 8017 kg ha^{-1}. The unfertilized turf without MC had the least DMY, 1037 kg ha^{-1} and the unfertilized turf with MC was intermediate with 3422 kg ha^{-1}.

DMY data in this study is similar to previous findings Wolton and Brockman, (1970) and Laidlaw (1980.) Both studies reported higher DMY in mixed swards of grass/ white clover than grass only turf receiving supplemental N fertilizer ranging from 0-134.5 kg N ha^{-1} yr^{-1}. DMY data was also similar to Wolton and Brockman's with respect to the grass only turf receiving 0 supplemental N decreasing with each succeeding year. Slight differences in this data includes Wolton and Brockman reporting higher DMY in each successive year at all N fertilizer rates in the white clover/ grass swards. This study did report a higher DMY in year two than year one for the turf with MC receiving supplemental N fertilizer, but by contrast the unfertilized turf with MC did not produce a higher DMY in year two than year one. However, the unfertilized turf with MC DMY data findings are similar to (Elgersma and Scheplers, 1997) who found in a three year study that annual DMY of unfertilized grass/ white clover mixtures declined each successive year, 12396, 10669, and 8840 kg ha^{-1}, respectively. Furthermore, DMY of turf with and without MC receiving supplemental N fertilizer in this study was consistent with the findings of (Kopp and Guillard, 2002). Kopp and Guillard reported using a similar cool-season lawn species mixture without MC consisting of bluegrass, ryegrass, and fescue on a fine sandy loam soil in a temperate humid climate similar to the Midwest in a two year field study examining the effects of N fertilizer rates and returned vs. removed clippings on DMY. Over their two year study the average DMY of turf receiving 0 and 98 kg N ha^{-1} with clippings returned was approximately 3000 and 6000 kg ha^{-1} respectively. DMY can also be compared to (Walker et al., 2007) who in a two year field study examined above ground responses of cool-season lawn species to different N fertilizer rates and application timings. Walker et al. (2007) reported substantially less DMY of grass only turf receiving supplemental N, but similar DMY values in unfertilized turf despite being in a temperate humid climate in the Midwest. Using similar grass species, unfertilized Kentucky bluegrass-only turf produced 1864 and 1785 kg ha^{-1} in year one and two, respectively, for a study total of 4561 kg ha^{-1} while in the present study, unfertilized grass only produced 2777 and 1037 kg ha^{-1} for year one and two respectively for a study total of 3815 kg ha^{-1}. Furthermore, Kentucky bluegrass receiving 123 kg N ha^{-1} yr^{-1} at different application timings did not produce as much DMY as the turf in the present study with or without MC receiving 98 kg N ha^{-1} yr^{-1}. Kentucky bluegrass receiving 123 kg N ha^{-1} yr^{-1} at different times of the year, had a DMY ranging from 3134 to 3496 kg ha^{-1} in year one and 3588 to 3725 kg ha^{-1} in year two, for a study total of 7842 to 8463 kg ha^{-1}. By contrast, in the current study studies turf with MC and grass-only turf in this study receiving 98 kg N ha^{-1} yr^{-1}, produced DMY values of 7177 and 5119 kg ha^{-1} in year one, and 8406 and 8017 kg ha^{-1} in year two, for a study total of 15583 and 13136 kg ha^{-1} producing roughly double the DMY. The differences in results could be due to N application timing. N fertilizer was applied evenly over the course of the growing season, compared to autumn focused fertility.

To better understand how MC and N fertilizer affected DMY, the DMY data into three different growing periods (e.g. spring, summer, fall) for each individual year (Table 2.) In this study, there were 37 total harvests with 22 and 15 in year one and two, respectively. Across all three growing periods in year one, fertilized turf with MC produced the most total DMY. By contrast, unfertilized turf without MC produced the least DMY. The unfertilized turf with MC produced more total DMY in spring and summer than fertilized turf without MC, but not in the fall.

In the spring of year one, fertilized and unfertilized turf with MC had more DMY than fertilized and unfertilized turf without MC. In the summer, fertilized turf with MC produced the most DMY, 3320 kg ha^{-1} and the unfertilized turf without MC the least 844 kg ha^{-1}. The fertilized turf without MC and unfertilized turf with MC were intermediate. In the fall, fertilized turf with and without MC produced the highest DMY, with 1704 and 1620 kg ha^{-1} respectively. By contrast, unfertilized turf without MC produced the lowest DMY with 495 kg ha^{-1}.

In year two, total DMY values ranged from 1037 to 8406 kg ha^{-1}. Across all three growing periods DMY results for unfertilized turf without MC and fertilized turf with MC were the same in year two as year one. Fertilized turf with MC produced the highest DMY across all three seasons and unfertilized turf without MC produced the least. What was different in year two was fertilized turf without MC produced significantly more DMY than in year one, as well as producing more DMY than unfertilized turf with MC which in year one produced statistically similar DMY. For example, in the spring, fertilized turf without MC and unfertilized turf with MC produced 2530 and 1184 kg ha^{-1}, respectively, and were not statistically similar ($P \leq 0.05$). In year one fertilized turf without MC produced 1964 kg ha^{-1} of DMY in the summer period, and in year two produced 3842 kg ha^{-1}. By contrast, fertilized turf with MC produced 3320 and 3864 kg ha^{-1} in summers of year one and two respectively. It is postulated that the large increase in total DMY for the fertilized turf without MC in the second year of the study, 5119 versus 8017 kg ha^{-1}, is due to the effects of the supplemental N fertilizer becoming realized to the grass plants. DMY values in the fall of year two, were nearly identical to year one even though the total number of harvests were fewer due to longer intervals between harvests. Turf receiving supplemental N with and without MC produced the highest DMY, 1657 and 1645 kg ha^{-1}, respectively. These values were similar to year one, 1620 and 1704 kg ha^{-1} for the turf receiving supplemental N with and without MC. By contrast, the unfertilized turf without MC produced the lowest DMY 161 kg ha^{-1}, and unfertilized turf with MC was the intermediate with 647 kg ha^{-1}.

By year two, fertilized turf with and without MC began to produce excessive clippings across all three seasons with respect to a low maintenance turf system. This suggests that the 98 kg N ha^{-1} yr^{-1} was sufficient N for a lower maintenance turf system on this soil type in this geographic region. On the other hand, the unfertilized turf with MC produced substantially less DMY in year two than the fertilized treatments, yet this turf still sustained sufficient growth, density and an ability to resist disease. These data suggest that a mixed sward of grass and MC appears to be a viable option for a persistent low maintenance lawn turf system.

