# Nitrogen Management in a Perennial Grain-Legume Intercropped System

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Master's Thesis

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### **Abstract**

Nitrogen pollution from intensified agricultural production is a major driver of ecosystem degradation, contributing to aquatic eutrophication and global greenhouse gas emissions.

Incorporating nitrogen-fixing leguminous intercrops into agricultural systems is an increasingly popular management strategy to mitigate those negative impacts. Using a perennial grain-legume intercropped agricultural system, this study aims to evaluate the potential for leguminous nitrogen fixers to support biomass yield of a well-developed perennial wheat, intermediate wheatgrass (Thinopyrum intermedium). The results of this study identified trends in improved forage quality, land use efficiency, and nitrogen fixation rates within the intermediate wheatgrass- alfalfa intercrop system. Findings also show that both white clover and alfalfa fix sufficient nitrogen to balance intermediate wheatgrass nitrogen uptake.

#### Introduction

Synthetic nitrogen fertilizer inputs to farms are major drivers of global change, damaging surrounding ecosystems through processes of nitrate leaching and aquatic eutrophication (Diaz and Rosenberg 2008), and emissions of greenhouse gasses such as nitrous oxide, which is particularly potent (IPCC 2019). Ecologically driven approaches to nitrogen management in agricultural systems focus on managing crop diversity and building soil organic matter to improve soil nitrogen cycling capacity, reducing the need for harmful external inputs (Drinkwater and Snapp, 2007). Perennial crops can play a key role in ecological nitrogen management because they have functional traits, such as deep root systems, that build soil organic carbon and retain nitrogen in soil (Glover et al. 2010). Incorporating perennial crops into agricultural systems may, therefore, provide a wider suite of ecosystem services than their annual counterparts (Dehaan et al. 2017).

Recent interest in the capacity for perennial cropping systems to mitigate the environmental consequences of annual crop production has led to greater investment in the development of perennial grain crops. Annual crops require continuous soil perturbations and fertilizer application, inefficiently provisioning nitrogen to the crops in those systems (Smith et al. 2013). Comparatively, multiple studies have found that perennial systems limit nitrate leaching and maximize the use of atmospheric nitrogen in the soil (Smith et al. 2013; Hauggaard-Nielsen et al. 2016; Culman et al. 2013; Dehaan et al. 2017). Intermediate wheatgrass (*Thinopyrum intermedium*) is among the most promising perennial wheat crops to date (Ryan et al. 2018), undergoing trait selection for increased grain yield and other agronomic traits by the Land Institute beginning in 1988 (Wagoner 1990). Although current yield potential of intermediate wheatgrass remains low relative to annual wheat, breeders expect intermediate wheatgrass to achieve comparable yields within the next 20 years (DeHaan et al. 2014). Grain

from improved lines is now sold as Kernza® to restaurants, bakeries, and other businesses in the United States for use in value-added products (Lubofsky 2016, Ryan et al. 2018).

Implementing multifunctional cropping practices could improve the efficiency of nitrogen management in perennial agroecosystems, grain yield of intermediate wheatgrass, and economic viability for farmers (Ryan et al. 2018). The functional traits of grass and legume plant species, for instance, are highly compatible in intercropping agricultural systems (Ryan et al. 2018, Brooker et al. 2014). Incorporating forage legumes into these systems as intercrops (i.e., the planting of multiple crop species in close spatial proximity) is a proposed method for sustaining intermediate wheatgrass yields while reducing or eliminating the need for nitrogen fertilizer inputs (Hauggaard-Nielsen et al 2016) and increasing farm enterprise diversity.

Intercropping can increase agricultural sustainability by supporting complementary interactions between species (Storkey et al. 2015, Finney and Kaye 2017, Blesh 2018). The nitrogen fixing capabilities of legumes and the nutrient scavenging capacity of perennial grass roots exhibit contrasting plant functional traits that could be optimized in an intercropping system (Booker et al. 2014). These interactions have the potential to increase multiple ecosystem services through the supply of new nitrogen to a field while increasing soil organic carbon and soil nutrient retention.