Additionally, changes in seasonal growth patterns of each lawn mixture as affected by N fertilization can be explained (Figure 1). In the spring of year one, unfertilized and fertilized turf with MC produced DMY at a faster rate than unfertilized and fertilized turf without MC (Figure 1). Progressing into the summer, fertilized turf with MC produced DMY at a faster rate than unfertilized turf with MC, and fertilized turf without MC produced DMY at a faster rate than unfertilized turf without MC. N fertilizer was very highly significant in the summer explaining why the fertilized plots with and without MC produced more than their counterparts, unfertilized turf with and without MC. Furthermore, MC was also had a significant impact on DMY in the summer, thus explaining why unfertilized turf with MC continued to produce DMY at a fast rate. In the fall, N fertilizer continued to have a very highly significant effect while MC only had a significant effect leading fertilized turf without MC to produce DMY at a faster rate than unfertilized turf with MC ending the year one with similar values for DMY. Fertilized turf with and without MC produced similar DMY values in the fall, however, for the first time in year one there was significant interaction, (PLO 0.05) between MC and N explaining why fertilized turf with MC produced DMY at a slightly faster rate in the fall than fertilized turf without MC.

By the first harvest in the spring of year two, fertilized turf without MC surpassed unfertilized turf with MC in cumulative DMY. Fertilized turf with and without MC produced DMY at the fastest and second fastest rate, respectively, while unfertilized turf with MC produced DMY at a slower rate than the fertilized turf but faster than unfertilized turf without MC. In the summer and fall of year two, fertilized turf with or without MC produced DMY at the fastest rate and statistically similar values. Furthermore, unfertilized turf with MC continued to produce DMY at a rate less than the fertilized turf regardless of MC inclusion and greater than unfertilized turf without MC. The rate of DMY produced in the unfertilized turf without MC decreased from year one to year two. The decrease in DMY production was a result of the unfertilized turf without MC inability to take up adequate nitrogen for growth. Furthermore, the stand density of the unfertilized turf without MC was afflicted in late May and June by low N diseases, red thread *L. fuciformis* and dollar spot *S. homoeocarpa*. Unfertilized turf with MC also produced DMY at a slower rate in year two than in year one. However, unfertilized turf with MC was not afflicted by disease, still produced an acceptable ground cover, and did not visually appear to lose stand density.

In year two, yield component analysis (YCA) was measured at three sampling dates (8 May, 28 July, and 7 Oct.) to understand how much MC foliage was contributing to the overall weight of the DMY harvests as a percentage, and how it was being affected by the N fertilizer (Table 4). Results showed that in year two yield components were comprised of 9 to 18 percent by weight MC foliage in unfertilized turf with MC, and 1 to 5 percent by weight in fertilized turf with MC across the three sampling dates. On 8 May and 7 Oct., yield components were 17 and 18 percent by weight MC foliage in the unfertilized turf with MC, and 5 and 1 percent by weight in the fertilized turf with MC respectively. At these sampling dates N fertilizer had a significant effect on MC percentage by weight contribution to DMY harvests. By contrast, on 28 July MC contributed 9 percent by weight to the DMY harvest in unfertilized turf with MC, and 2 percent by weight in the fertilized turf with MC. At this sampling date N fertilizer did not have a significant effect on MC's component of DMY harvests. It is believed that the lack of difference in MC percent by weight component of DMY could be due to the short interval between prior DMY harvest and the 28 July sampling date to determine YCA.

Visual Appearance

When evaluating the turf for appearance or visual turf quality (TQ), the mean TQ values for the study ranged from 5.6 to 8.3, with only the unfertilized grass only turf producing an unacceptable (< 6.0) quality (Table 3). When evaluating each individual year, mean visual quality in year one ranged from 6.6 to 8.1. The turf with MC receiving supplemental fertilizer N had the highest visual quality, 8.1, and the unfertilized turf without MC resulted in the lowest TQ, 6.6.

The unfertilized turf with MC, and grass only turf receiving 98 kg N ha^{-1} had similar TQ values, 7.6 and 7.5, respectively.

In year two, mean TQ values ranged from 4.6 to 8.6. Turf receiving supplemental N fertilizer either with or without MC resulted in the highest TQ, 8.6, and unfertilized turf without MC resulted in the lowest visual quality, 4.6. The unfertilized turf with MC produced a mean TQ rating of 7.2, falling in between the lowest and highest visual quality ratings, and was different than all other treatments.

When TQ is evaluated for the three different growing periods (e.g. spring, summer, fall) in each individual year the temporal and seasonal effects of treatments become apparent. Across all three growing periods in year one, turf with MC and receiving supplemental N produced the highest TQ, and unfertilized turf without MC produced the lowest TQ. All values, however, were deemed acceptable, $\geq$ 6.0. The seasonal TQ values of grass only fertilized turf improved over the year as the N fertilizer responses began to take effect. For example, in the spring of year one, the unfertilized turf with MC had a higher TQ than fertilized turf without MC, 7.2 versus 6.8, respectively. In the summer, however, they were similar, 7.8 and 7.6, respectively. In the fall, the fertilized grass-only turf was superior to the unfertilized turf with MC, 9.0 versus 8.3, respectively. The highest TQ values across all three growing periods was associated with the fertilized turf with MC which was superior to all treatments except in the fall when it was equivalent to the fertilized grass only turf.

In year two, across all three growing periods, the trends in TQ values were similar to year one for the unfertilized grass only turf and fertilized turf with MC. One difference in year two was that the values for the unfertilized turf without MC were slightly lower than year one and TQ was unacceptable in spring and summer. By contrast, the fertilized grass only turf had generally higher TQ values than in year one. The fertilized grass only turf had higher TQ values than the unfertilized turf with MC in all three growing periods and was similar to the fertilized turf with MC which had the highest TQ values for all treatments in both year one and two of the study. The increased TQ values for the fertilized grass-only turf was primarily due to the darker green color and density due to repeated supplemental fertilizer N applications.

The lower TQ values of the unfertilized turf without MC in year two was due to a lack of vigor in this turf as evidenced in the DMY data (Table 2) but also associated with the presence of turf disease. The weather in year two was characterized by cool, wet weather (Figure 1 and 2). In the spring and early summer red thread *L. fuciformis* and dollar spot *S. homoeocarpa* blighted the turf resulting in localized patches of dead, brown turf which negatively affected TQ ratings. For example on 18 June, 2015 percentage turf blight due to disease was 0.4% versus 9% for unfertilized grass with and without MC (data not shown). By contrast, there was no visible blight in the plots receiving supplemental N. Dollar spot and red thread are diseases commonly associated with turf that has low vigor and/or is N deficient (Smiley et al, 2005). Since this turf had not received supplemental fertilizer N since being planted in Aug. 2013, this response is not surprising.

TQ results of this study were similar to the findings of (Sincik and Acikgoz, 2014). Unfertilized grass/ white clover turf mixtures produced significantly higher TQ ratings than unfertilized grass only turf across all seasons in a three-year study.

Clover Population Changes

In year one MC populations ranged from 7 to 17 percent in fertilized turf while MC populations in unfertilized turf had less variation ranging from 11 to 14 percent (Table 5). MC populations were highest in mid-June in unfertilized turf, and late-May in fertilized turf.

Although MC populations decreased in each successive sampling in fertilized turf of year one, annual N rate did not have a significant effect on MC populations in year one when compared to unfertilized turf MC populations.

In year two, clover populations ranged from 1 to 5 percent in fertilized turf and 5 to 9 percent in unfertilized turf. Similar to year one, MC populations in fertilized turf continued to decrease throughout year two. On the first measurement date in June, the N fertilizer had a significant effect compared to the unfertilized turf. Furthermore, as the season progressed N fertilizer had a significant effect on MC populations in late July and October reducing MC populations to just one percent by the end of year two. Different from year one, MC populations were highest in unfertilized turf in May instead of June and steadily decreased from May to late July but remained constant from late July to October.

Flower Production

Flower production (FP) was measured from June to August by visually counting the number of MC flowers present in each plot prior to DMY harvests. In year one, FP ranged from 12 to 52 in unfertilized turf with MC, and 1 to 18 in fertilized turf with MC. For both fertilized and unfertilized turf with MC, FP was highest on 16 July and lowest on 20 Aug (Table 5). On 16 July of year one N fertilizer had a significant effect on FP and continued to have a significant effect the rest of the year. On average, unfertilized turf with MC produced 52 flowers while fertilized turf with MC only produced 18. This was further evident on 20 Aug. when unfertilized turf with MC on average produced 12 flowers and fertilized turf with MC produced only 1.

In year two, FP ranged from 26 to 80 in unfertilized turf with MC, and 1 to 6 in fertilized turf with MC. Similar to year one, FP for both fertilized and unfertilized turf with MC recorded its highest FP in mid-July and its lowest FP in Aug. In both year one and two, FP in fertilized turf with MC in Aug. only produced 1 flower. What was different in year two from year one was N fertilizer had a significant effect or very highly significant effect throughout all of year two on FP.

Canopy Greenness

Canopy greenness (CG) was measured as reflectance and data are presented as a unitless index value (Table 6). For the study, the CG values ranged from 0.714 to 0.749 with the unfertilized grass only turf having the lowest value and the fertilized turf with MC the highest value. When evaluating each individual year, in year one, CG ranged from 0.721 to 0.752. Turf with MC receiving supplemental N resulted in the highest CG, 0.752, and unfertilized turf without MC resulted in the lowest CG, 0.721. Grass only turf receiving supplemental N fertilizer and unfertilized turf with MC were intermediate and produced statistically similar CG values, 0.738 and 0.744 respectively. Furthermore, they both produced statistically significant higher CG values than unfertilized turf without MC. Grass only turf receiving supplemental N was not statistically similar to turf with MC receiving supplemental N while unfertilized turf with MC produced statistically similar CG values to fertilized turf with MC, 0.744 and 0.752 respectively.

In year two, CG ranged from 0.706 to 0.746. Same as year one, turf with MC receiving supplemental N fertilizer resulted in the highest CG. Unfertilized turf without MC resulted in the lowest CG, and grass only turf receiving supplemental N fertilizer and unfertilized turf with MC produced intermediate values, 0.743 and 0.738 respectively. What was different in year two, was grass only turf receiving supplemental N produced a higher CG in year two than year one and grass only turf receiving N fertilizer resulted in statistically similar CG values as fertilized turf with MC, 0.743 and 0.746, respectively. Unfertilized turf with MC produced a lower CG in year two than year one and was not statistically similar to fertilized turf with MC as it was in year one.

When CG is evaluated for the three different growing periods (e.g. spring, summer, fall) in each individual year the effects of MC and N on treatments became apparent. Across all three growing periods in year one, fertilized turf with MC produced the highest CG value. By contrast, grass-only turf receiving supplemental N produced the lowest CG value in the spring, and unfertilized grass only turf produced the lowest CG value in summer and fall.