Two studies based in Minnesota examined an intermediate wheatgrass-alfalfa intercrop system. One found significant competition between the two crops with benefits appearing in later years (Tautges et al. 2018), while the other demonstrated higher yields of intermediate wheatgrass in the intercropped systems when compared to an intermediate wheatgrass monoculture (Jungers et al. 2019). Only one study, conducted in Australia, has examined nitrogen inputs to intermediate wheatgrass in a clover intercropping system, finding that

intercropping increased nitrogen fixation and supported higher grain yields compared to monocropping (Hayes et al. 2017).

In annual grain rotations, legume cover crops and forages can supply substantial nitrogen to fields through the process of biological nitrogen fixation, which can reduce nitrogen losses from fields (Blesh and Drinkwater, 2013) and increase overall sustainability of the management system (Drinkwater and Snapp, 2007). Within the context of perennial grain cropping systems, however, we still lack an understanding of the potential nitrogen (N) supply from different legume intercrops, including how legume N sources will impact grain yields. By examining a multifunctional system, this study aims to address these gaps to better understand the impacts of two legume intercrops on intermediate wheatgrass growth and soil nutrient cycling processes. The perennial legumes alfalfa (*Medicago sativa* L.) and white clover (*Trifolium repens* L.) have significant potential in intercropping systems due to their capacity to fix large quantities of nitrogen and their use as forage crops. Through periodic mowing, aboveground litter inputs from the forage legumes and belowground nitrogen inputs from roots could significantly contribute to nitrogen inputs and soil carbon and nitrogen cycling processes.

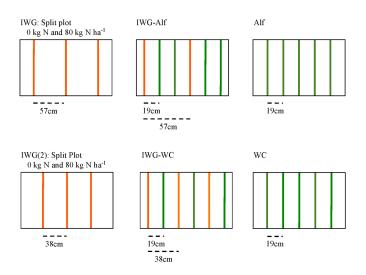
By applying ecological knowledge of interspecific interactions in intercropping systems (Brooker et al. 2014), this study will examine nitrogen cycling dynamics within two novel perennial intercropping systems: either white clover or alfalfa and intermediate wheatgrass. Specifically, we quantify and compare the nitrogen supply from legume N fixation by two forage species in intermediate wheatgrass intercrops and, further, begin to explore the effects of intercropped legumes (white clover and alfalfa) on intermediate wheatgrass yields and nitrogen content using a forage system framework. We expected to find that legume forages will have higher rates of nitrogen fixation in the intercropped treatments while overall nitrogen supply will be higher in the forage monocultures. Further, we expected higher yields of intermediate

wheatgrass from the intercropped treatments relative to the intermediate wheatgrass monocultures.

## Materials Methods

# Experimental Design

Using a randomized complete block design, the experiment was planted at the UM Campus Farm in September of 2019 with eight treatments and four replicates (Figure 1). The plot had previously been in an unmanaged fallow for 30 years, with only occasional mowing. A cover crop mixture of sorghum sudangrass and buckwheat was planted in the Summer of 2019 in order to suppress weed growth and build soil fertility. The experimental plot measured approximately 0.5 hectares with a Fox sandy loam soil series. Each plot measures four meters x eleven meters with a six-meter path on all four sides. The treatments include: i) intermediate wheatgrass monoculture (IWG); ii) intermediate wheatgrass intercropped with alfalfa (IWG-Alf); iii) alfalfa monoculture (Alf); iv) intermediate wheatgrass monoculture (IWG(2)); v) intermediate wheatgrass intercropped with white clover (IWG(2)-WC); vi) white clover monoculture (WC). Treatments i) and iv) are each intermediate wheatgrass monoculture treatments, but with different row spacings where treatment IWG was planted with 57 cm row spacing and treatment IWG(2) was planted with 38 cm row spacing. This was done so that each monoculture treatment could be paired with the respective legume intercrop (IWG-Alf and IWG(2)-WC) for analysis. The intermediate wheatgrass monoculture treatments were split plots, where ½ of the plot received N in starter manure with no additional inputs for the remainder of the experiment. The outstanding  $\frac{2}{3}$  of the plot was treated with additional N as bloodmeal at 80 kg N ha<sup>-1</sup>.



Schematic of the experimental

design, showing the six treatments.

Figure 1.

### Soil Sampling

Baseline soil samples were taken ahead of planting to twenty cm depth and were analyzed for total organic carbon and nitrogen by dry combustion on a Leco TruMac CN Analyzer (Leco Corporation, St. Joseph, Michigan). Baseline samples were also analyzed for soil moisture and inorganic nitrogen. Soil moisture was determined by drying the samples at 105 degrees Celsius for 48 hours. Soil samples were then sieved to 2 mm for analysis of extractable inorganic N (NO<sub>3</sub>- and NH<sub>4</sub>+) with 2 M KCL.