In the spring of year one, unfertilized turf with and without MC produced intermediate CG values, 0.722 and 0.708 respectively. These CG values were lower than fertilized turf with MC, but still remained statistically similar. However, grass only turf receiving supplemental N fertilizer was statistically different from the other three treatments and produced the lowest CG value, 0.705, in the spring of year one. In the spring of year one, MC had a significant effect on the treatments while the N fertilizer and the interaction between the two variables had not. This explains why the fertilized and unfertilized turf with MC produced the two highest CG values, but does not explain why grass only turf without supplemental N still produced a statistically similar CG value to both of the turf treatments with MC. In the summer, MC had a highly significant effect on the treatments. Same as in the spring, unfertilized and fertilized turf with MC produced the two highest CG values, 0.748 and 0.742 respectively, and were statistically similar. Furthermore, N fertilizer also had a significant effect in the summer. Grass only turf receiving supplemental N increased its CG value from 0.705 to 0.731 from the spring to summer season becoming statistically greater than unfertilized grass only turf and statistically similar to unfertilized turf with MC, but not statistically similar to fertilized turf with MC. Lastly, unfertilized grass only turf produced the lowest CG, 0.713, and was not statistically similar to any other treatment. In the fall of year one, N fertilizer had a very highly significant effect. Fertilized turf with MC and grass only turf receiving supplemental N fertilizer were statistically similar and produced the two highest CG values, 0.788 and 0.787 respectively. Unfertilized turf with MC produced an intermediate value of 0.770. For the first time in year one unfertilized turf with MC was not statistically similar to fertilized turf with MC. Lastly, similar to summer, unfertilized grass-only turf produced the lowest CG value, 0.751, and was not statistically similar to any other treatments. Similar to year one, fertilized turf with MC produced the highest CG values across all three seasons. Different from year one, unfertilized grass only turf produced the lowest CG values across all three seasons. In year two, N fertilizer continued to have a highly and very highly significant effect on CG causing seasonal CG of year one and year two to have multiple differences. For example, unfertilized and fertilized turf with MC and grass only turf receiving supplemental N all produced statistically similar CG values in the spring, 0.731, 0.739, and 0.739 respectively. By contrast, unfertilized grass only turf produced the lowest CG, 0.712. In the summer, all three treatments effects were very highly significant. Fertilized turf with MC continued to produce the highest CG value, 0.748. Grass only turf receiving supplemental N and unfertilized turf with MC produced intermediate values, 0.743 and 0.741 respectively. However, grass only turf receiving supplemental N CG value was still statistically similar to fertilized turf with MC while unfertilized turf with MC wasn’t. Same as in the summer, N fertilizer had a very highly significant effect and grass only turf receiving supplemental N and fertilized turf with MC produced statistically similar CG values, 0.770 and 0.766 respectively. Furthermore, MC alone did not have a significant effect in the fall concluding why unfertilized turf with MC was not statistically similar to fertilized turf with MC and grass only turf receiving supplemental N producing an intermediate CG value, 0.751. Lastly, unfertilized grass only turf produced the lowest CG value, 0.736, and was not statistically similar to any of the other treatments.

Summary and Conclusion

In conclusion, over the two growing seasons of this study, the turf with MC that received supplemental N, 98 kg N ha^{-1} yr^{-1}, produced the most desirable appearance and DMY or shoot growth, which was at times excessive. The grass-only turf receiving the supplemental N program also produced a high quality visual appearance and shoot growth nearly similar to the grass-MC turf with supplemental N. The unfertilized turf without MC produced the least shoot growth and least desirable appearance. The lack of vigor in this turf resulted in significant damage from leaf blighting diseases in year two and required fungicides. The unfertilized grass-MC turf resulted in vigorous but non-excessive shoot growth and a seasonal appearance that would be more than acceptable for green color and uniformity. Further, it was observed that supplemental N substantially reduced MC populations, suggesting that to maintain the potential benefits of MC, minimal supplemental N should be applied. Ultimately, this study demonstrated that a persistent grass-MC lawn species mixture could be maintained and appears to be an alternative to traditional grass-only lawns that receive supplemental fertilizer N. These grass-MC lawns would require substantially less supplemental N fertilization and reduce the risk potential for NPS pollution due to excess lawn nutrient applications.

Table 1. Overall summary of dry matter yield, visual turf quality and canopy greenness for a cool-season lawn species mixture grown with and without white clover (*Trifolium repens* L.-'Microclover') at two annual nitrogen (N) rates.

		Dry matter yield			Visual quality			Canopy greenness		
White clover†	Annual N rate‡	2014	2015	Study total	2014	2015	Study mean	2014	2015	Study mean
	kg N ha^{-1}	----------- kg ha^{-1}§ ----------			------- (0-10 rating scale) ¶ -------			--------- (color index) # -----------		
No	0	2777 c	1037 d	3815 d	6.6 c	4.6 c	5.6 d	0.721 c	0.706 c	0.714 c
No	98	5119 b	8017 b	13136 b	7.5 b	8.6 a	8.1 b	0.738 b	0.743 ab	0.741 b
Yes	0	5312 b	3422 c	8754 c	7.6 b	7.2 b	7.4 c	0.744 ab	0.738 b	0.741 b
Yes	98	7177 a	8406 a	15583 a	8.1 a	8.6 a	8.3 a	0.752 a	0.746 a	0.749 a
Source of variation										
Microclover (MC)		***	***	***	**	***	***	***	***	***
Nitrogen (N)		***	***	***	*	***	***	***	***	***
MC x N		NS	**	*	NS	***	***	NS	***	NS

† A commercially available cool-season lawn species mixture containing Kentucky bluegrass, perennial ryegrass, and fineleaf fescue was planted on 10 Aug., 2013 at 440 kg ha^{-2} 'Microclover' was included at 5% by weight, grass-only plots were established by applying a broad-spectrum broadleaf weed herbicide to remove clover following establishment.

‡ Granular N-fertilizer treatments began in May, 2014. Five individual 19.6 kg N ha^{-1} applications were evenly spaced across the growing season to emulate a traditional inorganic lawn care program using a combination of urea and sulfur coated urea products.

§ Dry matter yield was determined throughout each growing season by harvesting fresh clippings at a 6.4 cm cutting height using a rotary bagging mower.

¶ Turf quality was visually rated on a 0-10 scale where 10=optimal greenness, density and uniformity, 0=brown, dormant/dead turf and ≥6= acceptable lawn turf.

Canopy greenness was measured using a hand-held turf reflectance meter (Spectrum Technologies TCM-500) at ten representative locations in each plot which were averaged into a single plot value and expressed as a unitless color index.

Means in the same column followed by the same letter are not significantly different according to Fisher's protected least significance test (LSD P=0.05). *,**,*** and NS refer to significant at the 0.05, 0.001, 0.0001 and non-significant, respectively.

Table 2. Seasonal dry matter yield over two growing seasons for a cool-season lawn species mixture grown with and without white clover (*Trifolium repens* L.-‘Microclover’) and two annual nitrogen (N) rates.

		Dry matter yield								
		2014				2015				
White clover†	Annual N Rate‡	Spring¶	Summer	Fall	Total	Spring	Summer	Fall	Total	Study Total
	kg N ha^{-1}	-- kg ha^{-1}§ --								
No	0	1438 b	844 d	495 c	2777 c	503 d	373 c	161 c	1037 d	3815 d
No	98	1534 b	1964 c	1620 a	5119 b	2530 b	3842 a	1645 a	8017 b	13136 b
Yes	0	1943 a	2425 b	944 b	5312 b	1184 c	1591 b	647 b	3422 c	8754 c
Yes	98	2152 a	3320 a	1704 a	7177 a	2885 a	3864 a	1657 a	8406 a	15583 a
# of harvests		4	12	6	22	5	8	2	15	37
Source of variation										
Microclover (MC)		*	***	*	***	**	***	***	***	***
Nitrogen (N)		NS	***	***	***	***	***	***	***	***
MC x N		NS	NS	*	NS	NS	***	***	**	*

† A commercially available cool-season lawn species mixture containing Kentucky bluegrass, perennial ryegrass, and fineleaf fescue was planted on 10 Aug., 2013 at 440 kg ha^{-2} ‘Microclover’ was included at 5% by weight, grass-only plots were established by applying a broad-spectrum broadleaf weed herbicide to remove clover following establishment.

‡ Granular N-fertilizer treatments began in May, 2014. Five individual 19.6 kg N ha^{-1} applications were evenly spaced across the growing season to emulate a traditional inorganic lawn care program using a combination of urea and sulfur coated urea products.

§ Dry matter yield was determined throughout each growing season by harvesting fresh clippings at a 6.4 cm cutting height using a rotary bagging mower.

¶ Seasons were determined by astronomical seasons in the Northern Hemisphere. Spring: March 21 – June 19, Summer: June 20 – September 22, Fall: September 23 – December 20.

Means in the same column followed by the same letter are not significantly different according to Fisher’s protected least significance test (LSD P=0.05). *,**,*** and NS refer to significant at the 0.05, 0.001, 0.0001 and non-significant, respectively

Table 3. Seasonal changes in visual turf quality for a cool-season lawn species mixture grown with and without white clover (*Trifolium repens* L.-‘Microclover’) and two annual nitrogen (N) rates.

		Visual quality								
	Annual	2014				2015				
White clover†	N rate‡	Spring¶	Summer	Fall	Mean	Spring	Summer	Fall	Mean	Study Mean
	kg N ha^{-1}	----------------	----------------	----------------	----------------	(0-10 scale)§	----------------	----------------	----------------	----------------
No	0	6.7 d	6.6 c	7.0 c	6.6 c	5.2 c	4.1 c	6.0 c	4.6 c	5.6 d
No	98	6.8 c	7.6 b	9.0 a	7.5 b	7.8 a	9.0 a	9.0 a	8.6 a	8.1 b
Yes	0	7.2 b	7.8 b	8.3 b	7.6 b	6.6 b	7.5 b	7.9 b	7.2 b	7.4 c
Yes	98	7.3 a	8.3 a	8.9 a	8.1 a	7.8 a	9.0 a	9.0 a	8.6 a	8.3 a
Source of variation										
Microclover (MC)		***	***	***	**	**	***	***	***	***
Nitrogen (N)		***	***	***	*	***	***	***	***	***
MC x N		***	NS	***	NS	**	***	***	***	***

† A commercially available cool-season lawn species mixture containing Kentucky bluegrass, perennial ryegrass, and fineleaf fescue was planted on 10 Aug., 2013 at 440 kg ha^{-2} ‘Microclover’ was included at 5% by weight, grass-only plots were established by applying a broad-spectrum broadleaf weed herbicide to remove clover following establishment.

‡ Granular N-fertilizer treatments began in May, 2014. Five individual 19.6 kg N ha^{-1} applications were evenly spaced across the growing season to emulate a traditional inorganic lawn care program using a combination of urea and sulfur coated urea products.

§ Turf quality was visually rated on a 0-10 scale where 10=optimal greenness, density and uniformity, 0=brown, dormant/dead turf and ≥6= acceptable lawn turf.

¶ Seasons were determined by astronomical seasons in the Northern Hemisphere. Spring: March 21 – June 19, Summer: June 20 – September 22, Fall: September 23 – December 20.

Means in the same column followed by the same letter are not significantly different according to Fisher’s protected least significance test (LSD P=0.05).

*,**,*** and NS refer to significant at the 0.05, 0.001, 0.0001 and non-significant, respectively.

Table 4. Temporal variation of clover populations over two years using the line-intersect method, and yield component analysis of white clover present in dry matter yield harvests in a cool-season lawn species mixture grown with white clover (*Trifolium repens* L.-‘Microclover’).

Annual N rate†	Clover populations								Yield component analysis		
	2014				2015				2015		
	21 May	17 June	30 July	16 Oct	13 May	29 June	31 July	08 Oct	08 May	28 July	07 Oct
kg N ha^{-1}	Percentage (%)‡								(% MC by wt.)§		
0	13 a	14 a	13 a	11 a	9 a	7 a	5 a	5 a	17 a	9 a	18 a
98	17 a	15 a	13 a	7 a	5 a	2 b	1 b	1 b	5 b	2 a	1 b
Source of variation											
Nitrogen (N)	NS	NS	NS	NS	NS	*	**	**	*	NS	**

† Granular N-fertilizer treatments began in May, 2014 with five individual 19.6 kg N ha^{-1} applications evenly spaced across the growing season to emulate a traditional inorganic lawn care program. The N-sources used were urea and sulfur coated urea, and the percentage of each varied depending upon season of application. The fertilizers watered into the turf within 12 hours of application.

‡ Percentage ‘Microclover’ was quantitatively measured using the line-intersect method and a 0.9 x 1.82 m grid with 253 intersects..

§ Yield component analysis, percentage by weight ‘Microclover inclusion’, was determined from a biomass sample using a yield component analysis (YCA) technique. YCA was determined by harvesting tissue from above a 6.35 cm tall ring x 15.24 cm diameter in four locations per plot. Following harvest, grass and clover was separated from the samples, oven dried, and weighed.

Means in the same column followed by the same letter are not significantly different according to Fisher’s protected least significance test (LSD P=0.05). *,**,*** and NS refer to significant at the 0.05, 0.001, and non-significant, respectively.

Table 5. Visual white clover (*Trifolium repens* L.-‘Microclover’) flower counts for a cool-season lawn species mixture grown with and two annual nitrogen (N) rates.

	Visual Flower Counts							
	2014				2015			
Annual N rate‡	5 June	10 July	16 July	20 Aug	1 June	15 July	24 July	14 Aug
kg N ha^{-1}	--# of flowers ¶--							
0	25 a	33 a	52 a	12 a	45 a	80 a	66 a	26 a
98	9 a	16 a	18 b	1 b	4 b	6 b	4 b	1 b
Source of Variation								
Nitrogen (N)	NS	NS	*	*	*	***	***	***

† A commercially available cool-season lawn species mixture containing Kentucky bluegrass, perennial ryegrass, and fineleaf fescue was planted on 10 Aug., 2013 at 440 kg ha^{-2} ‘Microclover’ was included at 5% by weight, grass-only plots were established by applying a broad-spectrum broadleaf weed herbicide to remove clover following establishment.