These baseline samples were compared with samples collected in the summer of 2021 to determine changes in soil health over time after two years of legume and IWG growth. This includes changes in soil nitrogen cycling capacity in the alfalfa intercrops and monocrops using a field incubation method with buried soil cores (Robertson et al. 1999) after mowing biomass in late May and late July. Cores were extracted in triplicate with 2 M KCl and analyzed for NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>.

Nitrogen Fixation Analysis

N<sub>2</sub> fixation by alfalfa and white clover, respectively, was estimated based on the <sup>15</sup>N natural abundance method (Shearer and Kohl 1986). The natural abundance method requires an enriched soil N pool in order to accurately estimate the proportion of nitrogen derived from legume fixation. Legume biomass production was quantified by sampling aboveground biomass to 10 cm height from two 0.25 m<sup>2</sup> quadrats per plot in late May 2021 and again in late July 2021 immediately before intermediate wheatgrass harvest. Biomass was separated by species, dried to constant mass at 60 degrees C, and then ground to < 2 mm in a Wiley Mill. Samples collected in May of 2021 were further ground to a fine powder using a ball mill and analyzed using stable isotope methods to quantify N<sub>2</sub> fixation by the two legume species using nonlegume perennial weeds as a reference species (Shearer and Kohl, 2986; Blesh, 2019). This method was also used to quantify the amount of nitrogen transferred from the legume intercrops to intermediate wheatgrass in the intercropped treatments. Samples were analyzed for total C and N content, and for <sup>15</sup>N on a continuous flow Isotope Ratio Mass Spectrometer (Stable Isotope Facility, UC Davis).

%N from fixation =  $100*((\delta^{15}\text{Nref-}\delta^{15}\text{Nlegume})/(\delta^{15}\text{Nref-}B))$ 

In this equation, the B is used to account for isotopic fractionation within the legume and represents the  $\delta^{15}N$  value of a legume entirely dependent upon atmospheric N. In this study the B value was derived from the lowest collected  $\delta^{15}N$  values for alfalfa and white clover, set at -1.25 and -1.67, respectively.  $\delta^{15}N$  refrepresents a non-N fixing reference plant, for which we measured the  $\delta^{15}N$  content of weeds growing near the experimental plot.

Intermediate Wheatgrass harvest and grain analysis

Intermediate Wheatgrass was harvested on August 9, 2021. Grain yield was determined by sampling intermediate wheatgrass from two, 1m x 0.5m (0.5m²) sampling frames randomly placed within each plot. The sampling frames were then aligned to comprise a length of 1m in the intermediate wheatgrass treatment row. After counting all of the seed heads within the sampling area, the intermediate wheatgrass was cut to a 10cm stubble height. The samples were subsequently dried at 60°C for at least 72 hours, then weighed. Dried samples were threshed to remove grain from stems, and the resulting grain was then cleaned of debris and weighed with hulls to record grain yield. Grain weight was subtracted from the total sample weight for vegetative biomass weight, referred to as intermediate wheatgrass biomass. Intermediate wheatgrass biomass (IWG) was ground to < 2mm using a Wiley Mill and the grain was finely ground using a small grinder. IWG biomass and grain samples were analyzed for C and N by dry combustion using a Leco TruMac CN analyzer (Leco Corporation, St. Joseph, Michigan, USA). Land Equivalent Ratio

Using values averaged across the four experimental blocks, the partial land equivalent ratio for N (LER N) and the land equivalent ratio (LER) were calculated. LER values are a measure of land use efficiency between intercropped and monocropped systems. An LER greater than 1 indicates that crops grown in intercrop are more land efficient than when grown as separate monocrops. *Statistical analysis* 

In the programming software R-4.0.2, grain yield, biomass production, biomass quality (%N, C:N), aboveground nitrogen (kg N/ha), and nitrogen fixation rate were used as response variables in ANOVA mixed-effects models with treatment as a fixed effect and replicate block as

a random effect. Where effects are significant, Tukey's HSD was used to compare treatment means.