‡ Granular N-fertilizer treatments began in May, 2014. Five individual 19.6 kg N ha^{-1} applications were evenly spaced across the growing season to emulate a traditional inorganic lawn care program using a combination of urea and sulfur coated urea products.

¶ Number of flowers were visually counted in each plot prior to clipping harvests.

Means in the same column followed by the same letter are not significantly different according to Fisher’s protected least significance test (LSD P=0.05). *,*** and NS refer to significant at the 0.05, 0.0001 and non-significant, respectively.

Table 6. Seasonal changes in canopy greenness for a cool-season lawn species mixture grown with and without white clover (*Trifolium repens* L.-'Microclover') and two annual nitrogen (N) rates.

		Canopy greenness								
		2014				2015				
White clover†	Annual N rate‡	Spring¶	Summer	Fall	Mean	Spring	Summer	Fall	Mean	Study Mean
	kg N ha^{-1}	-- (color index)§ --								
No	0	0.708 ab	0.713 c	0.751 c	0.721 c	0.712 b	0.699 c	0.736 c	0.706 c	0.714 c
No	98	0.705 b	0.731 b	0.787 a	0.738 b	0.739 a	0.743 ab	0.770 a	0.743 ab	0.741 b
Yes	0	0.722 ab	0.742 ab	0.770 b	0.744ab	0.731 a	0.741 b	0.751 b	0.738 b	0.741 b
Yes	98	0.724 a	0.748 a	0.788 a	0.752 a	0.739 a	0.748 a	0.766 a	0.746 a	0.749 a
Source of variation										
Microclover (MC)		*	**	*	***	*	***	NS	***	***
Nitrogen (N)		NS	*	***	***	**	***	***	***	***
MC x N		NS	NS	*	NS	*	***	*	***	NS

† A commercially available cool-season lawn species mixture containing Kentucky bluegrass, perennial ryegrass, and fineleaf fescue was planted on 10 Aug., 2013 at 440 kg ha^{-2} 'Microclover' was included at 5% by weight, grass-only plots were established by applying a broad-spectrum broadleaf weed herbicide to remove clover following establishment.

‡ Granular N-fertilizer treatments began in May, 2014. Five individual 19.6 kg N ha^{-1} applications were evenly spaced across the growing season to emulate a traditional inorganic lawn care program using a combination of urea and sulfur coated urea products.

§ Canopy greenness was measured using a hand-held turf reflectance meter (Spectrum Technologies TCM-500 NDVI) at ten representative locations in each plot which were averaged into a single plot value and expressed as a unitless color index.

¶ Seasons were determined by astronomical seasons in the Northern Hemisphere. Spring: March 21 – June 19, Summer: June 20 – September 22, Fall: September 23 – December 20.

Means in the same column followed by the same letter are not significantly different according to Fisher's protected least significance test (LSD P=0.05). *,**,*** and NS refer to significant at the 0.05, 0.001, 0.0001 and non-significant, respectively

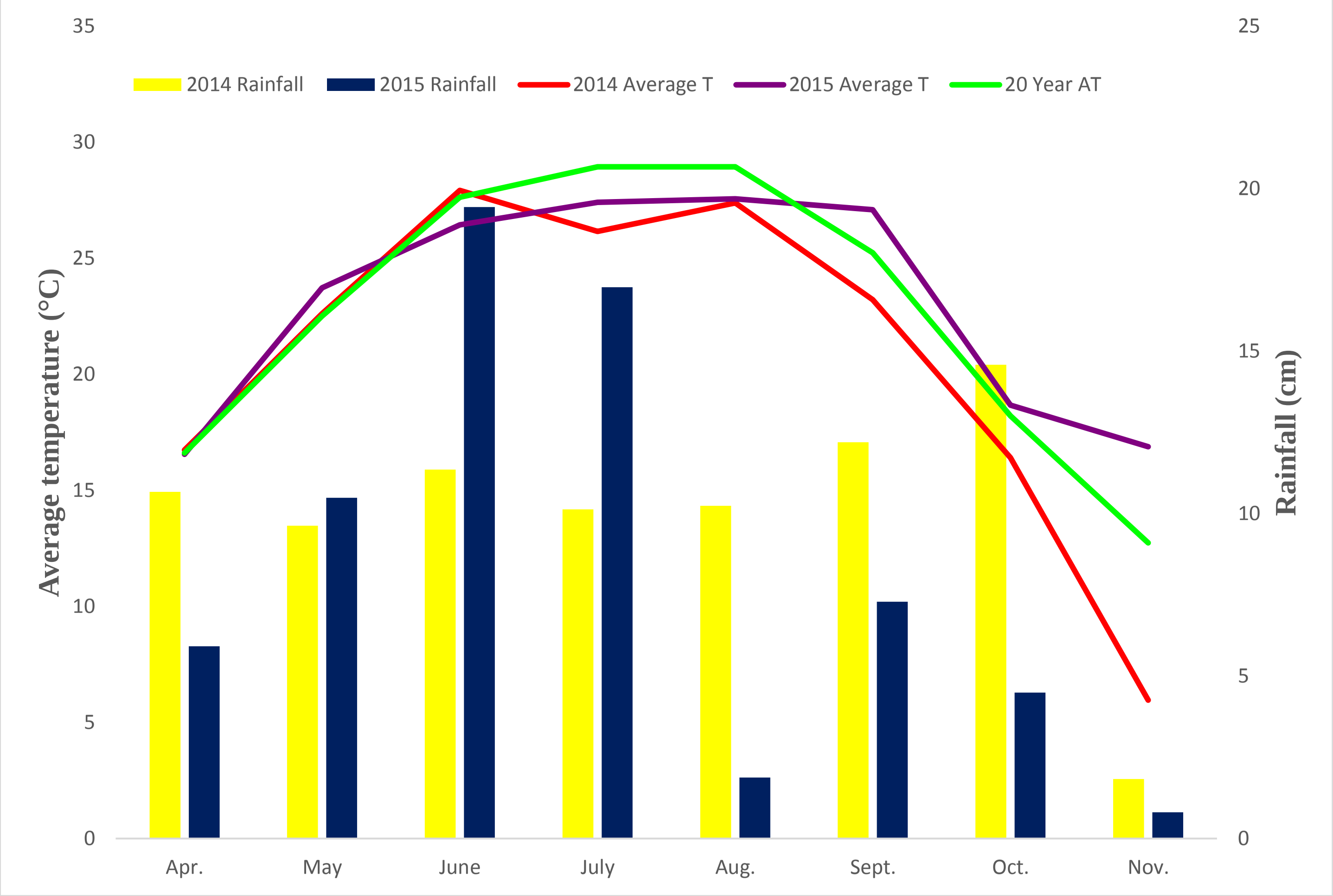

Figure 1. Average (A) temperature (T) (°C) and rainfall (R) (cm) from April to November in 2014 and 2015 compared to the 20 year average (1995-2015) in West Lafayette, IN