# Results

# Soil properties

Basic soil properties and soil health parameters, including total organic C and potentially mineralizable N (PMN), were analyzed in each of the four experimental blocks before planting in 2019. The soil is a sandy loam with high fertility levels due to its history as a grassland fallow for over 30 years.

Table 1 Baseline Soil Health Characteristics 2019

Soil Characteristic	Value
Soil Textural Classification	Sandy Loam
pН	6.05(0.09)
C (Mg/ha)	43.19 (2.30)
N (Mg/ha)	3.96 (0.14)
P (ppm)	33 (3.7)
PMN (mg N/kg dry soil/week)	13.745 (0.82)

*Note:* Values in parentheses are standard errors of the mean

Table 2 Mean Biomass

Treatment	Legume Biomass (Mg/ha)	IWG Biomass (Mg/ha)	Legume Total N (kg/ha)	IWG Total N (kg/ha)	Total Fixed N (kg/ha)	Total Fixed N, Literature Values (kg/ha)
IWG	!	4.16 (0.84) <sup>a</sup>	!	40.93 (9.57)	1	1
IWG-Alf	2.93 (0.32) <sup>a</sup>	1.99 (0.28) <sup>a</sup>	96.00 (9.77) <sup>a</sup>	22.47 (3.54)	88.51 (11.49) <sup>a</sup>	67.20 (6.84)
Alf	5.83(0.36) b		188.85 (12.07) <sup>b</sup>		150.90 (9.78) <sup>b</sup>	132.20 (8.45)
IWG(2)	1	9.68 (0.73) <sup>b</sup>	!	104.23 (9.22)	I	ł
IWG(2)-WC	1.68 (0.24) <sup>a</sup>	2.25 (0.78) <sup>a</sup>	$51.39 (6.60)^{a}$	23.69 (7.97)	38.32 (3.74) <sup>a</sup>	41.11 (5.28)
WC	7.02 (0.44) <sup>b</sup>	1	217.00 (18.6) <sup>b</sup>	!	183.70 (21.70) <sup>b</sup>	173.60 (14.88)

Note: Values in parentheses are standard errors of the mean.

# Nitrogen Fixation

For each treatment containing a legume, the proportion of aboveground N derived from biological nitrogen fixation exceeded that of N derived from the soil. IWG(2)-WC had the lowest mean total aboveground N and the lowest N supply from fixation (38.34 kg/ha) of all treatments. IWG-Alf had a greater total aboveground N than IWG(2)-WC, supplying on average 50.17 kg/ha more nitrogen to the system (p<0.1). There was no significant difference between IWG(2)-WC and IWG-Alf in the percent nitrogen derived from the atmosphere (p=0.147), where IWG-Alf provided 91% nitrogen from fixation and IWG(2)-WC provided 75% (Table 3). The Alf treatment fixed 62.39 kg N/ha more than IWG-Alf, though IWG-Alf fixed a greater proportion of its total aboveground N (11% more) than Alf. WC derived 84% of its nitrogen from fixation and provisioned 145.36 kg N/ha more total aboveground N than its intercropped counterpart (p<0.0001).