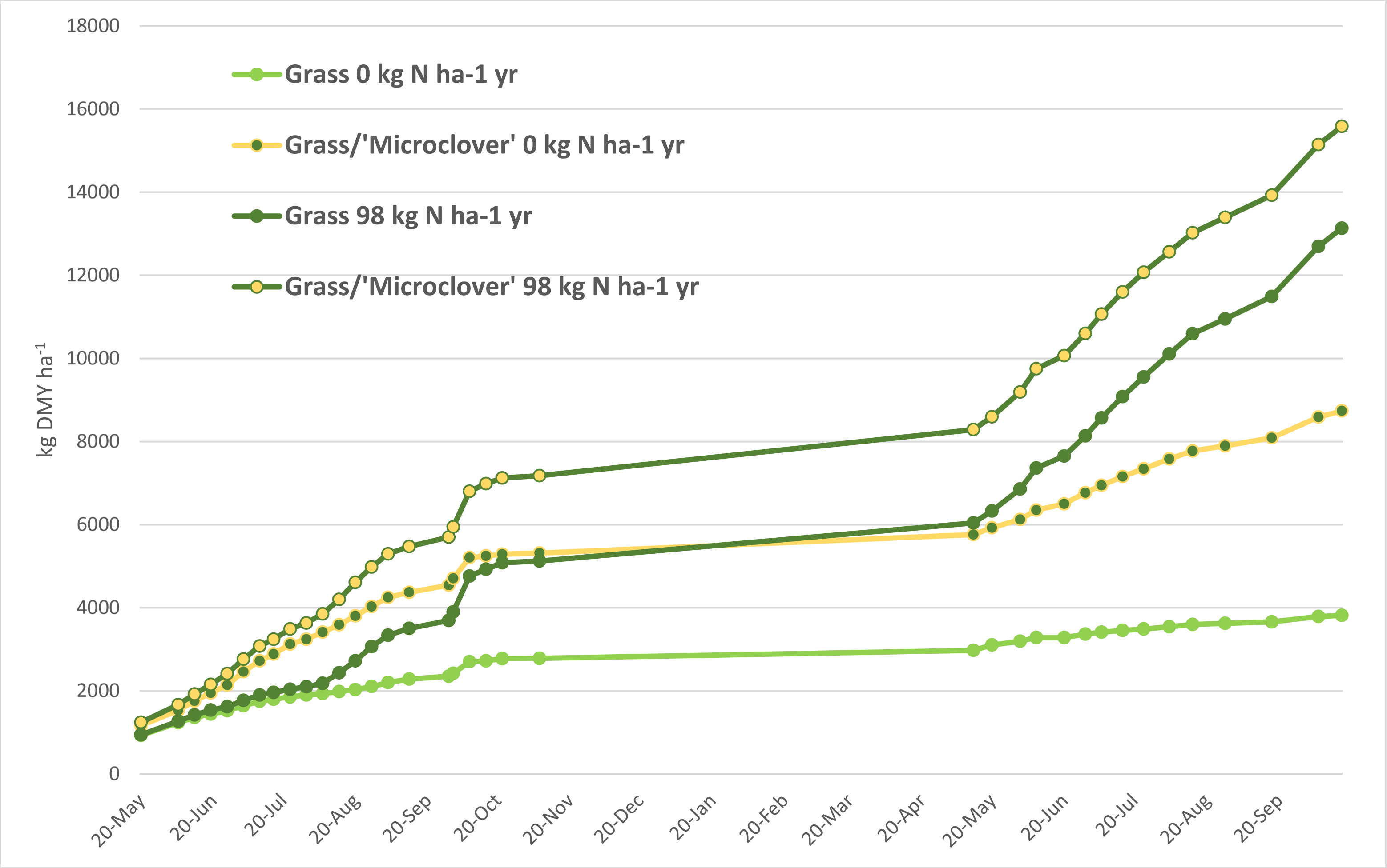

Figure 2. Cumulative seasonal dry matter yield (2014-2015) from a cool-season lawn turf.

CHAPTER THREE - SUMMARY, CONCLUSIONS AND FUTURE RESEARCH OPPORTUNITIES

Turf (lawns, roadsides, sports fields, golf courses, parks, cemeteries, etc.) in the United States occupies a large acreage of land which is estimated to be > 20.2 million ha (National Turfgrass Federation, 2009). Of the various turf segments, lawns dominate in most states, accounting for roughly 66% of all areas which is then followed by roadsides. To survive and persist, turf areas require at least some level of minimal supplemental nutrition which is often supplied by applying fertilizer often with nitrogen. The practice of lawn fertilization, if excess nitrogen (N) and/or phosphorus (P) is applied, has the capability to pollute surface water (Daniel et al., 1998), and ground water (Petrovic, 1990), disrupting the balance of rivers, lakes, and coastal estuary ecosystems. By contrast, there is also evidence that sod forming grasses, such as turfgrasses, provided with sufficient nutrition to form a dense shoot canopy and/or thatch layer can be an effective filter for reducing surface runoff and sediment loss, increasing filtration, and removing water from the soil reducing leaching (Linde et al., 1995, 1998; Ebdon et al., 1999). Furthermore, properly fertilized turf (e.g. not applying excessive rates of fertilizer and only applying fertilizer to actively growing turf) has been shown not to contribute to excessive nutrient losses (Easton and Petrovic, 2004; Bierman et al., 2010).

Even with proper fertilization practices, the general public, however, has a negative view toward contemporary lawn fertilization practices. Thus, large numbers of land-owners are strongly interested in more sustainable turf care practices that are perceived to be more “environmentally friendly” and/or require less inputs (i.e. mowing and fertilization). The challenge, however, is implementing these practices without compromising a high level of aesthetic or turf quality characteristics.

If excess N is a genuine concern, then alternative fertilizer management practices or novel turf systems need to be studied and proven before the public can adopt these alternatives. This thesis compared a tradition cool-season lawn mixture with a grass-legume lawn system where grass was combined with a novel white clover ‘Microclover’ (MC) and evaluated these turf areas receiving supplemental N fertilization (0 vs. 98 kg N $ha^{-1}yr^{-1}$). This MC, when compared to traditional forage-type white clover possesses smaller leaves and a more prostrate growth habit making it more compatible with most lawn systems. Specifically, the influence of MC and N were measured as dry matter yield (DMY) or clipping production, visual appearance characteristics, changes in MC populations, the expression of MC flowers, and canopy greenness (CG).

For the entire two-year study, both MC inclusion and supplemental N had very highly significant effects on DMY. DMY ranged from 3815 kg ha^{-1} in the unfertilized grass-only lawn mixture to 15583 kg ha^{-1} in the fertilized lawn mixture with MC. Furthermore, in both year one and year two of the study, and when broken up into seasons, unfertilized turf without MC consistently produced the least DMY, while fertilized turf with MC consistently produced the highest DMY.

In year two, when the components of DMY (e.g. percentage MC or grass in the DMY) were evaluated, N had a significant effect on the amount of MC that was present in the harvested samples in May and October. However, in July, N fertilization did not have a significant effect due to a shorter interval in between DMY harvests and lack of substantial growth in order for the treatments to separate.

Visual appearance ratings for the entire study ranged from 5.6 in unfertilized turf without MC to 8.3 in fertilized turf with MC. For comparison, a value of 6 equals an acceptable lawn turf appearance and 10 equals optimal green color, density and uniformity. Similar to DMY, both MC and N had very highly significant effects on visual appearance, and when split into seasons unfertilized turf without MC consistently had the lowest visual appearance and fertilized turf with MC had the highest appearance. For the overall study, unfertilized turf with MC and turf without MC receiving supplemental N had the same visual quality suggesting grass-MC mixtures may be a suitable alternative to traditional supplemental N fertilization practices.

Clover populations decreased at a much quicker rate over the entire two year study where supplemental N fertilization occurred. For example, there was 17 percent MC in May of year one and only 1 percent MC in October of year two. In general, N fertilization did not have a significant effect on MC populations until year two of the study in June and N fertilizer continued to have an effect through the end of the study. Clover populations in the unfertilized grass-MC turf also decreased during the study, but at a slower rate beginning at 13 percent in May of year one and ending at 5 percent in October of year two.

White clover flowers were most visible and prominent in the late-spring and summer months and when flowers were apparent, the highest flower counts occurred in mid-July and lowest in August. For the entire study, visual flower counts ranged from 12 to 80 flowers/plot in unfertilized turf with MC and 1 to 18 flowers/plot fertilized turf with MC. By mid-July of year one N fertilization had taken a significant effect on the number of flowers produced, reducing flower production in fertilized turf with MC compared to unfertilized turf with MC.

Canopy greenness values ranged from 0.714 in unfertilized turf without MC to 0.749 in fertilized turf with MC for the two year study. Similar to DMY and visual appearance measurements, MC and N fertilization effects were very highly significant for CG. Furthermore, across all seasons in both study years CG was highest in the fertilized turf with MC, while unfertilized turf without MC was lowest, except in the spring of year one where unfertilized turf without MC was slightly higher than fertilized turf without MC because the supplemental N fertilizer effects had not yet begun to take manifest.

Future recommendations to build on from this study include; possibly modifying the practice of N fertilization with respect to annual rate (e.g. slightly lower rates), application season, and alternative N-sources, while continuing to measure the responses of DMY and interspecific competition between grass and MC to maximize the potential benefits of MC inclusion in a lawn turf. In this study, 98 kg N ha^{-1} yr^{-1} produced excessive DMY, especially in year two. If the goal is a low-maintenance lawn system, rapid growth, excess clippings and frequent mowing is not a desirable characteristic. Instead of 98 kg N ha^{-1} yr^{-1}, annual rates could possibly be applied as low as 13, 24, and 49 kg N ha^{-1} yr^{-1}. Furthermore, the timing of N application could also be examined. While this study applied 98 kg N ha^{-1} yr^{-1} in five even applications across the growing season with a goal of providing consistent, slow, sustainable growth and balancing a desired population of grass/clover it negatively affected MC populations. Lower N-rates might achieve this allow for more MC persistence. Lastly, in this study inorganic granular urea-based N fertilizer products were applied, specifically urea and SCU which were applied as a mixture or alone. Other N sources such as polymer coated urea, an inorganic urea based fertilizer with a slower release rate, or slow release, natural organic fertilizers could be used to control excess DMY and perhaps minimize the loss of MC over time.

Ultimately, this field study demonstrated the feasibility of a grass-legume lawn system where MC was combined with a cool-season lawn mixture for the cool-humid region. It also showed that lower supplemental N-rates could be applied to these grass-legume systems than traditionally accepted lawn fertilization practices (e.g. > 98 kg N ha^{-1} yr^{-1}) for grass-only systems.

Many future ecological, environmental impact and soil health related studies could also be explored. The potential, however, for a grass-legume system to function as a refuge for pollinators in urban environments may be of particular interest

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APPENDIX

APPENDIX

Table A.1. Temporal variation of clover populations over two years using the line-intersect method of white clover present at four sampling dates in a traditional cool-season lawn species mixture grown with white clover (*Trifolium repens* L.-‘Microclover’) as affected by four annual organic nitrogen rates.

Annual N rate†	Clover Populations			
	2014		2015	
	16 June	29 July	28 May	31 July
kg N ha^{-1}	------------------Percentage (%)‡------------------			
0	20	21	15	11
98	22	23	17	12
146	23	24	19	11
195	31	18	16	7

† Granular N-fertilizer treatments began in May, 2014 with five individual applications of 0, 19.6, 29.2, and 39 kg N ha^{-1} spaced across the growing season to emulate an organic lawn care program. The N-source used was a 5-3-2 poultry manure based product. The fertilizer was watered into the turf within 12 hours of application.

‡ Percentage ‘Microclover’ was quantitatively measured using the line-intersect method and a 0.9 x 1.82 m grid with 253 intersects.