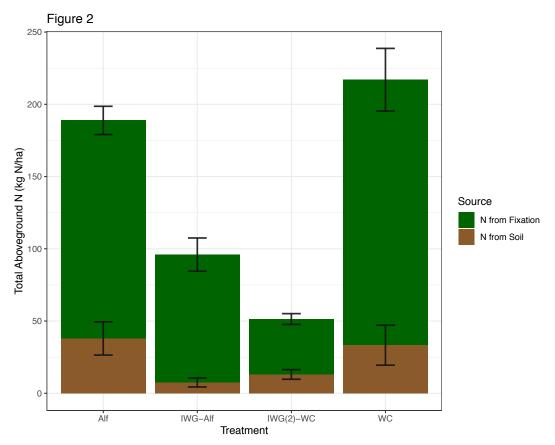


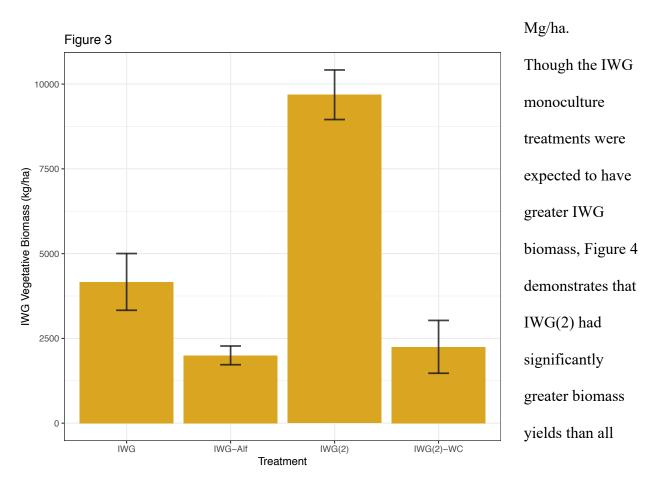
Table 3 Nitrogen from Fixation

Treatment	Mean Percent Nitrogen Derived	Mean Percent Nitrogen
	from Atmosphere	Derived from Atmosphere
		(literature values)
IWG-Alf	91(0.04)	70
Alf	80 (0.05)	70
IWG (2)-WC	75 (0.04	80
WC	84 (0.06)	80

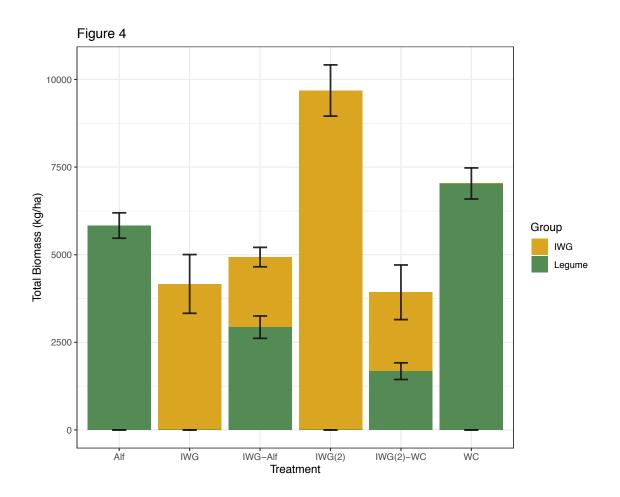
Note Values in parentheses are standard errors of the mean

# Intermediate Wheatgrass Yield

Across all treatments, IWG(2) had a significantly greater yield of intermediate wheatgrass vegetative biomass than all other treatments at 9.68 Mg/ha (p<0.05). No statistical significance in IWG biomass was found between treatments IWG, IWG-Alf and IWG(2)-WC. In IWG-Alf, the average IWG biomass yield was 1.99 Mg/ha, while IWG(2)-WC yielded an average of 2.25



other treatments when also accounting for legume biomass production. In legume biomass yields, IWG-Alf yielded 1250 kg/ha more biomass than IWG(2)-WC (p=0.1001).



# Land Equivalent Ratio

The IWG-Alfalfa intercrop system was more land-use efficient (LER=1.09) than these crops grown as sole crops. Conversely, the IWG-WC intercrop system was less land efficient (LER=0.46) than sole cropping. LER values calculated for N yield (LER N) followed similar patterns as the LER values for forage production, where LER N for alfalfa was 1.25 while LER N for white clover was 0.46.

Table 4 Mean Land Equivalent Ratios by Intercrop Treatment

	Alfalfa	White Clover
pLER (legume)	0.51 (0.07)	0.24 (0.02)
pLER (IWG)	0.58 (0.17)	0.23 (0.08)
LER	1.09 (0.22)	0.46 (0.06)
pLER N (legume)	0.52(0.07)	0.24 (0.02)
pLER N (IWG)	0.73 (0.26)	0.22 (0.08)
LER N	1.25 (0.30)	0.46 (0.06)

Note Values in parentheses are standard errors of the mean

# Potentially Mineralizable Nitrogen

Using potentially mineralizable nitrogen as an indicator of nitrogen availability from organic matter (Drinkwater et al 1996), the 2021 values measured indicate that white clover increases soil N availability more than alfalfa with 14.41 mg/kg of PMN. There is, however, no significant difference between the treatments.

**Table 5** Potentially Mineralizable Nitrogen by Treatment

Treatment	PMN (mg/kg soil/week) 2021
IWG	11.57 (1.29) <sup>a</sup>
IWG-Alf	9.69 (0.14) <sup>a</sup>
Alf	9.69(0.82) <sup>a</sup>
IWG (2)	9.74 (1.89) <sup>a</sup>
IWG (2)-WC	13.83 (2.01) <sup>a</sup>
WC	14.41 (2.95) <sup>a</sup>

*Note* Values in parentheses are standard errors of the mean

# Discussion

The use of leguminous intercrops is an established management strategy to mitigate nitrogen pollution from intensive agricultural production by addressing the need for external nitrogen inputs (Drinkwater and Snapp 2007). The complementary traits of the perennial grain, intermediate wheatgrass and leguminous nitrogen fixers have the potential to further improve

nitrogen management in agricultural systems. Intercropping this perennial grain crop with legumes may also improve the viability of the perennial grain by improving biomass production and forage quality. To improve the understanding of perennial grain production and yield in these intercropped systems, we tested hypotheses on the interactions between intermediate wheatgrass and both alfalfa and white clover. In this system, we predicted that rates of biological nitrogen fixation would be higher in the intercropped plots, and thus intercropping would increase intermediate wheatgrass yield and multiple soil health parameters compared to monocropping.

### White clover versus Alfalfa

Biological nitrogen fixation, intermediate wheatgrass biomass production, and the land equivalent ratio calculation were the key response variables in examining intercrop treatment performance. Overall, the intercrop alfalfa performed better than the white clover intercrop, supplying significantly more total aboveground nitrogen to the system (Figure 2). The values recorded for both percent nitrogen derived from the atmosphere (Table 3) and intermediate wheatgrass vegetative biomass (kg/ha) (Figure 3), though not significantly different in the intercrops, suggest a trend of improved soil health and intermediate wheatgrass biomass production in the alfalfa intercrop relative to white clover intercrop treatment. This study analyzes only one season of growth in each treatment; thus, the temporal scale of this analysis is small relative to the accrual of benefits observed in perennial grain systems over time.

From our calculations of IWG, alfalfa, and white clover forage production, IWG and alfalfa grown as sole crops would require 1.09 ha of land total to achieve the same forage production as 1 ha of the IWG-Alf intercrop. IWG and white clover, in contrast, would only require 0.46 ha of

land when grown as sole crops to achieve the same forage production as 1 ha of the IWG-WC intercrop. Partial LER Ns for alfalfa and IWG in the alfalfa system indicate that the higher LER N was partially driven by higher N in IWG when intercropped with alfalfa compared to the IWG treatment (Table 4).

To replicate on-farm conditions, two different row spacings were used for the IWG plantings. The IWG-Alf treatment used the 57cm row spacing, while IWG(2)-WC used the 37cm row spacing. We expected the denser row spacing to yield greater quantities of IWG biomass; however, in the intercrop it is likely that this closer spacing facilitated competition between IWG and white clover. Both treatments used a 19cm row spacing between the legume plantings. As is indicated in the total biomass production from each treatment, the 38 cm row spacing was advantageous for IWG but may have been detrimental to white clover production. Competition for resources and suppressed white clover growth would explain why the higher rates of PMN (Table 5) in the white clover monocrop and increased IWG biomass production in the IWG(2) treatment were not reflected in the IWG(2)-WC treatment.

### Nitrogen Fixation and Uptake

Treatments Alf and WC, the two legume monoculture plots, had the highest rates of nitrogen fixation and the most total aboveground nitrogen relative to their corresponding intercropped treatments (Figure 2). Between the legume monocultures, no significant differences were found in nitrogen fixation quantities, implying that each legume crop has the potential to support IWG growth in a forage system. In the intercropped plots, IWG-Alf fixed significantly more nitrogen to the system compared to IWG(2)-WC. The low amounts of fixed N in IWG(2)-WC may have been the result of competition between the two crops, where white clover growth was

suppressed. It was observed that each legume monocrop fixed more nitrogen than its corresponding intercrop, supporting this inference.

The measured value of fixed nitrogen in the intercrop plots of both alfalfa and white clover indicate that these legumes fix a sufficient amount of atmospheric nitrogen to balance nitrogen uptake by intermediate wheatgrass (Table 2). This information, coupled with the collected data suggesting that each legume species supplied a significant amount of nitrogen to the system, indicates that a rotational design with an intercrop may be an efficient nitrogen management strategy. It is important to note that the conditions of this study site were ideal to support both legume and intermediate wheatgrass growth. Relative to typical on-farm conditions, the experimental plots in this experiment had higher fertility and may impact the performance of these systems on farms (Table 1). This study was also spatially specific to Southeastern Michigan; therefore, it is likely that these systems would exhibit different interactions and performance in other ecoregions.

# Intermediate Wheatgrass Biomass Yield

The IWG(2) treatment had significantly greater vegetative biomass production than all other treatments, where no statistical significance was found between the other treatments included in this analysis. This result is likely due to the 38 cm row spacing used in this treatment, where the other IWG monocrop plot used a 57 cm row spacing and, thus, had fewer plantings of IWG. Despite each IWG monocrop treatment receiving fertilizer applications, the closer row spacing yielded significantly more vegetative biomass and acts as a reference to IWG yield potential more generally.

# Intermediate Wheatgrass as a Forage Crop

Grain yields in each of these plots were low relative to annual grain yields in industrial agricultural production (Dehaan et al. 2014). As such, intermediate wheatgrass is not an economically competitive crop. Implementing the proposed intercrop as a forage system would improve economic viability for farmers while introducing nitrogen management benefits (Dick et al 2018r). The presence of forage legumes is beneficial to soil nitrogen content, which is another economically viable option given the reduced need for synthetic nitrogen fertilizers (Hauggaard-Nielsen et al. 2016). The most consistent thread found throughout previous studies on these systems is that the implementation of perennial cropping systems necessitates multifunctionality in the form of vegetative biomass collection, intercropping, or functionally diverse systems (Dick et al. 2018). Those findings were consistent with the data collected in this study.

### Intermediate Wheatgrass Forage Quality

The final metric of intercrop treatment performance was to determine changes in forage quality. This was quantified using measures of the carbon to nitrogen ratio and percent nitrogen found in IWG forage by treatment. Though no significant differences were found in this first year of growth, the results indicate a potential trend of improved IWG forage quality in the IWG-Alf intercrop relative to the IWG monocrop treatment (p=0.11).

#### Future Directions

Research on improving nitrogen management through the implementation of leguminous intercrops, and multifunctional systems, will accelerate the widespread application of these management practices. This also contributes significantly to efforts to address the global

sustainability problem of nitrogen pollution from synthetic fertilizer applications on agricultural landscapes. Our approach of mimicking on-farm conditions through row spacings, and both additive and replacement treatment designs, furthers this objective. Our field experiment yielded several results indicating improved IWG performance when intercropped with the legume alfalfa. We also found that both legume study species, alfalfa and white clover, supply sufficient nitrogen to the system to meet IWG nitrogen demand, but low IWG biomass yields in the intercrops suggest IWG was still N limited. This indicates that N synchrony may be more critical in supplying nitrogen to IWG in legume intercrop systems than total N in the system.

These data suggest the importance of long-term studies to more accurately capture the accrual of benefits to soil health, biomass yield, and nutrient cycling dynamics that are likely to occur in this system. This includes exploring in-season synchrony that may occur between nitrogen mineralization and IWG nitrogen uptake. Similarly, expanding the ecoregions represented in the literature on IWG systems will better inform farm practices by establishing more comprehensive data on the viability of IWG cultivation on a broader geographic scale.

#### Conclusion

The continued intensification of agricultural production has exasperated conditions of GHG emissions and nutrient pollution, perpetuating the degradation of natural habitats and issues of environmental injustice. Increasing farm functional diversity through implementing leguminous intercrops is an important strategy for addressing these sustainability challenges. Utilizing the nitrogen-fixing capacities of legume crops has the potential to reduce the demand for synthetic nitrogen fertilizers, and so addresses excess nitrogen fertilizer runoff from agricultural

landscapes. Furthermore, incorporating perennial grains into these systems has significant potential to improve farm soil health conditions (Hauggaard-Nelson et al 2016).

A forage system framework may improve the economic viability of the perennial grain-legume intercrop system, where grain production is currently not competitive with that of annual wheat. Though grain production is not yet competitive, the results of this analysis show that an intercropped system with intermediate wheatgrass and alfalfa is a viable management strategy for farmers in southeastern Michigan. Our findings on white clover were also promising and may indicate future trends of improved IWG biomass production in IWG-white clover intercropped

systems.

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# Appendix: Photographs of Treatment Plots



Intermediate wheatgrass intercropped with alfalfa.



Intermediate wheatgrass forage growth.